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ORIGINAL LITERARY WORK DECLARATION

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BIOACTIVE EXTRACTS AND PEPTIDES FROM MORINDA CITRIFOLIA,
ANNONA SQUAMOSA, ALSTONIA ANGUSTILOBA AND LACTIC ACID
BACTERIA”

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 anthraquinones, 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 rapati pengekstrakan metanol tisu tumbuh-tumbuhan menunjukkan aktiviti antimikrob lebih tinggi daripada ekstrak akues 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 mergguntttan GC-MS ekstrak mendedahkan sebatian bioaktif (diterpena, anthraquinones, alkaloid, asid organik) dalam ekstrak ini.


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


Dibahagian ketiga kajian, peptida antibakteria dari bakteria asid laktik, diasingkan dengan kaedah methanoli berasid, menunjukkan keattifan terhadap bakteria patogen termasuk MRSA and *H. pylori* dan mempunyai aktiviti 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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<tr>
<td>ADP</td>
<td>Adenosine diphosphate</td>
</tr>
<tr>
<td>AGC target</td>
<td>Automatic gain control</td>
</tr>
<tr>
<td>APM</td>
<td>Australian plant mixture</td>
</tr>
<tr>
<td>ATCC</td>
<td>American Type Culture Collection</td>
</tr>
<tr>
<td>B. cereus</td>
<td>Bacillus cereus</td>
</tr>
<tr>
<td>BHT</td>
<td>Butylated Hydroxyl Toluene assay</td>
</tr>
<tr>
<td>CCl4</td>
<td>Carbon tetrachloride</td>
</tr>
<tr>
<td>CFU</td>
<td>Colony forming units</td>
</tr>
<tr>
<td>CO₂</td>
<td>Carbon dioxide</td>
</tr>
<tr>
<td>DMPD</td>
<td>N.N. dimethyl-p-phenyldiamine</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>DPPH</td>
<td>2, 2'-diphenyl-1-picrylhydrazyl solution</td>
</tr>
<tr>
<td>E. coli</td>
<td>Escherichia coli</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionization</td>
</tr>
<tr>
<td>FDR</td>
<td>The false discovery rate</td>
</tr>
<tr>
<td>FTC</td>
<td>Ferric thiocyanate assay</td>
</tr>
<tr>
<td>FT-CID method</td>
<td>Fourier transform - Collision-induced dissociation method</td>
</tr>
<tr>
<td>FT-ICR</td>
<td>Fourier transform ion cyclotron resonance</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared spectroscopy</td>
</tr>
<tr>
<td>g</td>
<td>Gram</td>
</tr>
<tr>
<td>GAE</td>
<td>Gallic acid equivalence</td>
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<td>GC-MS</td>
<td>Gas chromatography–mass spectrometry</td>
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\begin{itemize}
    \item \textit{H. pylori} \textit{Helicobacter pylori}
    \item HCl \textit{Hydrochloric acid}
    \item HPLC \textit{High-performance liquid chromatography}
    \item hr \textit{Hour}
    \item IC\textsubscript{50} \textit{The half maximal inhibitory concentration}
    \item IR \textit{Infrared spectroscopy}
    \item KB cells \textit{KERATIN-forming tumor cell line}
    \item KDa \textit{Kilodaltons}
    \item kV \textit{Kilovolt}
    \item \textit{L. casei} \textit{Lactobacillus casei}
    \item \textit{L. paracasei} \textit{Lactobacillus paracasei}
    \item LC-MS \textit{Liquid chromatography–mass spectrometry}
    \item LTQ Orbitrap \textit{Linear ion trap and the proprietary Orbitrap}
    \item $m/z$ \textit{Mass-to-charge ratio}
    \item MBC \textit{Minimum bactericidal concentration}
    \item mg \textit{Milligram}
    \item MIC \textit{Minimum inhibitory concentration}
    \item min \textit{Minute}
    \item \textit{$\mu$m} \textit{Micrometer}
    \item mm \textit{Millimeter}
    \item MRS broth \textit{De Man-Rogosa-Sharpe broth}
    \item MRSA \textit{Methicillin-resistant \textit{Staphylococcus aureus}}
    \item ms \textit{Microscan}
    \item MTT \textit{3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide}
    \item NaCl \textit{Sodium chloride}
    \item NADPH \textit{Nicotinamide adenine dinucleotide phosphate}
\end{itemize}
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<tr>
<td>NCIM</td>
<td>National Collection of Industrial Microorganisms</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometer</td>
</tr>
<tr>
<td>O$_2^-$</td>
<td>superoxide anion</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
</tr>
<tr>
<td>OH</td>
<td>hydroxyl radical</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td><em>Pseudomonas aeruginosa</em></td>
</tr>
<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
</tr>
<tr>
<td>rRNA</td>
<td>Ribosomal RNA</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td><em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SDE</td>
<td>Simultaneous distillation – solvent extraction</td>
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<tr>
<td>SEM</td>
<td>Scanning Electron Microscopy</td>
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<tr>
<td>SOD assay</td>
<td>Superoxide dismutase assay</td>
</tr>
<tr>
<td>TBA</td>
<td>Thiobarbituric acid assay</td>
</tr>
<tr>
<td>TEM</td>
<td>Transmission Electron Microscopy</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>3 D</td>
<td>Tertiary structure</td>
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<td>TLC</td>
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