

ABSTRACT

Sinusitis can be defined as an acute or chronic inflammation of the sinuses. One of the most potent inflammatory mediators are free radicals that can be neutralized by antioxidants. The present study was carried out to evaluate *in vitro* antioxidant activities of *Ervatamia coronaria* and *Tinospora crispa* of petroleum ether, chloroform, methanol and water extracts using four different assays namely DPPH Radical Scavenging Assay, Metal Chelating Assay, Reducing Power Assay and Haemolysate Catalytic Assay. Total Phenolic Content Assay revealed that chloroform and methanol extracts exhibited higher phenolic content compared to petroleum ether and water extracts. It agrees with the preliminary screening on *Ervatamia coronaria* which exhibited pronounced antioxidant activities especially in chloroform and methanol extracts except for Metal Chelating Assay for *Ervatamia coronaria* (roots) which revealed that water extract exhibited pronounced antioxidant activity. Meanwhile, methanol extract of *Tinospora crispa* exhibited pronounced antioxidant activities in all four assays. Significant positive correlation was observed in DPPH Assay ($r=0.788$, $p<0.01$) and Reducing Power Assay ($r=0.556$, $p<0.05$) while weak correlation or no significant difference between Total Phenolic Content and Metal Chelating and Haemolysate Catalytic Assays. The extracts were further evaluated for antimicrobial activity using Disc Diffusion Assay followed by Minimal Inhibitory Concentration (MIC) and Minimal Bactericidal Concentration (MBC) against ten common sinusitis-causing microorganism namely *Streptococcus pneumoniae* (ATCC49619), *Haemophilus influenza* (ATCC49247), *Moraxella catarrhalis* (ATCC23296) *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Candida albicans*. Most of the extracts

inhibited at least one of the microorganisms except for *Tinospora crispa* water extract which did not inhibit any of the microorganism tested. The lowest Minimal Inhibitory Concentration (3.12 mg/ml) was observed on chloroform extract of *Ervatamia coronaria* (stems) against *Moraxella catarrhalis*, methanol extract of *Tinospora crispa* against *Streptococcus pneumoniae* and petroleum ether extract of *Tinospora crispa* against *Staphylococcus aureus*. However when evaluated using the Minimal Bactericidal Concentration (MBC) Assay, only certain extracts exhibited killing against the microorganisms. Thin Layer Chromatography (TLC) was carried out to screen the chemical composition of the plant. Among the compounds identified were alkaloid, terpenoid, phenol, saponin, flavonoid and conjugated bond compound. Liquid Chromatography Mass Spectrometry (LCMS) was also carried out to identify and determine the compound in both plants. Initial investigation using LCMS/MS analysis of all sample extracts revealed the presence of large amount of alkaloids which correlated with the result from Thin Layer Chromatography (TLC). Findings of this study indeed justified the potentials of *Ervatamia coronaria* and *Tinospora crispa* as source of antioxidant agents by preventing free radicals from causing damage and subsequent inflammation to healthy tissue such as the sinus and also as source of antimicrobial agents against bacteria that cause sinusitis.

ABSTRAK

Sinusitis boleh diertikan sebagai keradangan sinus yang akut dan kronik. Salah satu mediator inflamasi yang paling poten adalah radikal bebas yang boleh dineutralkan oleh antioksidan. Kajian ini telah dijalankan untuk menilai aktiviti antioksidan secara *in vitro* *Ervatamia coronaria* dan *Tinospora crispa* dalam ekstrak petroleum eter, klorofom, metanol dan air menggunakan empat ujian berbeza iaitu ‘DPPH Radical Scavenging Assay’, ‘Metal Chelating Assay’, ‘Reducing Power Assay’ dan ‘Haemolysate Catalytic Assay’. ‘Total Phenolic Content Assay’ mendedahkan bahawa ekstrak klorofom dan metanol menunjukkan kandungan fenolik yang lebih tinggi berbanding dengan ekstrak petroleum eter dan air. Ia selaras dengan saringan awal antioksidan untuk *Ervatamia coronaria* yang menunjukkan aktiviti antioksida yang ketara terutama dalam ekstrak klorofom dan metanol kecuali ‘Metal Chelating Assay’ untuk *Ervatamia coronaria* (akar) yang mendedahkan ekstrak air menunjukkan aktiviti antioksidan ketara. Sementara itu, ekstrak metanol *Tinospora crispa* menunjukkan aktiviti antioksidan yang ketara dalam kesemua empat ujian. Korelasi positif yang signifikan telah diperhatikan dalam ‘DPPH assay’ ($r = 0.788$, $p < 0.01$) dan ‘Reducing Power Assay’ ($r = 0.556$, $p < 0.05$) manakala korelasi yang lemah atau tiada perbezaan yang signifikan antara ‘Total Phenolic Content’ dan ‘Metal Chelating Assay’ dan ‘Haemolysate Catalytic Assay’. Kajian juga dilakukan untuk aktiviti antimikrobial menggunakan ‘Disc Diffusion Assay’ diikuti oleh ‘Minimal Inhibitory Concentration’ (MIC) dan ‘Minimal Bactericidal Concentration’ (MBC) terhadap sepuluh mikroorganisma yang biasa menyebabkan sinusitis iaitu *Streptococcus pneumoniae* (ATCC49619), *Haemophilus influenza* (ATCC49247), *Moraxella catarrhalis* (ATCC23296) *Staphylococcus aureus*, *Streptococcus faecalis*, *Escherichia coli*,

