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ORAL PRESENTATIONS

COMMERCIALISATION, MARKETING AND REGULATORY AFFAIRS

SCREENING OF SELECTED FUNGI FOR BIOLOGICAL CONTROL AGENT POTENTIAL TOWARDS WOOD-DEGRADING FUNGI OF MALAYSIA

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Introduction: Wood-degrading fungi are serious threat to rubberwood. Conventional chemical control has been a successful method of preserving staining fungal growth, but the effects of these chemicals are of concern because they create problems for the environment and public health.

Objectives: This study is part of a screening programme, where a total of 10 extracts prepared from broth cultures of selected fungi belonging to several species of the genera *Trametes* and *Lentinus* were evaluated for their biological control potential against selected wood-degrading fungi.

Materials and Methods: This study was carried out by minimum inhibitory concentration (MIC) assay associated with broth dilution method.

Results: The MICs of extracts determined by the broth dilution method ranged from 0.31 to 5.00 mg/mL.

Conclusion: The differences in antifungal activities observed in different fungal genera suggested that the ability to produce bioactive compounds is not homogenously distributed.

RAW MATERIAL AND POST-HARVESTING

RAUNITICINE-*ALLO*-OXINDOLE B, A NEW NATURAL PENTACYCLIC OXINDOLE ALKALOID FROM MALAYSIAN *UNCARIA LONGIFLORA* VAR *PTEROPODA*

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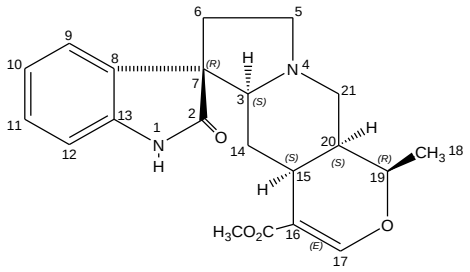
Introduction: Uncaria, a woody climber, is a genus belonging to the family Rubiaceae. Previously, we have reported the isolation of four heteroyohimbine-type oxindole alkaloids namely, pteropodine, isopteropodine, uncarine F and isopteropodic acid from the stems extract of *Uncaria longiflora* var. *pteropoda*, locally known as *kekai*.

Objectives: To further isolate and elucidate bioactive compounds from the methanolic stem extract of Malaysian *U. longiflora* var. *pteropoda* using various chromatographic and spectroscopic techniques.

Materials and Methods: Crude methanolic stem extract of the plant was acidified with 5% hydrochloric acid. Filtration to remove non-alkaloidal material followed by basification with 37% ammonium hydroxide released the alkaloids, which were taken into chloroform to give the crude alkaloid fraction. The crude alkaloid fraction was then dissolved in methanol and subjected to radial chromatography (4 mm thickness silica gel plate) with dichloromethane:ethyl acetate:methanol (DCM:EtOAc:MeOH) in gradient elution. Fractions with identical retention factor (R_f) on the analytical thin layer chromatography (TLC) were pooled. Alkaloids were separated and purified by repeated preparative column chromatographic techniques.

Results: Further isolation and purification of alkaloidal crude extract from the stems of the plant have afforded rauniticine-*allo*-oxindole B, a pentacyclic oxindole alkaloid (POA) which has never been isolated from a natural source. Along with this, two coumarins were also successfully isolated and characterised. Structure elucidation of these compounds was accomplished by spectroscopic methods of NMR (^{1}H, ^{13}C and DEPT), 2D-NMR (COSY, HMQC and HMBC), UV, IR and MS, as well as by comparison with literature.



Rauniticine-*allo*-oxindole B

SYNTHESIS OF SILVER NANOPARTICLES (AGNPS) BY *PYCNOPORUS SANGUINEUS* IN SHAKE FLASK CULTURE

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Introduction: The development of environmental friendly and sustainable techniques for the production of silver nanoparticles (AgNPs) to be used in medical field is a big challenge. Reports showed that it can be synthesised by microorganism (such as bacteria, fungi, actinomycete, yeast, and viruses) either intra- or extra-cellularly. Earlier this decade, the potential of various microbes for the synthesis of nanoparticles were explored. The possibility of using microbes and plant materials as nano-factories were examined. Since then, various microorganisms have been employed for the synthesis of nanoparticles. However, to the best of our knowledge no studies were ever done using the white rot fungi. In this study, the wood rotting fungus, *Pycnoporus sanguineus* was chosen for the synthesis of the AgNPs.

