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Restoration of Antibiotic Effectiveness with *P. hartigii* Extract Against Multidrug-Resistant *E. coli*

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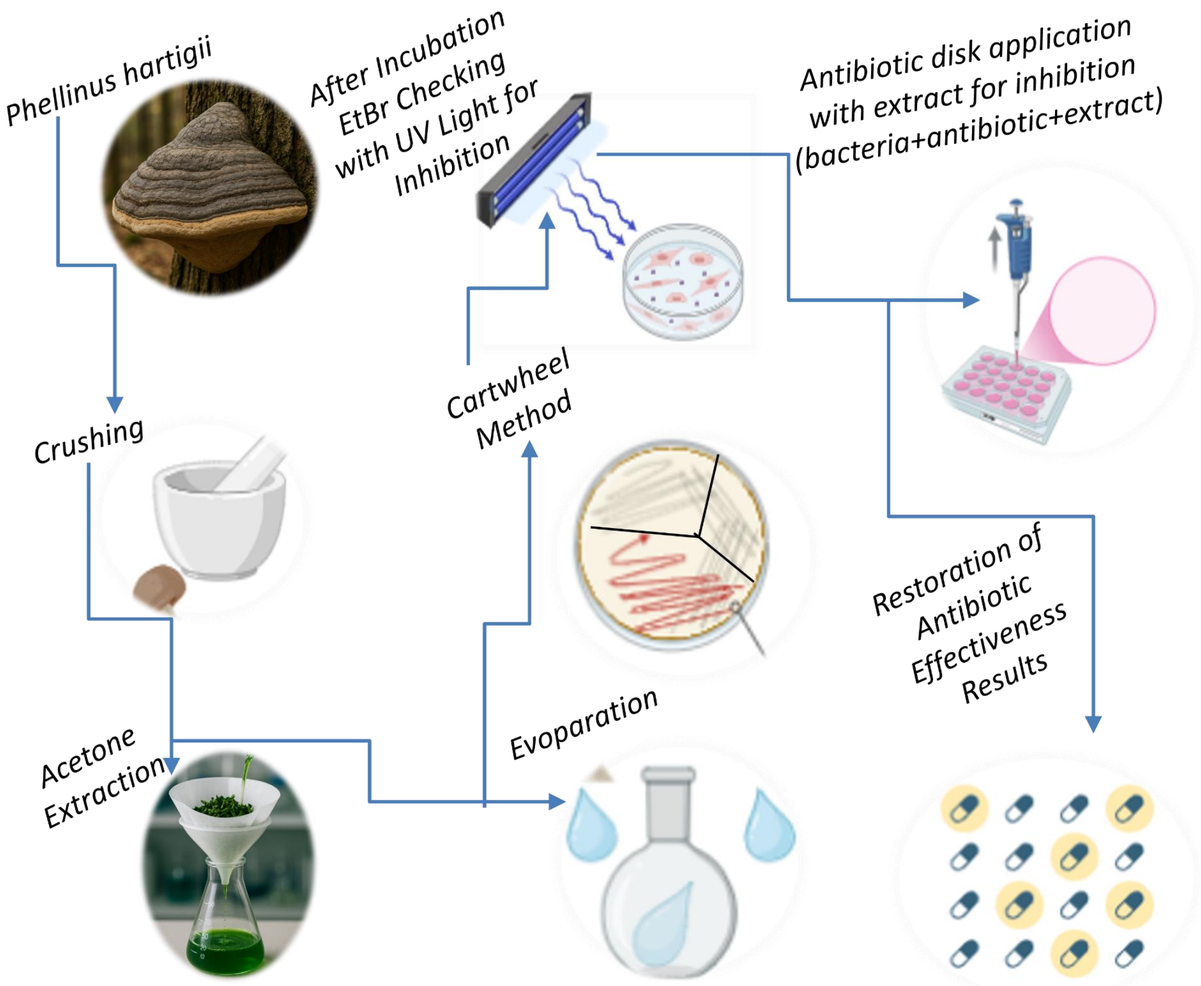
GRAPHICAL ABSTRACT



ABSTRACT

Studies on multidrug-resistant microorganisms, especially *Escherichia coli* (*E. coli*) strains, continue worldwide. In addition to the discovery of new antibiotics, there is also significant research on resistance mechanisms. One such mechanism is the efflux pump, which rapidly expels substances (antibiotics) from the bacterial cell or reduces their concentration. Therefore, the effectiveness of antibiotics can be restored by preventing this mechanism, and studies on potential inhibitors in this direction are ongoing.

