

EXTRACTION, CHARACTERIZATION AND  
PURIFICATION OF FLAVONOIDS FROM  
FILICOPHYTES

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Doctor of Philosophy

UNIVERSITI MALAYSIA PAHANG



## SUPERVISOR'S DECLARATION

We hereby declare that we have checked this thesis and in my opinion, this thesis is adequate in terms of scope and quality for the award of the degree of Doctor of Philosophy in Faculty of Engineering and Technology.

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I hereby declare that the work in this thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at Universiti Malaysia Pahang or any other institutions.

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## LIST OF SYMBOLS

°C	Degree celcius
cm <sup>2</sup>	Square centimeter
g	Gram
H	Height
h	Hour
min	Minute
mg	Milligram
mL	Milliliter
SD	Standard deviation
nm	Nanometer
t	Triplet
s	Singlet
d	Doublet
Rpm	Revolution per minute
UV	Ultraviolet
SEM	Standard error of mean

## LIST OF ABBREVIATIONS

DNA	Deoxyribonucleic acid
ELISA	Enzyme linkage immuno sorbent assay
RO	Reactive oxygen
A	Absorbance
MFES	Methanolic fern extracts
AFES	Alcoholic fern extracts
PDA	Potato Dextrose Agar
PBS	Phosphate Buffer Saline
Sp.	Species
MH	Muller Hinton
DMSO	Dimethylsulfoxide
FTIR	Fourier Transform Infrared Spectroscopy
HPLC	High Performance Liquid Chromatography
TLC	Thin Layer Chromatograph
TPC	Total phenolic content
TFC	Total flavonoid content
CC	Column Chromatography

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## ABSTRAK

Tumbuh-tumbuhan adalah sumber yang kaya dengan fitokimia seperti flavonoid, terpenoid, alkaloid dan fenol. Produk semulajadi ini telah memainkan peranan penting dalam penyelidikan farmasi, kerana kebanyakan ubat-ubatan adalah sintesis daripada produk semula jadi atau terbitannya. Kajian ini adalah mengealpasti jenis paku-pakis yang telah dipilih (*Drynaria rigidula*, *Chelianthes tenuifolia*, *Asplenium nidus*, *Dipteris conjugata* dan *Antrophyum callifolium*) sebagai sumber alternatif baru semula jadi melalui proses pengekstrakan, pencirian dan penulenan. Ekstrak methanol paku-pakis (MFE) dari semua lima jenis paku-pakis adalah bergatung kepada jumlah kandungan fenolik (TPC), jumlah kandungan flavonoid (TFC), aktiviti antibakteria dan antioksidan. TPC telah dikenalpasti menggunakan kaedah kolorimetri Folin-Ciocalteu dan TFC telah ditentukan dengan menggunakan kaedah kolorimetri cerakan aluminium klorida. Jumlah kandungan tertinggi TFC telah dicatatkan dalam *C. tenuifolia* manakala kandungan TPC terbaik berada dalam *D. rigidula*. MFEs telah dinilai melalui tindakannya sebagai antibakteria dan antikulat dengan mengukur zon perencatan terhadap panel strain bakteria dan kulat patogenik menggunakan kaedah penyebaran cakera. *C. tenuifolia* menunjukkan zon perencatan maksimum terhadap *Staphylococcus aureus* (26 mm). Daripada semua paku-pakis, *C. tenuifolia* menunjukkan keupayaan memerangkap aktiviti radikal 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) antioksidan yang tinggi dengan nilai  $IC_{50}$  50.37 mM. Walau bagaimanapun, bagi sel hepatoma manusia (HepG2) dan sel karsinoma manusia (HeLa), *C. tenuifolia* (MFE) menunjukkan aktiviti antikanser yang tinggi menggunakan 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium bromida (MTT) berbanding dengan paku-pakis yang lain. Data yang menunjukkan bahawa ekstrak metanol *C. tenuifolia* mempunyai aktiviti anti-oksidan yang lebih tinggi yang mungkin disebabkan oleh polifenol di dalam ekstrak mentah. Paku-pakis terbaik (*C. tenuifolia*) telah dipilih untuk proses penulenan flavonoid selanjutnya. Dua flavonoid telah dikenalpasti iaitu rutin (2.8 mg) dan quercetin (3.34 mg). Nilai MIC flavonoid yang dituliskan terhadap *S.aureus* dan *Enterobacter sp.*, masing-masing adalah 2.25 dan 0.45 ug/ml. Rutin dan quercetin menunjukkan aktiviti antioksidan in vitro yang ketara, potensinya memerangkap quercetin (86.1%) lebih tinggi daripada rutin (73.2%) diukur berbanding radikal bebas yang bukan -biologi seperti DPPH. Walau bagaimanapun, bagi sel hepatoma manusia (HepG2) dan sel karsinoma manusia (HeLa), quercetin ( $80,91 \pm 1.93$ ) menunjukkan aktiviti anti-kanser labih yang tinggi daripada rutin ( $11.10 \pm 2.10$ ). Hasil kajian mencadangkan bahawa ekstrak methonal *C. tenuifolia* berpotensi digunakan dalam perubatan tradisional kerana sebatian yang diekstrak ini kaya dengan antioksidan, antibakteria dan antikanser.



