Appendix A

Preparation of Solutions

TLC Analysis (Visualizing Reagents)

i) Preparation of Dragendorff's Reagent

This reagent was used to detect the presence of alkaloid compound by orange spot would be visible on the TLC plate after sprayed with the reagent.

Procedure:

Solution A : A 1.7 gram bismuth nitrate in 100 ml mixture of water-acetic acid, H_2O-HOAc (80:20)

Solution B : A 40 gram of potassium iodide in 100 ml distilled water.

The reagent was prepared by mixing 5 ml solution A with 5 ml solution B together with 20 ml of acetic acid and 70 ml of distilled water into reagent bottle.

ii) Preparation of Vanillin-Sulphuric Acid Reagent

This reagent was used to detect the presence of terpenoid and phenol derivatives. Purple and pink/red coloured spots would be visible on the TLC plates after sprayed and heated to 110 °C. Purple colour indicated the presence of various terpenoid derivatives while pink/red colour indicated phenol derivatives.

Procedure:

The reagent was prepared by adding 1 gram vanillin to 100 ml absolute ethanol with 1.5 ml concentrated sulphuric acid into reagent bottle.

iii) Preparation of Anisaldehyde-Sulphuric Acid Reagent

This reagent was used to detect flavanoid derivatives. Purple coloured spots will be visible on the TLC plates after sprayed and heated to 110 °C. Purple colour indicated the presence of various flavanoid derivatives.

Procedure:

The reagent was prepared by adding 10 ml of acetic acid glycial to 0.5 ml of anysaldehyde followed by adding 85 ml of methanol and 5 ml concentrated sulphuric acid into reagent bottle.

iv) Preparation of Iodine Vapour

This iodine vapour was used to detect conjugated compound by brownish colour appeared after exposed to the iodine in closed tank.

Procedure:

The iodine in solid form was bought ready-made from supplier. The iodine was put into closed tank together with TLC plate and let the iodine evaporated.

Total Phenolic Content (TPC)

i) Preparation of Folin-ciocalteu

A folin-ciocalteu reagent was diluted to 10 fold of distilled water.

Procedure:

A 2 ml of folin-ciocalteu reagent was pipetted in measuring cylinder containing 18 ml distilled water.

ii) Preparation of 6% (w/v) sodium carbonate

A stock of 1.2 g sodium carbonate was dissolved in 20 ml distilled water.

Procedure:

A 1.2 g of sodium carbonate powder was weighted using a balance. The sodium carbonate powder was poured into a graduated cylinder containing 18 ml of distilled water. Once the sodium carbonate was dissolved completely (swirls the cylinder gently if necessary), distilled water was added to bring the volume up to the final volume of 20 ml.

iii) Preparation of 0.05 mg/ml standard gallic acid

A stock of 0.0005 g gallic acid was dissolved in 10 ml distilled water. The stock solution was kept in centrifuge tube and wrapped with aluminium foil.

Procedure:

A 0.0005 g of gallic acid powder was weighted using a balance. The gallic acid powder was poured into a graduated cylinder containing 8 ml of distilled water. Once the gallic acid was dissolved completely (swirls the cylinder gently if necessary), distilled water was added to bring the volume up to the final volume of 10 ml.

Antioxidant Assays

(I) 2,2-Diphenyl-1-picryl-hydrazyl (DPPH) Free Radical Scavenging Activity Assay

i) Preparation of 1 mg/ml Ascorbic Acid

A stock of 0.01 g of ascorbic acid was dissolved in 10 ml methanol. The stock solution was kept in universal bottle and wrapped with aluminium foil.

Procedure:

A 0.01 g of ascorbic acid powder was weighted using a balance. The ascorbic acid powder was poured into a graduated cylinder containing 8 ml of methanol. Once the ascorbic acid was dissolved completely (swirls the cylinder gently if necessary), methanol was added to bring the volume up to the final volume of 10 ml.

ii) Preparation of 1mM DPPH

A stock of 0.00394 g of DPPH (Sigma) was dissolved in 10 ml methanol. The stock solution was kept in universal bottle and wrapped with aluminium foil.

Procedure:

A 0.00394 g of DPPH powder was weighted using a balance. The DPPH powder was poured into a graduated cylinder containing 8 ml of methanol. Once the DPPH was dissolved completely (swirls the cylinder gently if necessary), methanol was added to bring the volume up to the final volume of 10 ml. (MW=394.32 g/ml)

(II) Reducing Power Assay

i) Preparation of 1% (10 mg/ml) potassium ferricyanide [$K_3Fe(CN)_6$]

A stock of 0.6 g of potassium ferricyanide was dissolved in 60 ml distilled water. The stock solution was kept in centrifuge tube and wrapped with aluminium foil.

Procedure:

A 0.6 g of potassium ferricyanide was weighted using a balance. The potassium ferricyanide powder was poured into a graduated cylinder containing 55 ml of distilled water. Once the potassium ferricyanide was dissolved completely (swirls the cylinder gently if necessary), distilled water was added to bring the volume up to the final volume of 60 ml.

ii) Preparation of 10% (100 mg/ml) Trichloroacetic acid (TCA)

A stock of 6 g of trichloroacetic acid was dissolved in 60 ml distilled water. The stock solution was kept in centrifuge tube and wrapped with aluminium foil.

Procedure:

A 6 g of trichloroacetic acid crystal was weighted using a balance. The trichloroacetic acid crystal was poured into a graduated cylinder containing 55 ml of distilled water. Once the trichloroacetic acid was dissolved completely (swirls the cylinder gently if necessary), distilled water was added to bring the volume up to the final volume of 60 ml.

iii) Preparation of 0.1 % (1mg/ml) ferric chloride ($FeCl_3$)

A stock of 0.01 g of ferric chloride was dissolved in 10 ml distilled water. The stock solution was kept in centrifuge tube and wrapped with aluminium foil.

Procedure:

A 0.01 g of ferric chloride crystal was weighted using a balance. The ferric chloride crystal was poured into a graduated cylinder containing 8 ml of distilled water. Once the ferric chloride was dissolved completely (swirls the cylinder gently if necessary), distilled water was added to bring the volume up to the final volume of 10 ml.

(III) Metal Chelating Assay

i) Preparation of 5mM of Ferrozine (MW: 492.45)

A stock of 0.0246 g of ferrozine was dissolved in 10 ml deionized water. The stock solution was kept in centrifuge tube and wrapped with aluminium foil.

