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**ORIGINAL LITERARY WORK DECLARATION**

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Field of Study: Biology-Microbiology

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# ABSTRACT

Medicinal plants and lactic acid bacteria are used to treat a wide range of disease conditions. The aim of the study was to determine antimicrobial and antioxidant activities of bioactive compounds and peptides from different morphological parts of common medicinal plants namely *Morinda citrifolia*, *Annona squamosa*, *Alstonia angustiloba*, an Australian plant mixture and lactic acid bacteria. In the first part of the study, different methods were used to standardize the extraction of antimicrobial and antioxidant compounds. It was found that methanol extraction of plants tissue showed higher antimicrobial activity than aqueous extracts against the test bacteria *Staphylococcus aureus* (RF 122), *Escherichia coli* (UT181), *Bacillus cereus* (ATCC 14579), *Pseudomonas aeruginosa* (PA7), methicillin-resistant *Staphylococcus aureus* (ATCC BA-43) and *Helicobacter pylori* (ATCC 43504). Furthermore, plant tissues showed significant antioxidant activities using DPPH and SOD assays. GC-MS analysis of extracts revealed bioactive compounds (diterpenes, anthraquinones, alkaloids, organic acids) in these extracts.

In the second part of the study, bioactive compounds were fractionated into anthroquinones, alkaloids, diterpenes and phenolic compounds. Anthraquinones extracts from the fruit, leaf and root of *M. citrifolia* exhibited significant antibacterial activity against all strain of test bacteria. Anthraquinones extracted from the fruit have higher level of antioxidant activities compared to another parts of the plant. IR spectra of the anthraquinones extracts of *M. citrifolia* indicated the presence of O-H, C=O, C-H groups. A significant morphological change in cell wall, membrane and destruction of *B. cereus* was observed in the presence of anthraquinones.

Alkaloid extracts from the medicinal plants showed antibacterial activity against pathogenic bacteria including MRSA and *H. pylori* while *P. aeruginosa* was resistant to alkaloids extracted from *M. citrifolia* fruit. Alkaloid extracts from *A. squamosa* leaves have a high level of antioxidant activities. IR spectra of the alkaloid extracts indicated the presence of O-H, C=O, C-H and N-H groups. SEM observations of the action of alkaloids on bacterial cell wall showed rupture and cell lysis.

Phenolic compounds extract from plant mixture gave antibacterial and antioxidant activities. Diterpens extracts from *A. squamosa* fruit had significant antibacterial activity against pathogenic bacteria and MRSA and significant antioxidant activity. SEM observation of the action on bacterial cells showed disruption of cell wall and swelling of the cells. IR spectra of diterpenes and phenolic compounds indicated the presence of O-H, C-H, C=O and C-H groups. LC-MS analysis of bioactive compounds plants identified specific compounds.

In the third part of the study, antibacterial peptides extracted from lactic acid bacteria by the acidic methanolic method were shown to have activity against pathogenic bacteria including MRSA and *H. pylori* and had antioxidant activity. LC-MS analysis of peptide of *Lactobacillus paracasei* subsp. *paracasei* 8700:2 identified a novel bacteriocin in this extract.

Peptides extracts from the medicinal plants had significant antibacterial and antioxidant activities. LC-MS analysis of Australian plant mixture indicated the presence of Pathogenesis-related protein 2 of *Phaseolus vulgaris*. SEM and TEM analysis of the mechanism of action of purified peptides from lactic acid bacteria and APM showed membrane disruption with bubble-like formations and cell lysis.

# ABSTRAK

Tumbuhan ubatan dan bakteria asid laktik digunakan untuk merawat pelbagai penyakit. Tujuan kajian ialah untuk menentukan aktiviti agen antimikrob dan antioksidan sebatian bioaktif dan peptida dari bahagian-bahagian morologikal berbeza tumbuhan ubatan (*Morinda citrifolia*, *Annona squamosa*, *Alstonia angustiloba* dan tumbuhan Australia campuran) dan bakteria asid laktik. Dibahagian pertama kajian, pelbagai kaedah digunakan untuk menstandardkan pengekstrakan sebatian agen antimikrob dan antioksidan. Di dapati pengekstrakan metanol tisu tumbuh-tumbuhan menunjukkan aktiviti antimikrob lebih tinggi daripada ekstrak akueus terhadap bakteria ujian, *Staphylococcus aureus* (RF 122), *Escherichia coli* (UT181), *Bacillus cereus* (ATCC 14579), *Pseudomonas aeruginosa* (PA7), *Staphylococcus aureus* tahan methicillin (ATCC BA-43) dan *Helicobacter pylori* (ATCC 43504). Tambahan pula, tisu tumbuh-tumbuhan menunjukkan aktiviti penting antioksidan dengan menggunakan ujian DPPH and SOD. Analisis mergguntan GC-MS ekstrak mendedahkan sebatian bioaktif (diterpena, anthraquinones, alkaloid, asid organik) dalam ekstrak ini.

Dibahagian kedua kajian, sebatian bioaktif diasingkan kepada anthroquinones, alkaloid, diterpena dan sebatian fenol. Anthraquinones dari buah, daun dan akar *M. citrifolia* menunjukkan aktiviti antibakteria yang mynrbtan terhadap semua bakteria ujian. Anthraquinones dari buah mempunyai aktiviti-aktiviti antipengoksidan lebih tinggi berbanding dengan bahagian-bahagian lain tumbuhan. Spektrum IR ekstrak anthraquinones *M. citrifolia* menunjukkan kehadiran kumpulan-kumpulan O-H, C=O, C-H. Satu perubahan morfologi penting di dinding sel, membran dan kemusnahan sel bakteria *B. cereus* telah diperhatikan seuase kehadiran anthraquinones.