Objectives: This study involves identifying the optimum parameter for biological synthesis of AgNPs by locally isolated fungus, *P. sanguineus.*

Materials and Methods: Locally isolated fungus, *P. sanguineus* was obtained from Forest Research Institute of Malaysia (FRIM). Malt extract broth (MEB) comprised of malt extract (3 g/L), yeast extract (3 g/L), peptone from meat (3 g/L) and glucose (10 g/L) were used as a seed culture media. Five percent of the tested cell suspension was then inoculated and incubated at 28°C, 200 rpm for 48 h in shake flask culture. The mycelium thus formed was transferred aseptically into a cultivation broth which contains KH_2PO_4 (7 g/L), K_2HPO_4 (2 g/L), $MgSO_4.7H_2O$ (0.1 g/L), $(NH_4)_2SO_4$ (1 g/L), yeast extract (0.6 g/L) and glucose (10 g/L), respectively. The flasks were then incubated at 28°C, 200 rpm for 96 h. The harvested culture broth was centrifuged at 4500 rpm, 10°C for 15 min. The mycelium was washed with deionised water and inoculated into an aqueous $AgNO_3$ solution. The extracellular formations of AgNPs were monitored by measuring the optical density (OD) at 380 nm. For intracellular identification, the mycelium was re-suspended in phosphate buffer saline (PBS) and homogenised using a sonicator at a frequency of 6.5 Hz for 5 min. The culture supernatant were inoculated into the $AgNO_3$ solution and cultured using previous similar condition. The particle-size distributions were determined using Zetasizer Nano ZE and the concentrations of AgNPs were identified using inductive couple plasma (ICP).

Results: Results showed that the AgNPs produced intracellularly were monodisperse, and the size was distributed linearly in a range of 30–100 nm. However a reverse trend was observed for the AgNPs produced extracellularly, in which the distribution was polydisperse with larger radius size of 45–80 nm. The concentration of AgNPs were found to be higher (400–5000 mg/L) as compared to the particles that were produced intracellularly.

Conclusion: P. sanguineus, a wood rotting fungus is able to produce AgNPs either intra- or extracellularly. Different environment condition influenced the characteristics and concentration of the nanoparticles production in shake flask culture.

ISOLATION AND IDENTIFICATION OF ACTIVE TRITERPENOID COMPOUND AS ANTIFUNGI FROM METHANOL EXTRACT OF BINAHONG [*ANREDERA CORDIFOLIA* (TEN.) STEENIS] LEAVES

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Objectives: Isolation, identification, and antifungal activity of triterpenoid compound from methanol extract of *Anredera cordifolia* (Ten.) Steenis had been done.

Materials and Methods: Dried leaves powder of the plant (387.8 g) was extracted successively with *n*-hexane (11.16 g) and methanol (33.1 g). The methanol extract was partitioned between water and *n*–butanol. The *n*–butanol fraction was separated respectively by vacuum liquid and column chromatography to get the X compound. The X compound was identified using UV–Vis, IR, ^{1}H–RMI and ^{13}C–RMI spectroscopy.

Results: Based on the spectra, we conclude that X compound is similar with boussingosida A_2. Antifungal activity of compound X showed growth inhibition of *Candida albicans* at 250–1000 μg/mL.

COMPARATIVE STUDY OF STANDARDISED EXTRACTS OF *VINCA ROSEA* LEAVES FROM PERLIS HERBAL FARM AND WILDLY COLLECTED EXTRACTS FROM BALIK PULAU USING ANTICANCER MARKERS ON HPLC

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Introduction: *Catharanthus roseus* (*Vinca rosea*) is locally known as *kemuning cina,* a perennial plant commonly seen in tropical countries. In order to maintain consistent supplies of quality raw materials for processing, cultivation and harvesting of *V. rosea* as per the guidelines of Good Agriculture Practices (GAP) were attempted.

Objectives: To compare and quantify the anticancer markers present in *V. rosea* leaf extracts from Perlis Herbal Farm (PHF) with *V. rosea* leaf extracts wildly collected plants from Balik Pulau (BP) using high performance liquid chromatography (HPLC) method.

Materials and Methods: *V. rosea* leaves harvested from Perlis Herbal Farm and collected from Balik Pulau were air dried, powdered and extracted using conventional method. A reversed phase-HPLC (RP-HPLC) validated method was used to determine quantitatively anticancer markers (vindoline, vincristine, catharanthine and vinblastine) using an Agilent Technologies Series 1100 system equipped with degasser, an auto sampler, a column heater, quaternary pump and UV detector. Column (Eclipse plus C_{18}, 250 mm x 4.6 mm, 5 µm i.d.) was maintained at 40ºC and samples (20 µl) were eluted by an isocratic mobile phase consisting of methanol:acetonitrile:ammonium acetate buffer (15:45:40 v/v) with 0.1% triethylamine (25 mM) at 297 nm.