Procedure:

A 0.0246 g of ferrozine powder was weighted using a balance. The ferrozine powder was poured into a graduated cylinder containing 8 ml of deionized water. Once the ferrozine was dissolved completely (swirls the cylinder gently if necessary), deionized water was added to bring the volume up to the final volume of 10 ml.

ii) Preparation of 2mM Ferum Chloride ($FeCl_2$) – (MW:198.81)

A stock of 0.0025 g of ferum chloride was dissolved in 10 ml deionized water. The stock solution was kept in centrifuge tube and wrapped with aluminium foil.

Procedure:

A 0.0025 g of ferum chloride was weighted using a balance. The ferum chloride was poured into a graduated cylinder containing 8 ml of deionized water. Once the ferum chloride was dissolved completely (swirls the cylinder gently if necessary), deionized water was added to bring the volume up to the final volume of 10 ml.

iii) Preparation of 1 mg/ml Ethylenediaminetetraacetic acid (EDTA)

A stock of 0.01 g of EDTA was dissolved in 10 ml deionized water. The stock solution was kept in centrifuge tube and wrapped with aluminium foil.

Procedure:

A 0.01 g of EDTA was weighted using a balance. The EDTA was poured into a graduated cylinder containing 4 ml of distilled water. The pH was adjusted to pH 8 while stirring with Sodium Hydroxide (NaOH) solution until most of EDTA dissolved. Once the EDTA dissolved, total volume of the solution was added with deionized water until the final volume of 10 ml.

(IV) Haemolysate Catalytic Assay

i) Preparation of haemolysate

A stock of haemolysate was prepared in 500 ml of scott bottle and kept in ice at 4°C. The haemolysate stock was freshly prepared every time used.

Procedure:

A 250 µl blood from vacutainer tube was added into scott bottle containing 500 ml distilled water using P1000 micropipetter.

ii) Preparation of 1 M hydrogen peroxide (H_2O_2)

A 10 ml of 1 M hydrogen peroxide was prepared in universal bottle and kept in ice at 4°C. The hydrogen peroxide stock used was freshly prepared.

Procedure:

A 910 µl of 35 % 11 M hydrogen peroxide was poured in graduated cylinder containing a little less than 9090 µl of distilled water. After that, more distilled water was added to bring final volume of solution up to 1000 µl in order to eliminate any error because the final volume of the solution may not equal the calculated sum of the individual components.

iii) Preparation of 0.1 M sodium azide

A stock of 0.006501 g of sodium azide was dissolved in 10 ml distilled water. The stock solution was kept in universal bottle.

Procedure:

A 0.006501 g of sodium azide powder was weighted using a balance. The sodium azide powder was poured into a graduated cylinder containing 8 ml of distilled water. Once the sodium azide was dissolved completely (swirls the cylinder gently if necessary), distilled water was added to bring the volume up to the final volume of 10 ml.

Antimicrobial Assays

i) Preparation of Mueller Hinton Agar (Difco ^{TM})

Procedure:

A 38 gram of the powder was suspended in 1 liter of purified or sterile water and was mixed thoroughly. The solution was heated with frequent agitation and boiled for 1 minute to completely dissolve the powder before autoclaved at 121 °C for 15 minutes. The agar media was cool to room temperature followed by pouring into sterile petri dishes on a level pouring surface to a uniform depth of 4 mm and allowed it to solidify.

ii) Preparation of Mueller Hinton Broth (Difco ^{TM})

Procedure:

A 22 gram of the powder was suspended in 1 liter of purified or sterile water and was mixed thoroughly. The solution was heated with frequent agitation and boiled for 1 minute to completely dissolve the powder before autoclaved at 121 °C for 10 minutes. The broth media was cool to room temperature followed by pouring into sterile bijou bottle or universal bottle.

iii) Preparation of Nutrient Agar (Difco ^{TM})

Procedure:

A 23 gram of the powder was suspended in 1 liter of purified or sterile water and was mixed thoroughly. The solution was heated with frequent agitation and boiled for 1 minute to completely dissolve the powder before autoclaved at 121 °C for 15 minutes. The agar media was cool to room temperature followed by pouring into sterile petri dishes on a level pouring surface to a uniform depth of 4 mm.

iv) Preparation of Nutrient Broth (Difco ^{TM})

Procedure:

A 8 gram of the powder was suspended in 1 liter of purified or sterile water and was mixed thoroughly. The solution was heated with frequent agitation and boiled for 1 minute to completely dissolve the powder before autoclaved at 121 °C for 15 minutes. The broth media was cool to room temperature followed by pouring into sterile bijou bottle or universal bottle.

v) Preparation of Sabouraud Dextrose Agar (Difco ^{TM})

Procedure:

A 65 gram of the powder was suspended in 1 liter of purified or sterile water and was mixed thoroughly. The solution was heated with frequent agitation and boiled for 1 minute to completely dissolve the powder before autoclaved at 121 °C for 15 minutes. The agar media was cool to room temperature followed by

pouring into sterile petri dishes on a level pouring surface to a uniform depth of 4 mm and allowed it to solidify.

vi) Preparation of Tryptic Soy Agar (Difco ™)

Procedure:

A 40 gram of the powder was suspended in 1 liter of purified or sterile water and was mixed thoroughly. The solution was heated with frequent agitation and boiled for 1 minute to completely dissolve the powder before autoclaved at 121 °C for 15 minutes. The agar media was cool to room temperature followed by pouring into sterile petri dishes on a level pouring surface to a uniform depth of 4 mm and allowed it to solidify.