Alkaloid dari tumbuhan ubatan menunjukkan aktiviti antibakteria terhadap bakteria patogen termasuk MRSA and *H. pylori* manakala *P. aeruginosa* resistan kepada

alkaloid dari *M. citrifolia*. Alkaloid dari daun-daun *A. squamosa* mempunyai peringkat tinggi aktiviti-aktiviti antipengoksida. Spektrum IR ekstrak alkaloid menunjukkan kehadiran kumpulan-kumpulan O-H, C=O, C-H dan N-H. Pemerhatian-pemerhatian SEM menunjukan tindakan alkaloid pada dinding sel bakteria menunjukkan kepecahan dan lisis.

Sebatian fenolik dari campuran tumbuhan menunjukan aktiviti antibakteria dan antioksidan. Diterpena dari buah *A. squamosa* mempunyai aktiviti penting antibakteria terhadap bakteria patogen dan MRSA dan kegiatan antioksidan yay fignrttan. Pemerhatian SEM menunjukkan gangguan dinding sel bakteria dan pembengkakan sel. Spektrum IR diterpena dan sebatian fenol menunjukkan kehadiran O-H, C-H, C=Kumpulan-kumpulan O and C H. Analisis LC-MS sebatian bioaktif telah mengenal pasti sebatian-sebatian khusus dalam ekstrak.

Dibahagian ketiga kajian, peptida antibakteria dari bakteria asid laktik, diasingkan dengan kaedah methanolik berasid, menunjukkan keattifan terhadap bakteria patogen termasuk MRSA and *H. pylori* dan mempunyai activiti antioksidan. Analisis LC-MS peptida *Lactobacillus paracasei* subsp. *paracasei* 8700:2 mengenal pasti satu bakteriosin novel dalam ekstrak ini.

Ekstrak peptida dari tumbuhan ubatan mempunyai aktiviti antibakteria penting dan aktiviti-aktiviti antipengoksida. Analisis LC-MS tumbuhan Australia campuran menunjukkan kehadiran protein berkaitan dengan Pathogenesis 2 *Phaseolus vulgaris*. Analisis SEM and TEM menunjukkan mekanisme tindakan peptida tulen dari bakteria asid laktik dan APM menunjukkan gangguan membran bakteria dengan formasi seperti gelembung dan lisis sel.

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# LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| ADP | Adenosine diphosphate |
| AGC target | Automatic gain control |
| APM | Australian plant mixture |
| ATCC | American Type Culture Collection |
| *B. cereus* | *Bacillus cereus* |
| BHT | Butylated Hydroxyl Toluene assay |
| CCl4 | Carbon tetrachloride |
| CFU | Colony forming units |
| $CO_2$ | Carbon dioxide |
| DMPD | N.N. dimethyl-p-phenylendiamine |
| DMSO | dimethyl sulfoxide |
| DNA | Deoxyribonucleic acid |
| DPPH | 2, 2- diphenyl-1- picrylhydrazyl solution |
| *E. coli* | *Escherichia coli* |
| ESI | Electrospray ionization |
| FDR | The false discovery rate |
| FTC | Ferric thiocyanate assay |
| FT-CID method | Fourier transform - Collision-induced dissociation method |
| FT-ICR | Fourier transform ion cyclotron resonance |
| FTIR | Fourier transform infrared spectroscopy |
| g | Gram |
| GAE | gallic acid equivalence |
| GC-MS | Gas chromatography–mass spectrometry |

| | |
|---|---|
| *H. pylori* | *Helicobacter pylori* |
| HCl | Hydrochloric acid |
| HPLC | High-performance liquid chromatography |
| hr | Hour |
| $IC_{50}$ | The half maximal inhibitory concentration |
| IR | Infrared spectroscopy |
| KB cells | KERATIN-forming tumor cell line |
| KDa | Kilodaltons |
| kV | Kilovolt |
| *L. casei* | *Lactobacillus casei* |
| *L. paracasei* | *Lactobacillus paracasei* |
| LC-MS | Liquid chromatography–mass spectrometry |
| LTQ Orbitrap | Linear ion trap and the proprietary Orbitrap |
| *m/z* | Mass-to-charge ratio |
| MBC | Minimum bactericidal concentration |
| mg | Milligram |
| MIC | Minimum inhibitory concentration |
| min | Minute |
| µm | Micrometer |
| mm | Millimeter |
| MRS broth | De Man-Rogosa-Sharpe broth |
| MRSA | Methicillin- resistant *Staphylococcus aureus* |
| ms | Microscan |
| MTT | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide |
| NaCl | Sodium chloride |
| NADPH | Nicotinamide adenine dinucleotide phosphate |

| | |
|---|---|
| NCIM | National Collection of Industrial Microorganisms |
| nm | Nanometer |
| $O_2^-$ | superoxide anion |
| OD | Optical density |
| OH | hydroxyl radical |
| *P. aeruginosa* | *Pseudomonas aeruginosa* |
| PCR | polymerase chain reaction |
| rRNA | Ribosomal RNA |
| *S. aureus* | *Staphylococcus aureus* |
| SD | Standard deviation |
| SDE | Simultaneous distillation – solvent extraction |
| SEM | Scanning Electron Microscopy |
| SOD assay | Superoxide dismutase assay |
| TBA | Thiobarbituric acid assay |
| TEM | Transmission Electron Microscopy |
| TFA | Trifluoroacetic acid |
| 3 D | Tertiary structure |
| TLC | Thin layer chromatography |

# LIST OF APPENDICES