# BIOLOGICAL ACTIVITIES OF *ALPINIA PAHANGENSIS* AND *ALPINIA MUTICA* EXTRACTS

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## Abstract

The aim of the present study is to investigate the biological activities of two selected wild *Alpinia* species namely, *Alpinia pahangensis* Ridl. and *Alpinia mutica* Roxb. Extraction of the rhizomes and leaves was done successively using three solvents with different polarity: hexane, dichloromethane and methanol. The crude extracts were tested for their antimicrobial and antioxidant activities. The antioxidant activity was determined using the DPPH free radical scavenging method. The antimicrobial activity was investigated based on disc-diffusion assay and minimum inhibition concentration (MIC) against 12 microorganisms consisting of six gram positive bacteria: *Bacillus cerues, Bacillus subtilis, Staphylococcus aureus, Streptococus mutans, Streptococcus sanguis* and *Streptococus mitis* (Streptococci species are oral bacteria), three gram negative bacteria: *Proteus vulgaris, Pseudomonas aeruginosa* and *Escherichia coli* and three unicellular fungi: *Candida parapsilosis, Candida albican* and *Schizosaccharomyces pombe*.

The yield of crude extracts from the rhizomes for both species of *Alpinia* was higher compared to those from the leaves with the highest yield (7.16 %) obtained from the rhizome of *A. mutica* (methanol extract). In comparing the effect of solvents on crude extract yield, it was found that the increase in yield correlated directly with the solvent polarity except for the leaves of *A. pahangensis* where the yield was higher (3.16 %) in dichloromethane extract compared with the methanol extract (1.78 %).

In the antimicrobial assay, the rhizome hexane and dichloromethane extracts of *A. pahangensis* showed strong inhibition against gram positive bacteria namely *Bacillus cerues* and *Bacillus subtilis* with an MIC value (3120 µg/ml) close to the standard reference, Streptomycin (2500 µg/ml), except for the dichloromethane extract against *Bacillus cerues* with MIC at 12,500 µg/ml. All extracts failed to exhibit any inhibition against all the tested unicellular fungi and two gram positive oral bacteria namely *Streptococus mutans* and *Streptococcus sanguis*.

In DPPH free radical scavenging assay, *A. pahangensis* rhizome methanol extract demonstrated the lowest $IC_{50}$ value (1.71 mg/ml) but less scavenging activity compared to ascorbic acid (0.085 mg/ml). The results obtained suggest that the antimicrobial compounds in both plants maybe found in the extract of the less polar solvents (hexane and dichloromethane) while the antioxidant compounds, particularly in the rhizomes, maybe found in the extract of the polar solvent (methanol).

Therefore, it can be summarized that of the two *Alpinia* species studied, *A. pahangensis* showed greater response and potent biological activities compared to *A. mutica*, which may due to the synergistic effects of the compounds present in *A. pahangensis*, an unexploited wild ginger species.

## Abstrak

Penyelidikan ini bertujuan untuk mengkaji aktiviti biologi dua spesies *Alpinia* liar iaitu *Alpinia pahangensis* Ridl. dan *Alpinia mutica* Roxb. Pengekstrakkan rizom dan daun telah dijalankan secara berturutan menggunakan tiga pelarut dengan kepolaran berbeza: heksana, diklorometana dan metanol. Kesemua ekstrak mentah kemudiannya diuji untuk aktiviti antimikrobial dan antioksidan. Aktiviti antioksidan telah dinilai menggunakan esei penghapusan radikal bebas DPPH. Aktiviti antimikrobial esei difusi disk dan esei kepekatan perencatan yang minimum telah dijalankan terhadap 12 mikroorganisma yang terdiri daripada enam bakteria gram positif: *Bacillus cerues, Bacillus subtilis, Staphylococcus aureus, Streptococus mutans, Streptococcus sanguis* dan *Streptococus mitis*: spesies Streptococci ialah bakteria dalam mulut (oral), tiga bakteria gram negative: *Proteus vulgaris, Pseudomonas aeruginosa* and *Escherichia coli* dan tiga fungi unisel: *Candida parapsilosis, Candida albican* dan *Schizosaccharomyces pombe*