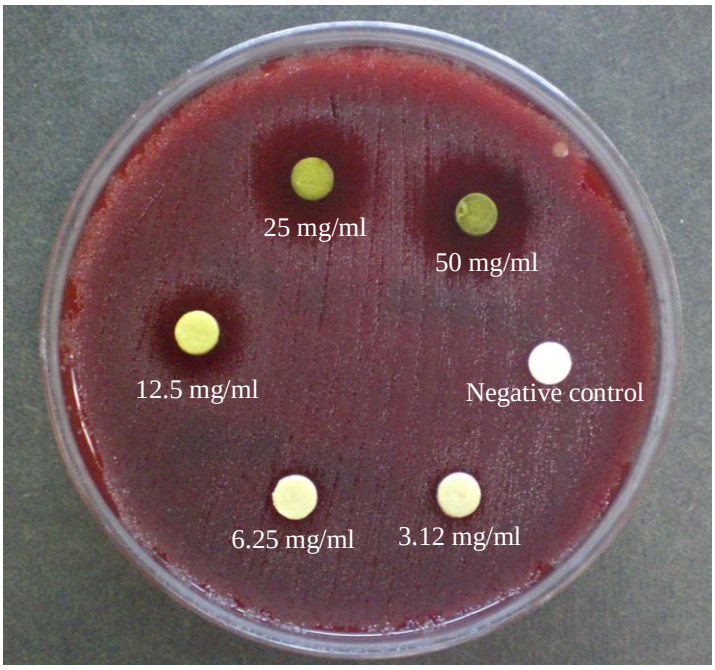
vii) Preparation of Tryptic Soy Broth (Difco ™)

Procedure:

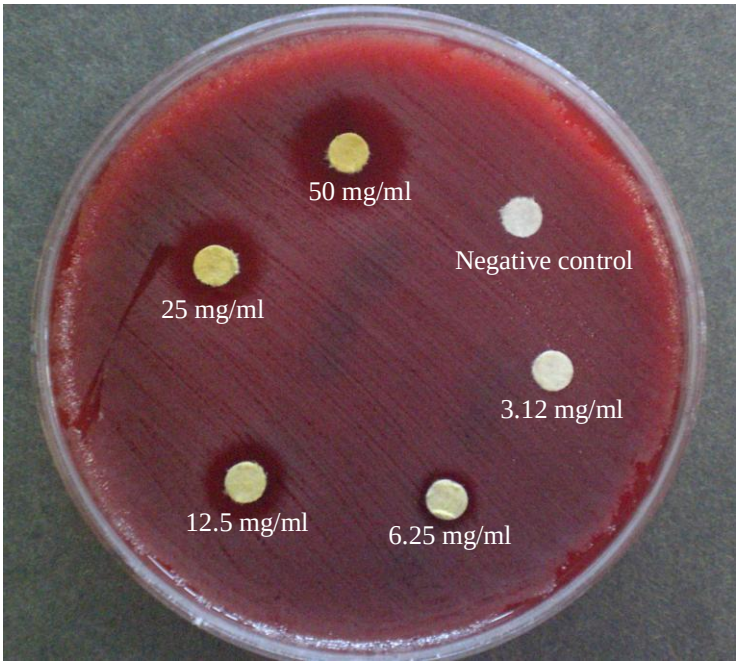
A 30 gram of the powder was suspended in 1 liter of purified or sterile water and was mixed thoroughly. The solution was heated with frequent agitation and boiled for 1 minute to completely dissolve the powder before autoclaved at 121 °C for 15 minutes. The broth media was cool to room temperature followed by pouring into sterile bijou bottle or universal bottle.

Appendix B

Antimicrobial Assay (Paper Disc Diffusion Method)



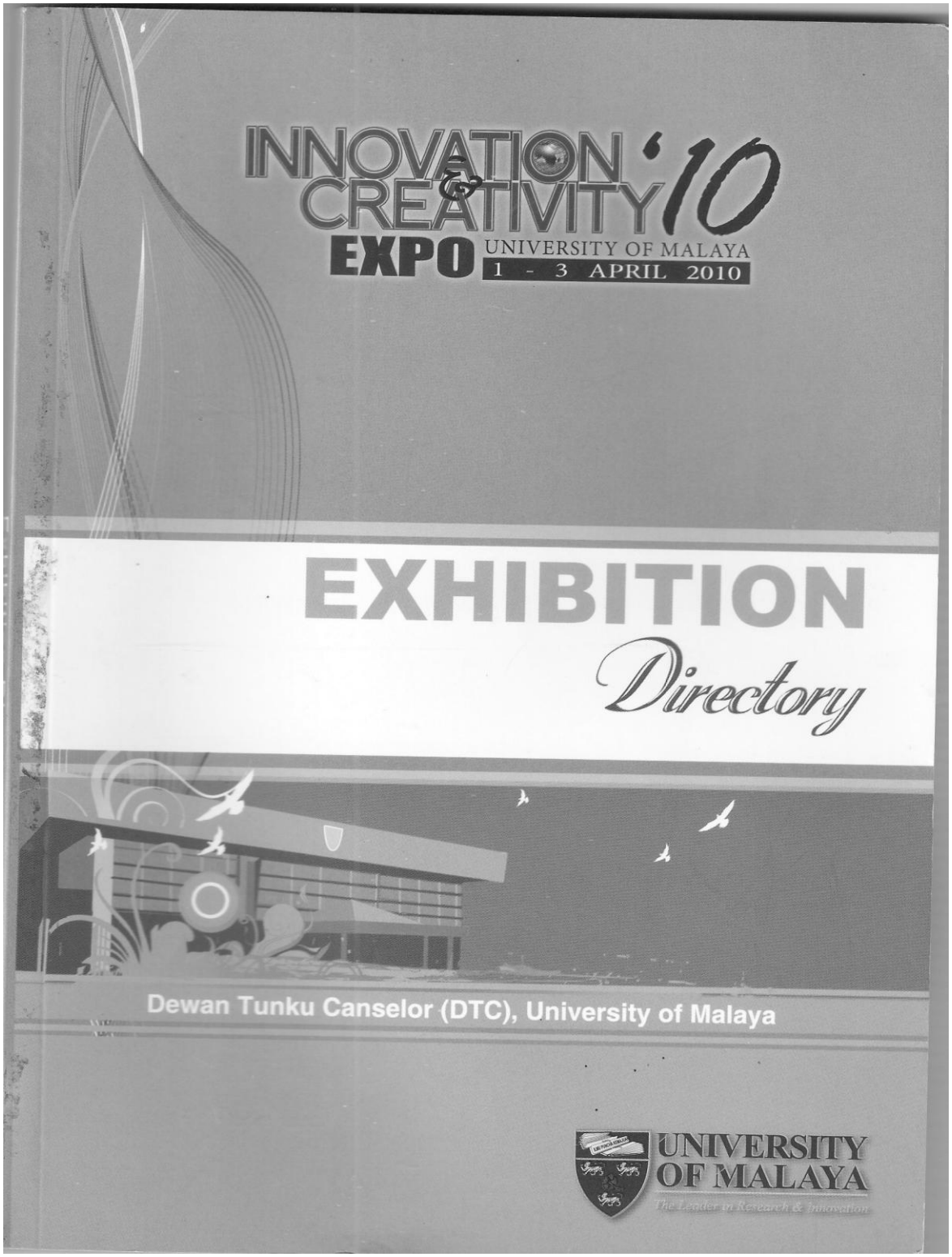
Inhibition zone diameter of *Ervatamia coronaria* (stems) chloroform extract against *Moraxella catarrhalis*