## ABSTRACT

Plants are rich source of phytochemicals (flavonoids, terpenoids, alkaloids, phenols). These natural products have played a key role in pharma research, as many medicines are either natural products or derivatives. The present study is the exploration of selected ferns (*Drynaria rigidula*, *Cheliantes tenuifolia*, *Asplenium nidus*, *Dipteris conjugata* and *Antrophyum callifolium*) as a new candidate of natural compounds. This was done through a series of plant extraction, characterization and purification protocols. The methanolic fern-extracts (MFEs) from all five ferns were subjected to determine total phenolic content (TPC), total flavonoid content (TFC), antibacterial and antioxidant activities. TPC was determined following the Folin-Ciocalteu colorimetric method and TFC was determined using aluminium chloride colorimetric assay. Highest amount of TFC was noted in *C. tenuifolia* whereas best TPC was in *D. rigidula*. The MFEs of these ferns were assessed for antibacterial and antifungal activities by measuring inhibition zones against a panel of pathogenic bacterial and fungal strains using agar well diffusion method. The *C. tenuifolia* showed maximum inhibition zone against *Staphylococcus aureus* (26 mm). Out of all ferns, *C. tenuifolia* showed a significant antioxidative activity in vitro i.e. 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging potential of *C. tenuifolia* (MFE) with IC<sub>50</sub> value of 50.37 mM. However, for human hepatoma HepG2 and human carcinoma HeLa cells *C. tenuifolia* (MFE) exhibited high anticancer activity using 3-(4, 5-Dimethylthiazol-2-yl)-2, 5-Diphenyltetrazolium Bromide (MTT) assay than others ferns. The data revealed that MFE of *C. tenuifolia* had higher antioxidant and antibacterial activities that might be due to the polyphenols present in the crude extract. Among the five ferns we selected the best fern (*C. tenuifolia*) for purification of flavonoids. Two flavonoids were identified as rutin (2.8 mg) and quercetin (3.34 mg). The MIC values of purified flavonoids were 2.25 and 0.45 µg/ml against *S. aureus* and *Enterobacter sp.*, respectively. Rutin and quercetin showed significant *in vitro* antioxidant activity, the scavenging potential of quercetin (86.1%) was higher than that of rutin (73.2%) were measured against stable, non –biological radicals such as DPPH. However, for human hepatoma HepG2 and human carcinoma HeLa cells, quercetin (80.91 ± 1.93) exhibited high anticancer activity than rutin (11.10 ± 2.10). The results suggested that MFE of *C. tenuifolia* could potentially be employed in traditional medicine as they are rich in compounds with antioxidant, antimicrobial and anticancer properties.

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