Results: PHF methanol extracts samples showed vindoline, vincristine, catharanthine and vinblastine concentration in the range 0.50%, 0.02%, 0.15% and 0.02% respectively, whilst samples from BP contains 0.19%, 0.02, 0.12% and 0.01% respectively. Similarly, this trend was also observed in other methanol: water solvent mixtures i.e. 75:25; 50:50; 25:75 and water extracts.

Conclusion: In order to obtain standardised extracts, good quality raw materials are essential. This can be obtained through cultivation under controlled agronomical conditions in compliance with GAP.

THE APPLICATION OF THE NINE STAR HALO-N THEORY IN THE NATURAL PRODUCT RESEARCH

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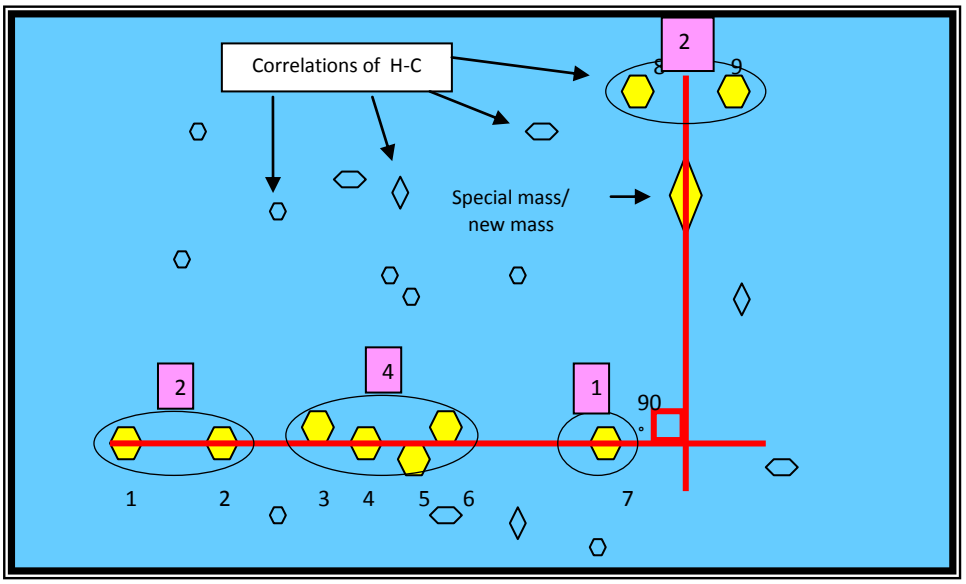
Introduction: The Nine Star Halo-N and the related theories have been created by Dr. Hj. Ahmad Laksamana Omar (Dr. Halo-N) and these theories were deposited in his book titled *Al-Fathun Nawa Jilid 1*.

Objectives: The study was undertaken to prove the Nine Star and related theories and its application in the field of natural products.

Materials and Methods: The HMBC spectra of various alkaloids have been used in order to prove the above theories.

Results: Nine Stars Halo-N theory stated that: each specialty of mass of bio-nature will occur under the arrangement conducted by nine stars (called *Code Nine Stars L System: 2.4.1.2.*) in righteous equilibrium coordinate**.** This theory can be pictured as shown below.



Nine Stars L System: 2.4.1.2.

According to this teory, any compound which has a the 2.4.1.2 arrangement in the HMBC spectrum and has special point between the two correlation points in the vertical line as shown above, thus this compound must have bioactivity and can be used as a vaccine to treat certain disease.

Conclusion: The present study showed that the HMBC spectra of alkaloid (-) tavoyanine; (-) norboldine; (-) thalifoline; and (+) pallidinine have shown the positive results according to the Nine Star Halo-N theory.

REVIEW ON THE EFFECTS OF PROCESS PARAMETERS OF PROTEIN EXTRACTION FROM PLANT

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Introduction: The development of eco-friendly material in resin production has been realised due to the shrinking earth's natural petrochemical resources, increasing demand of adhesives, and the increasing environmental concern. Nowadays, utilisation of plant protein in biopolymers and composites production has been necessitated in order to enhance the global sustainability.

Objectives: The review aims to understand the effects of process parameters on protein extraction from various plants.