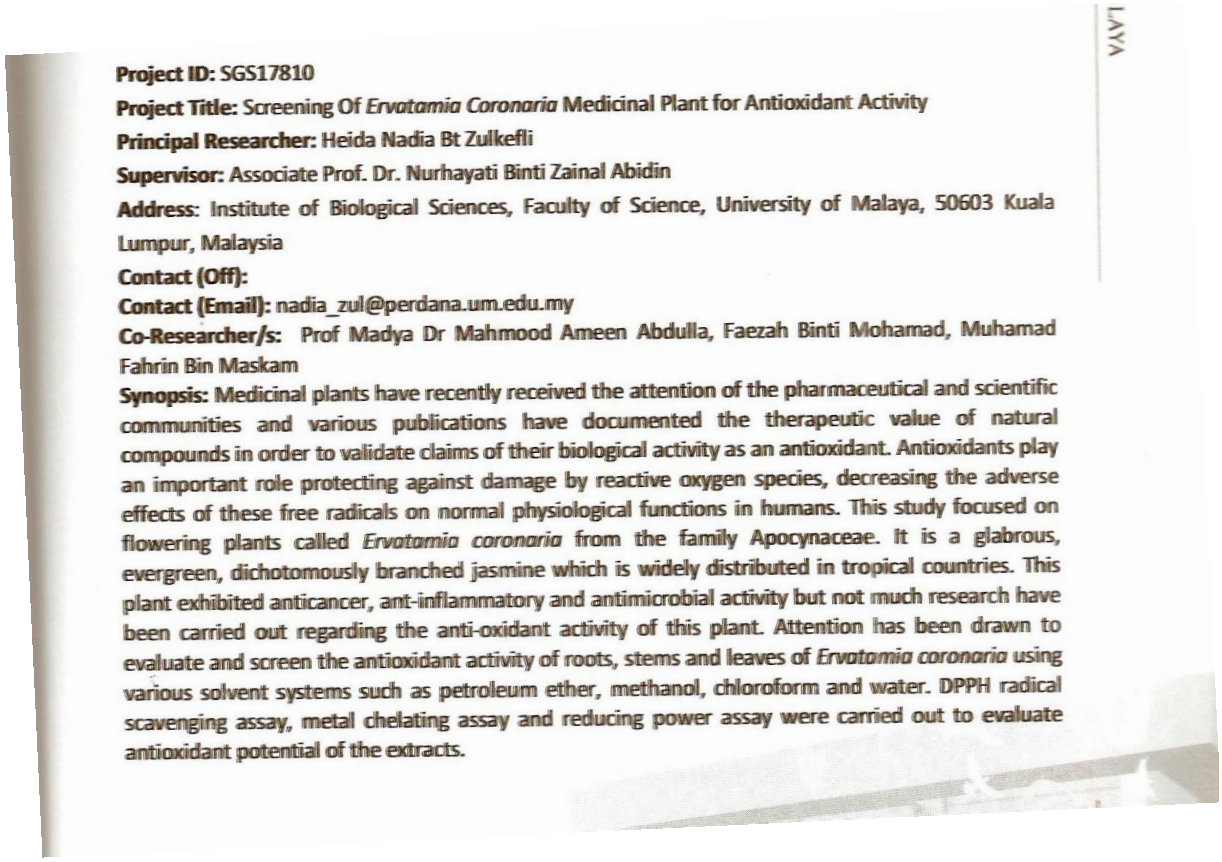
Inhibition zone diameter of *Ervatamia coronaria* (stems) methanol extract against *Moraxella catarrhalis*

Appendix C

Publication / Conference / Expo



Abstract in Innovation and Creativity Expo (University Malaya)



LAYA

Project ID: SGS17810

Project Title: Screening Of *Ervatamia Coronaria* Medicinal Plant for Antioxidant Activity