Pseudomonas aeruginosa, *Klebsiella pneumoniae*, *Proteus mirabilis* dan *Candida albicans*. Kebanyakan ekstrak berupaya merencat sekurang-kurangnya salah satu daripada mikroorganisma kecuali ekstrak air *Tinospora crispa* yang tidak boleh merencat sebarang mikroorganisma yang diuji. 'Minimal Inhibitory Concentration' yang terendah (3.12 mg / ml) dapat diperhatikan pada ekstrak klorofom *Ervatamia coronaria* (batang) terhadap *Moraxella catarrhalis*, ekstrak metanol *Tinospora crispa* terhadap *Streptococcus pneumoniae* dan ekstrak petroleum eter *Tinospora crispa* terhadap *Staphylococcus aureus*. Walaubagaimanapun, apabila diuji menggunakan 'Minimal Bactericidal Concentration Assay', hanya ekstrak tertentu boleh membunuh mikroorganisma. Kromatografi lapisan nipis (TLC) telah dijalankan untuk menyaring komposisi kimia tumbuhan. Antara sebatian yang dikenal pasti ialah alkaloid, terpenoid, fenol, saponin, flavonoid dan sebatian yang mempunyai ikatan konjugat. 'Liquid Chromatography Mass Spectrometry' (LCMS) juga telah dijalankan untuk mengenal pasti dan menentukan sebatian dalam kedua-dua tumbuhan. Siasatan awal menggunakan analisis LCMS / MS ke atas kesemua ekstrak mendapati banyak sebatian alkaloid yang sejajar dengan kajian daripada kromatografi lapisan nipis (TLC). Hasil kajian ini menunjukkan *Ervatamia coronaria* dan *Tinospora crispa* mempunyai potensi sebagai sumber agen antioksidan dengan menghalang radikal bebas daripada menyebabkan kerosakan dan seterusnya menyebabkan keradangan kepada tisu yang sihat seperti sinus dan juga sebagai sumber agen antimikrobial terhadap bakteria yang menyebabkan resdung.

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Figure 5.1 : Structure of DPPH and its reduction form by the antioxidant RH

Figure 5.2 : Metal chelating reaction by antioxidant RH

LIST OF ABBREVIATIONS

%	= Percentage
>	= More than
≥	= More than or equal
ANOVA	= Analysis of Variance
ATCC	= American Type Culture Collection
CAT	= Catalase
CHD	= Coronary Heart Disease
cm	= Centimetre
CNS	= Central Nervous System
CO ₂	= Carbon Dioxide
cps	= Count per Second
DNA	= Deoxyribonucleic Acid
EDTA	= Ethylenediaminetetraacetic acid
Et al.	= Et alia (and other)
Fe ²⁺	= Ferrous Ion
Fe ³⁺	= Ferric Ion
FeCl ₂	= Ferrous Chloride
FeCl ₃	= Ferric Chloride
g	= Gram
GAE	= Gallic Acid Equation
GSSG	= Glutathione
H ₂ O	= Water
H ₂ O ₂	= Hydrogen Peroxide
H ₃ PMo ₁₂ O ₄₀	= Phosphomolybdic

$H_3PW_{12}O_{40}$	= Phosphotungstic
HOA ₂	= Acetic Acid
HPLC	= High Performance Liquid Chromatography
HTM	= Haemophilus Test Medium
IC ₅₀	= Inhibition Concentration at 50%
IL-1	= Interleukin 1
IL-6	= Interleukin 6
IZD	= Inhibition Zone Diameter
$K_3Fe(CN)_6$	= Pottasium Ferricyanide
LCMS	= Liquid Chromatography Mass Spectrometry
LDL	= Low Density Lipoprotein
m/z	= Mass to Charge Ratio
MEP/DOXP	= 2-C-methyl-D-erythritol 4-phosphate/1-deoxy-D-xylulose 5-phosphate
mg	= Miligram
ml	= Mililiter
mm	= Milimetre
mM	= Milimolar
Mo_8O_{23}	= Molybdene
MW	= Molecular Weight
NaOH	= Sodium Hydroxide
NCCLS	= National Committee for Clinical Laboratory Standards.
NIDDM	= Non-insulin Dependent Diabetes Mellitus
Nm	= Nanometer
NTHI	= Non-typable <i>Haemophilus Influenzae</i>
° C	= Degree Celcius
O ₂	= Oxygen

OD	= Optimal Density
pH	= Power of Hydrogen
r	= Correlation Coefficient
r^2	= Correlation of Determination
R_f	= Retardation Factor
RNS	= Reactive Nitrogen Species
ROS	= Reactive Oxygen Species
rpm	= Round per Minute
SD	= Standard Deviation
SOD	= Superoxide Dismutase
SPSS	= Statistical Package for the Social Sciences
TCA	= Trichloroacetic Acid
TLC	= Thin Layer Chromatography
TNF- α	= Tumor Necrosis Factor Alpha
UV	= Ultraviolet
w/v	= Weight per Volume
W_8O_{23}	= Tungstene
WHO	= World Health Organization
α	= Alpha
β	= Beta
μ l	= Microliter