In this study, the potential inhibitory effect of acetone extract obtained from *Phellinus hartigii* (*P. hartigii*) against drug-resistant *E. coli* strains was investigated. This was done by using the extract in combination with the antibiotics aztreonam (ATM 30 µg), cefixime (CFM 5 µg), amoxicillin–clavulanate (AMC 30 µg), piperacillin–tazobactam (TZP 36 µg), and ceftriaxone (CRO 30 µg) (Oxoid, UK).

For the first stage of inhibition, the Ethidium Bromide (EtBr) test was used with *P. hartigii* acetone extract. After observing inhibition according to the test results, antibiotic disks were applied together with the extract, antibiotic, and resistant strains. As a result of this study, it was observed that activities against *E. coli*#3 strain ATM (MIC 4), *E. coli*#10 strain ATM (MIC 8), *E. coli*#7 strain TZP (MIC >16/4), and *E. coli*#8 strain TZP (MIC >16/4) were restored. Based on these findings, *P. hartigii* acetone extract may have a potential efflux pump inhibitory effect, but further studies are required to explain this effect more broadly.

Key Words: Efflux pumps, antibiotic resistance, *E. coli*, *P. hartigii*

INTRODUCTION

- Efflux pumps are present in both eukaryotic organisms—such as molds, fungi, yeasts, and humans and prokaryotic organisms like bacteria (1).
- Some natural efflux pump inhibitors used in *in vitro* studies include reserpine (2, 3), piperine (4,3), berberine (5, 6), and geraniol (7,5).

MATERIAL & METHOD

***P. hartigii*:** Macrofungus / **Extraction:** Provided by acetone extraction / **EtBr Test:** First stage of inhibition / **Antibiotic Discs:** ATM 30 µg, CFM 5 µg, AMC 30 µg, TZP 36 µg, CRO 30 µg (Oxoid, UK).

- ✓ The ethidium bromide cartwheel method was employed to evaluate the inhibition of efflux pumps by the extracts. Initially, TSB agar plates containing different EtBr concentrations (0.0–2.5 mg/L) were used to determine the maximum EtBr level that *E. coli* could expel. Next, extracts at MIC/2 were applied to these plates to test for inhibition. After incubating for 24 hours at 37°C, the plates were exposed to UV light to check for EtBr retention, which indicated the inhibition of the efflux pumps (Martins et al. (2010).
- ✓ Last step, extracts that showed efflux pump inhibition were mixed with antibiotics the strains were resistant to. Each well of a 24-well plate received 1 mL of Mueller Hinton Broth, the resistant antibiotic, 100 µL of extract (at MIC/2 concentration), and the microorganism inoculum, followed by incubation at 37°C for 24 hours.

RESULTS & CONCLUSION

Restoration of Antibiotic Effectiveness

Microorganism	*Antibiotic*
E. coli#3	ATM
E. coli#10	ATM
E. coli#7	TZP
E. coli#8	TZP

After observing inhibition according to the test results, antibiotic disks were applied together with the extract, antibiotic, and resistant strains. As a result of this study, it was observed that activities against *E. coli*#3 strain ATM (MIC 4), *E. coli*#10 strain ATM (MIC 8), *E. coli*#7 strain TZP (MIC >16/4), and *E. coli*#8 strain TZP (MIC >16/4) were restored. Based on these findings, *P. hartigii* acetone extract may have a potential efflux pump inhibitory effect, but further studies are required to explain this effect more broadly.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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