Principal Researcher: Heida Nadia Bt Zulkefli

Supervisor: Associate Prof. Dr. Nurhayati Binti Zainal Abidin

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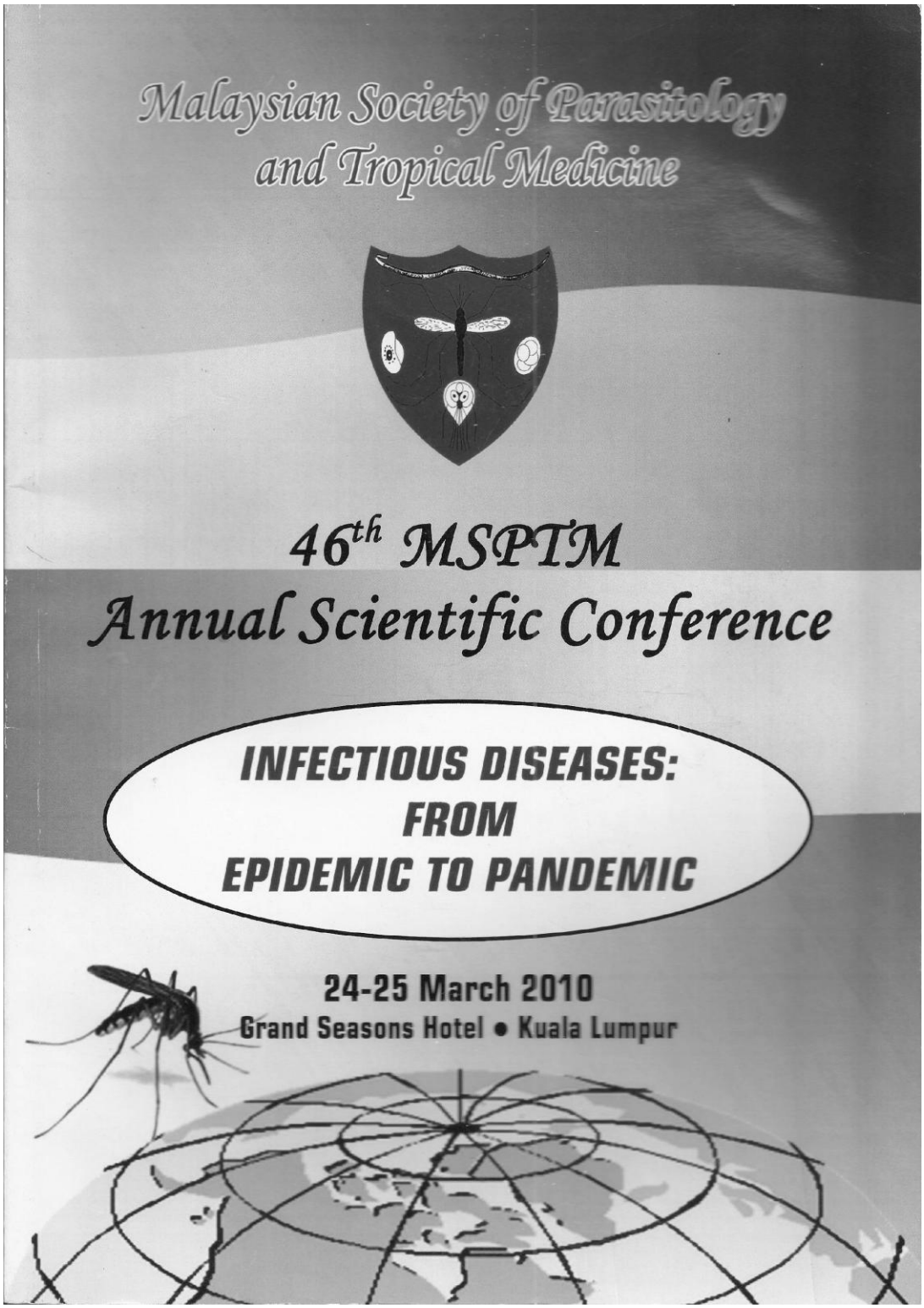
Co-Researcher/s: Prof Madya Dr Mahmood Ameen Abdulla, Faezah Binti Mohamad, Muhamad Fahrin Bin Maskam

Synopsis: Medicinal plants have recently received the attention of the pharmaceutical and scientific communities and various publications have documented the therapeutic value of natural compounds in order to validate claims of their biological activity as an antioxidant. Antioxidants play an important role protecting against damage by reactive oxygen species, decreasing the adverse effects of these free radicals on normal physiological functions in humans. This study focused on flowering plants called *Ervatamia coronaria* from the family Apocynaceae. It is a glabrous, evergreen, dichotomously branched jasmine which is widely distributed in tropical countries. This plant exhibited anticancer, ant-inflammatory and antimicrobial activity but not much research have been carried out regarding the anti-oxidant activity of this plant. Attention has been drawn to evaluate and screen the antioxidant activity of roots, stems and leaves of *Ervatamia coronaria* using various solvent systems such as petroleum ether, methanol, chloroform and water. DPPH radical scavenging assay, metal chelating assay and reducing power assay were carried out to evaluate antioxidant potential of the extracts.


Malaysian Society of Parasitology and Tropical Medicine
46th MSPTM
Annual Scientific Conference
INFECTIOUS DISEASES:
FROM
EPIDEMIC TO PANDEMIC
24-25 March 2010
Grand Seasons Hotel • Kuala Lumpur

Abstract in 46th MSPTM Annual Scientific Conference

P69

Screening of *Ervatamia coronaria* medicinal plant for antioxidant activity

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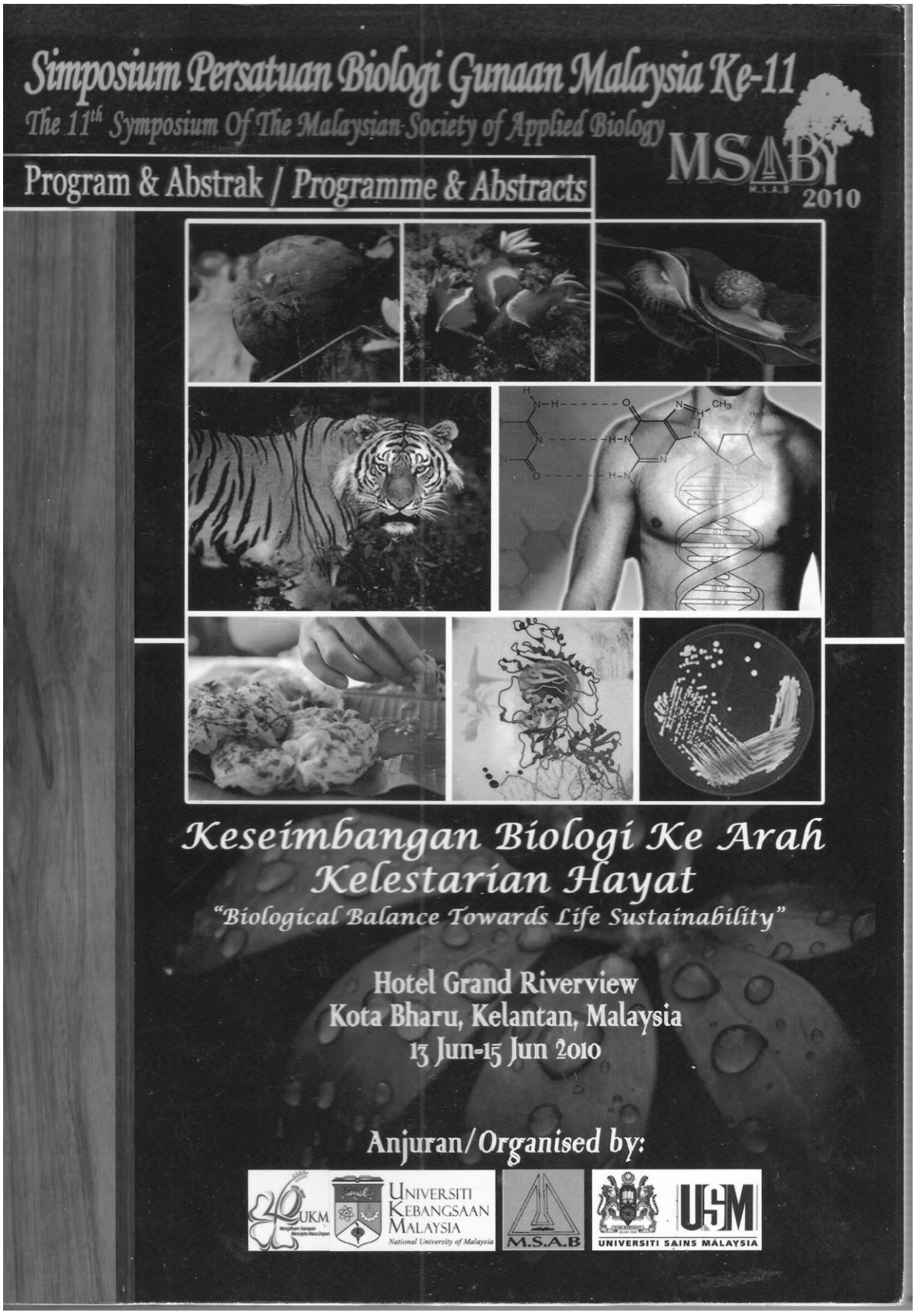
In recent years the use of natural antioxidants has been promoted because of concerns regarding the safety of synthetic ones. Antioxidants play an important role protecting against damage by reactive oxygen species. This study focused on flowering plants called *Ervatamia coronaria* from family Apocynaceae which is widely distributed in tropical country. This plant exhibited anticancer, ant-inflammatory and antimicrobial activity but not many researches have been carried out regarding anti-oxidant activity of this plant. Attention has been drawn to screen the antioxidant activity of roots, stems and leaves of *E. coronaria* using various solvent systems such as petroleum ether, methanol, chloroform and water. DPPH radical scavenging assay, metal chelating assay and reducing power assay were carried out to evaluate antioxidant potential of the extracts. The IC_{50} for standard reference ascorbic acid in DPPH radical scavenging assay was 0.004 mg/ml. The assay showed that the crude chloroform extracts for roots, crude methanol extracts for stems and leaves exhibited active scavenging activity on the stable 2,2-diphenyl-1-picrylhydrazyl with IC_{50} 0.160, 0.176 and 0.162 respectively. In the metal chelating assay, crude water extracts for roots and chloroform extracts for stems showed active inhibition of the formation ferrozine-Fe^{2+} complex in 1 mg/ml with 92.62% and 85.34% respectively when compared to standard reference ethylenediaminetetra acetic acid which was 98.51%. However, all the extracts showed low activity in reducing power assay when compared to standard reference butylated hydroxyanisole. The extracts of *E. coronaria* that showed active antioxidant activity are worth of further investigation in order to identify the active compounds.