Hasil ekstrak mentah untuk kedua-dua spesis *Alpinia* dari bahagian rizom adalah lebih tinggi berbanding dengan bahagian daun dengan hasil tertinggi (7.16 %) diperolehi daripada rizom *A. mutica* (ekstrak metanol). Dalam membandingkan pengaruh pelarut ke atas hasil ekstrak mentah, pertambahan hasil ekstrak berkorelasi secara langsung dengan kepolaran pelarut kecuali untuk bahagian daun *A. pahangensis* di mana ekstrak diklorometana (3. 16 %) adalah lebih tinggi jika dibandingkan dengan ekstrak metanol (1.78 %).

Untuk esei antimikrobial, ekstrak heksana dan diklorometana rizom *A. pahangensis* menunjukkan perencatan yang tinggi menentang gram positif bacteria iaitu *B. cereus* dan *B. subtilis* dengan nilai perencatan minimum (3120 µg/ml) yang tidak jauh

daripada nilai kawalan standard, Streptomycin (2,500 µg/ml), kecuali ekstrak diklorometana menentang *B. cereus* dengan nilai perencatan minimum pada 12,500 µg/ml. Kesemua ekstrak gagal menunjukkan sebarang perencatan menentang kesemua fungi unisel yang dikaji serta dua bakteria gram positif (oral) iaitu *Streptococus mutans* dan *Streptococcus sanguis*.

Di dalam esei penghapusan radikal bebas DPPH, ekstrak metanol rizom *A. pahangensis* memberikan nilai $IC_{50}$ terendah (1.71 mg/ml) tetapi kurang aktiviti penghapusan berbanding asid askorbik (0.085 mg/ml). Keputusan yang diperolehi menunjukkan bahawa sebatian antimikrob yang hadir dalam kedua-dua tumbuhan mungkin berada di dalam ekstrak pelarut kurang polar (heksana dan diklorometana) sementara sebatian antioksidan juga mungkin hadir dalam ekstrak pelarut polar (metanol) khususnya untuk bahagian rizom.

Oleh itu, kesimpulannya, di antara dua spesis *Alpinia* yang dikaji, *A. pahangensis* menunjukkan tindak balas dan potensi aktiviti biologi yang lebih baik berbanding *A. mutica*, di mana ia mungkin disebabkan oleh kesan sinergistik sebatian yang hadir dalam *A. pahangensis*, suatu spesies halia liar yang belum di eksploitasi.

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## LIST OF SYAMBOLS AND ABBREVIATIONS

| | |
|---|---|
| m | meter |
| cm | centimeter |
| mm | millimeter |
| E° | solvent strength parameter |
| ° C | celcius |
| g | gram |
| µg | microgram |
| ml | milliliter |
| µl | microliter |
| µg/ ml | microgram/mililiter |
| mg/ml | milligram/milliliter |
| cfu/ml | colony forming unit/milliliter |
| nm | nanometer |
| % | percent |
| ICU | Intensive Care Unit |
| $SO_2$ | sulphur dioxide |
| UV | ultraviolet |
| ROS | reactive oxygen species |
| $HO^{\bullet}$ | hydroxyl radical |
| $O2^{\bullet-}$ | superoxide radical anion |
| $ROO^{\bullet}$ | peroxyl radical |
| DPPH | 2,2-diphenylpicrylhydrazyl |
| FRAP | ferric reducing antioxidant assay |
| DCM | dichloromethane |
| MIC | Minimum Inhibition Concentration |

| | |
|---|---|
| DMSO | dimethylsulfoxide |
| MHA | Muller Hinton agar |
| MHB | Muller Hinton broth |
| BHIA | Brain Heart Infusion agar |
| BHIB | Brain Heart Infusion broth |
| SDA | Sabauroud Dextrose agar |
| SDB | Sabauroud Dextrose broth |
| YPA | Yeast Potato agar |
| YPB | Yeast Potato broth |
| VISA | *Staphylococcus aureus* with intermediate resistance to vancomycin |
| VRSA | *Staphylococcus aureus* with complete resistance to vancomycin |
| NCCLS | National Committee for Clinical Laboratory Standards |