Simposium Persatuan Biologi Gunaan Malaysia Ke-11
The 11th Symposium Of The Malaysian Society of Applied Biology
MSAB 2010
Program & Abstrak / Programme & Abstracts
Keseimbangan Biologi Ke Arah Kelestarian Hayat
"Biological Balance Towards Life Sustainability"
Hotel Grand Riverview
Kota Bharu, Kelantan, Malaysia
13 Jun-15 Jun 2010
Anjuran/Organised by:
UKM
Universiti Kebangsaan Malaysia
National University of Malaysia
M.S.A.B
USM
Universiti Sains Malaysia

Abstract in The 11th Symposium of the Malaysian Society of Applied Biology

P132

Evaluation of antioxidant activity in *Ervatamia coronaria* roots

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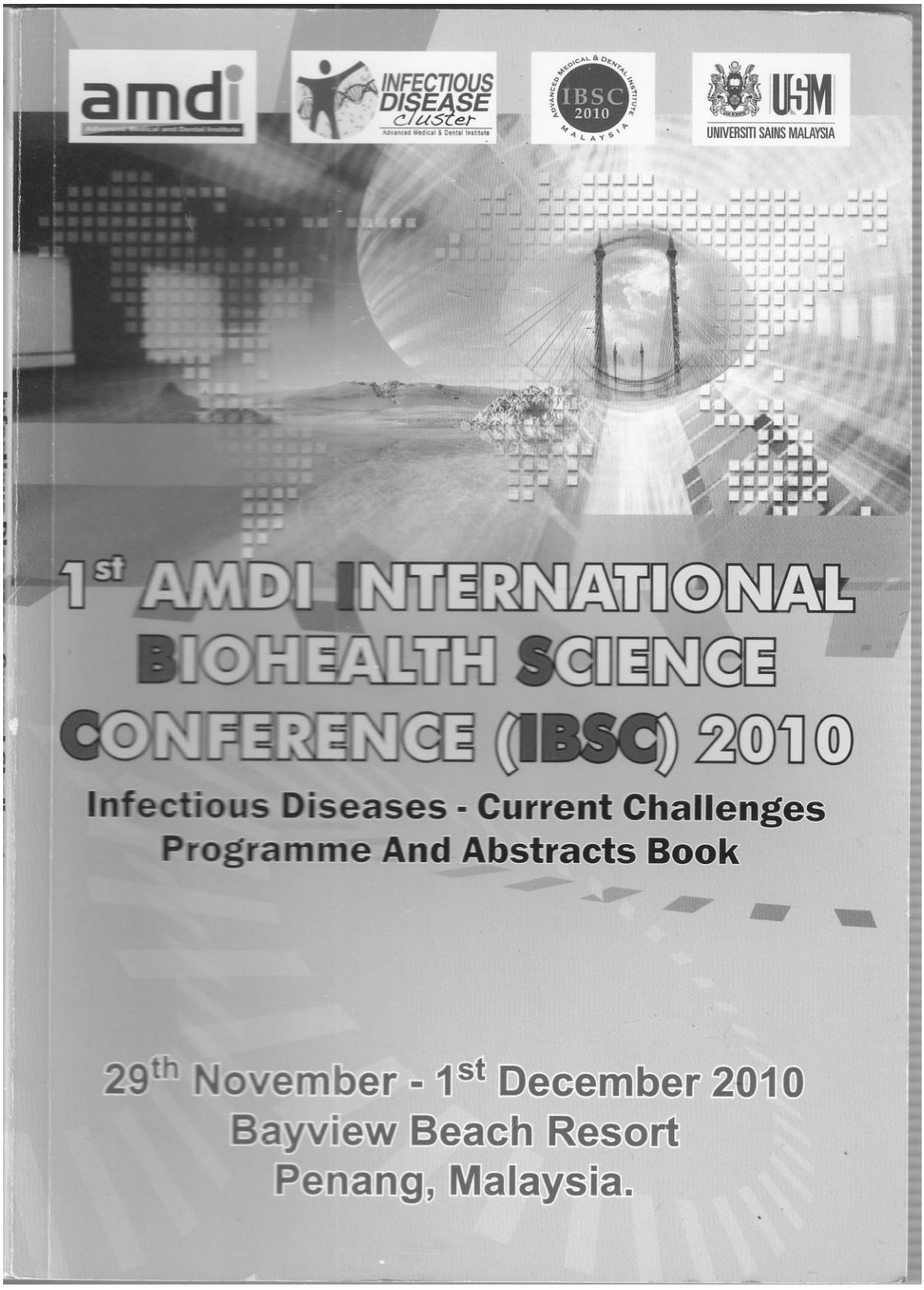
There has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants because of concerns regarding the safety of synthetic ones. The present study was carried out to evaluate the antioxidant activity of *Ervatamia coronaria* roots using various solvent systems such as petroleum ether, methanol, chloroform and water. *Ervatamia coronaria* is a member of Apocynaceae family which is widely distributed in tropical countries. The antioxidant activity was determined using three assays, namely DPPH radical scavenging assay, metal chelating assay and reducing power assay. The IC_{50} is concentration required to obtain 50% antioxidant capacity. The IC_{50} for ascorbic acid (positive control) in the DPPH radical scavenging assay was 0.004 mg/ml. Chloroform extract exhibited active scavenging activity against the stable 2,2-diphenyl-1-picrylhydrazyl with IC_{50} 0.16 mg/ml and 76.53% inhibition in 0.5 mg/ml concentration compared to other extracts. Water extract showed active inhibition (92.62%) of the formation ferrozine-Fe^{2+} complex at 1 mg/ml when compared with the inhibition activity of the standard EDTA which was 98.51%. All extracts showed slightly low activity in reducing power assay when compared to standard BHA. 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 due to positive result with dragendorff reagent, vanillin reagent, anisaldehyde reagent and iodine vapour. The extracts of *Ervatamia coronaria* that showed active antioxidant activity are worth of further investigation in order to isolate and identify the active compounds.


amdi
INFECTIOUS DISEASE cluster
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ADVANCED MEDICAL & DENTAL INSTITUTE MALAYSIA
USM
UNIVERSITI SAINS MALAYSIA
1st AMDI INTERNATIONAL BIOHEALTH SCIENCE CONFERENCE (IBSC) 2010
Infectious Diseases - Current Challenges
Programme And Abstracts Book
29th November - 1st December 2010
Bayview Beach Resort
Penang, Malaysia.

Abstract in 1st AMDI International Biohealth Science Conference (IBSC)

IBSC-2010 POSTER

T007.P

The effects of *Ervatamia coronaria* (roots) on microorganism causing sinusitis

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Background/Aim: The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. The present study was carried out to evaluate antimicrobial activity and phytochemical contents of *Ervatamia coronaria* (roots) using various solvent systems such as petroleum ether, chloroform, methanol and water extracts.

Methodology: Thin layer chromatography (TLC) was used to screen phytochemical contents of the plant extracts while the antimicrobial activity of each extract against three common fastidious bacteria causing sinusitis namely *Streptococcus pneumonia* (ATCC49619), *Haemophilus influenza* (ATCC19247) and *Moraxella catarrhalis* (ATCC23246) was evaluated using disc diffusion method followed by minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). MIC is the lowest concentration of an antimicrobial that will inhibit the visible growth of a microorganism while MBC is the lowest concentration of antimicrobial required to kill a microorganism.

Results: Positive reactions of TLC with dragendorff reagent, vanillin reagent, anisaldehyde reagent and iodine vapour indicated the presence of alkaloid, terpenoid, phenol, flavanoid, saponin and conjugated bond. Chloroform and methanolic extract was seen to inhibit the growth of all test microorganisms while petroleum ether only inhibited the growth of *Moraxella catarrhalis*. In contrast, water extract did not show any antimicrobial activity against the test organisms. The lowest MIC value was observed in chloroform extract of *Streptococcus pneumonia* with 6.25mg/ml followed by 12.5mg/ml in chloroform and methanol extracts of *Moraxella catarrhalis*. The chloroform extract of *Moraxella catarrhalis* also exhibited lowest MBC compared to other extracts with 12.5mg/ml. Standard antimicrobial disc used were imipinem and penicillin.

Conclusions: These findings justify the traditional use and potential usefulness of this plant as treatment for sinusitis.

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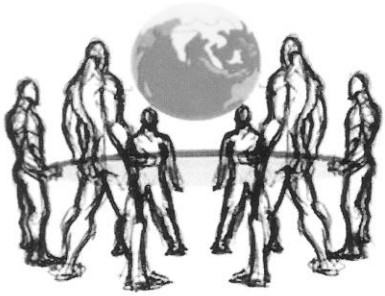
15th Biological Sciences Graduate Congress

"Bridging Ideas, Building Talents"

Programme & Abstracts



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Chulalongkorn University
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Abstract in 15th Biological Sciences Graduate Congress

15th Biological Sciences Graduate Congress
15-17 December 2010
University of Malaya

PT1- 01

The Effects of *Ervatamia coronaria* (stems) on Microorganism Causing Sinusitis

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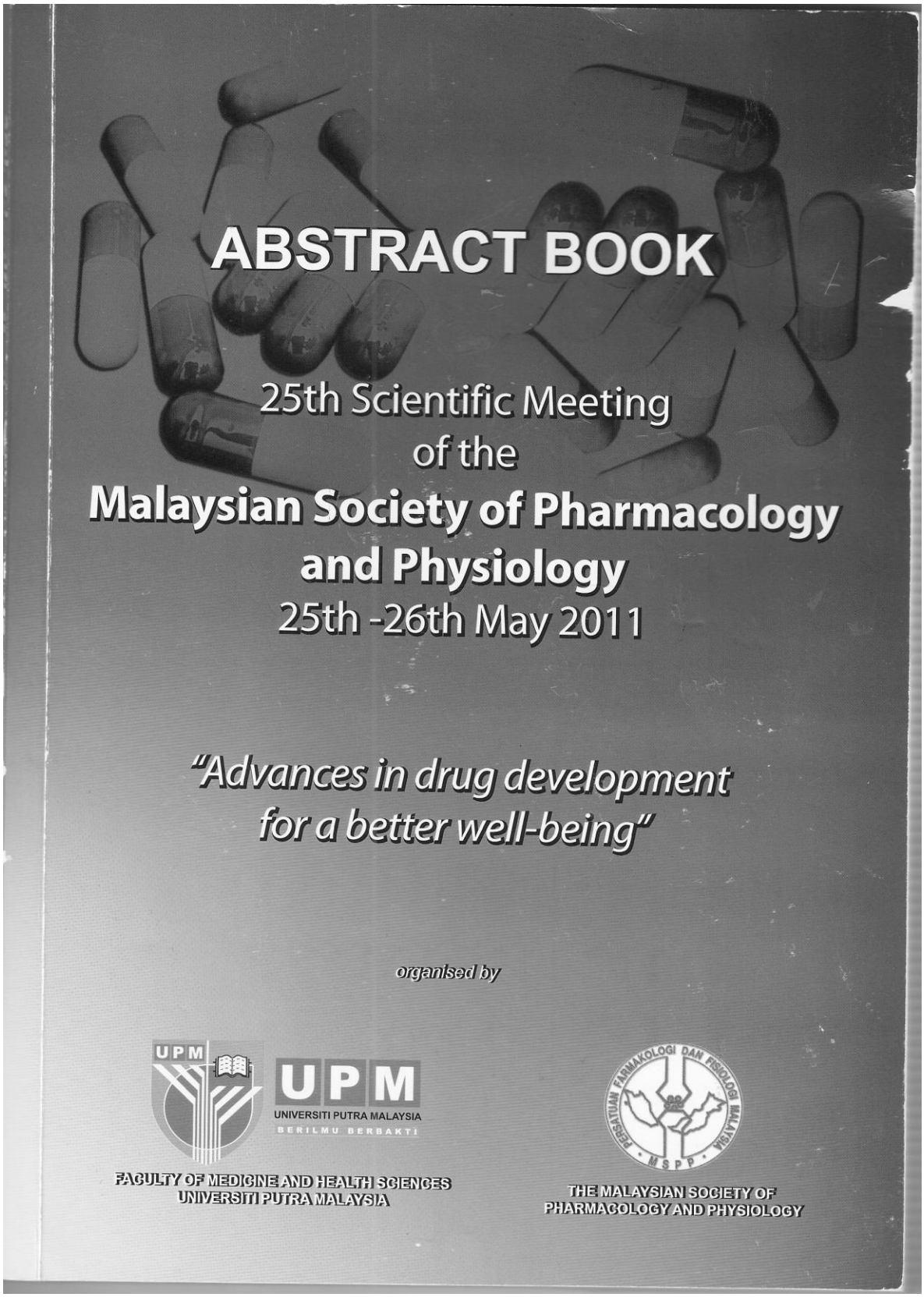
The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other sources including plants. The present study was carried out to evaluate antimicrobial activity and phytochemical contents of *Ervatamia coronaria* (stems) using various solvent systems such as petroleum ether, chloroform, methanol and water extracts. Thin Layer Chromatography (TLC) was used to screen phytochemical contents of the plant extracts. Positive reactions with dragendorff reagent, vanillin reagent, anisaldehyde reagent and iodine vapour indicate the presence of alkaloid, terpenoid, phenol, flavanoid and conjugated bond. The antimicrobial activity of each extract against three common fastidious bacteria causing sinusitis namely *Streptococcus pneumonia* (ATCC49619), *Haemophilus influenza* (ATCC19247) and *Moraxella catarrhalis* (ATCC23246) was evaluated using disc diffusion method followed by minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). Chloroform and methanolic extract was seen to inhibit the growth of all test microorganisms while petroleum ether only inhibited the growth of *Moraxella catarrhalis* and *Streptococcus pneumoniae*. In contrast, water extract did not show any antimicrobial activity against the test organisms. The lowest MIC value was observed for chloroform extract of *Moraxella catarrhalis* with 3.12 mg/ml followed by 6.25 mg/ml methanol extracts of *Streptococcus pneumoniae* and *Moraxella catarrhalis*. The methanol extract of *Moraxella catarrhalis* exhibited the lowest MBC compared to other extracts with 12.5 mg/ml. Positive control antimicrobial disc used were Imipinem and Penicillin. These findings justify the traditional use and potential usefulness of this plant as treatment for sinusitis.


ABSTRACT BOOK
25th Scientific Meeting
of the
Malaysian Society of Pharmacology
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"Advances in drug development
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PHARMACOLOGY AND PHYSIOLOGY

Abstract in Malaysian Society of Pharmacology and Physiology

P035

In Vitro Antioxidant And Antimicrobial Activity Of _Tinospora crispa_ On The Treatment Of Sinusitis

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Sinusitis is a condition characterized by inflammation of the sinuses resulting from infection, allergy or autoimmune issues. Among 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 activity of *Tinospora crispa* (stems) of petroleum ether, chloroform, methanol and water extracts using three different assays namely DPPH radical scavenging assay, metal chelating assay and reducing power assay. The extracts were further evaluated for antimicrobial activity using disc diffusion method followed by minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) against four common bacteria causing sinusitis namely *Streptococcus pneumonia* (ATCC49619), *Haemophilus influenza* (ATCC49247), *Moraxella catarrhalis* (ATCC23296) and *Staphylococcus aureus*. Methanol extract exhibited good radical scavenging and metal chelating activities with IC_{50} 0.118 mg/ml (90.85% inhibition) and 81.97% inhibition respectively. All extracts showed slightly low activity in reducing power assay (0.39 A-0.96 A) when compared to standard BHA (2.79 A). Assays on antimicrobial activity showed chloroform extract inhibited all test microorganisms. Petroleum ether and methanol extracts inhibited the growth of three organisms except *Streptococcus pneumonia* and *Staplylococcus aureus* respectively while water extract did not show antimicrobial activity against any of the test microorganisms. The lowest MIC observed was 3.12 mg/ml and the lowest MBC observed was 6.25 mg/ml. Findings of this study indeed justified the potential of *Tinospora crispa* as antioxidant by preventing free radical from damaging healthy tissue causing inflammation to sinus and also as antimicrobial by preventing the bacteria causing sinusitis infection.

Keywords: *Tinospora crispa*, sinusitis, antioxidant, antimicrobial, DPPH