Materials and Methods: Few research papers were reviewed and the results from journals were rediscussed and reanalysed by simulating responses, and using the regression equations from journals. This concluded review among the decision of using alkaline, saline, or water treatment method is based on the bulk of protein containing either highest alkaline-soluble fraction, salt-soluble fraction, or alcohol-soluble fraction. Finally, this study suggests possible future works that could enhance the contribution of protein extraction to minimise local environmental degradation and sustainable livelihoods erosion.

Results: Most of the proteins were solubilised during the first 30 minutes of extraction. Further extraction time may cause a decrease in extractable protein due to possible coagulation of soluble protein with other components. There are few main parameters that will affect the protein extraction from plant: solvent:meal ratio, pH of solution, extraction temperature, and extraction time. Solvent:meal ratio which always seems to exert quadratic effect on protein extraction, also seems to have the largest effect on protein extraction.

Conclusion: Decision of using alkaline, saline, or water treatment method for plant protein extraction is actually based on the bulk of protein containing either highest alkaline-soluble fraction, salt-soluble fraction, or alcohol-soluble fraction.

CHEMISTRY OF NATURAL PRODUCTS INCLUDING QUALITY CONTROL, STANDARDISATION AND GOOD MANUFACTURING PRACTICE (GMP)

DETERMINATION OF FLAVONOIDS AND CHLOROGENIC ACID IN *GYNURA PROCUMBENS* BY A SIMPLE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD WITH ULTRAVIOLET DETECTION (HPLC-UV)

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Introduction: Gynura procumbens (Lour.) Merr. (Compositae), is cultivated in most of the South-east Asian countries including Malaysia, Thailand and Indonesia for its medicinal purposes such as blood sugar and lipid lowering, antihypertensive and antioxidant. This plant was known to contain various flavonoids and polyphenolic compounds, however to date there is no report related to quantification of these compounds in *G. procumbens.*

Objectives: To develop and validate a RP-HPLC-UV method for the quantification of chlorogenic acid, rutin and kaempferol-3-O-rutinoside in different ethanolic aqueous (95%, 75%, 50%, and 25% v/v) and water extract of *G. procumbens.*

Methods: HPLC analysis was performed on a LiChroCART RP-18 column (250 X 4.6 i.d. mm, 5 µm particle size) with a isocratic mobile phase of acetonitrile:0.25% aqueous acetic acid (20:80 v/v) at the flow rate of 1.0 mL/min in room temperature.

Results: The method recorded a limit of detection of 0.195 µg/mL and limit of quantification of 0.781 µg/mL for chlorogenic acid and kaempferol-3-O-rutinoside while for rutin, the limit of detection and limit of quantification are 0.351 µg/mL and 1.563 µg/mL respectively. The calibration curves of all 3 compounds were linear over the concentration range of 0.781–50 µg/mL for chlorogenic acid and kaempferol-3-O-rutinoside and 1.56–50 µg/mL for rutin with squared correlation coefficient of 0.9992 or better. The intra-day and inter-day precision for the 3 compounds were below 4.7%, while accuracies were between 96.92% and 103.55%. This method was successfully applied for the quantification of the three compounds in *G. procumbens*.

Conclusion: A simple HPLC-UV method was developed and validated for simultaneous determination of chlorogenic acid, kaempferol-3-O-rutinoside and rutin in *G. procumbens.* The method was found to be accurate and precise. The method was successfully applied for the quantification determination of flavonoids and chlorogenic acid in *G. procumbens*.

ANTIANGIOGENIC ACTIVITY OF *MORINDA CITRIFOLIA* EXTRACTS AND ITS CHEMICAL CONSTITUENTS

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Introduction: Morinda citrifolia L. (*noni*) has been used in folk remedies by Polynesians for over 2000 years, and has been used for the treatment of wide variety of diseases including cancer. The antiangiogenic effect of *M. citrifolia* was evaluated by using chicken chorioallantoic membrane (CAM) assay. The chick embryo chorioallantoic membrane assay is one of the widely used in vivo tools for evaluating antiangiogenic potential of drugs including herbal extracts.

Objectives: The study was undertaken to evaluate antiangiogenic effect of different extracts, fractions and chemical constituents of *M. citrifolia* fruits and leaves.

Materials and Methods: Antiangiogenic activity was evaluated in vivo using the CAM assay. Bioactivity-guided fractionation and isolation were performed to identify the active

constituent and subsequently, high performance liquid chromatography (HPLC) analysis was carried out to quantify the amount of active constituent in the active extracts and fraction.

Results: The methanol extracts of fruits and leaves of *M. citrifolia* and the subsequent chloroform fraction of the fruits methanolic extract were found to have potential antiangiogenic activity and were more potent compared to suramin. Scopoletin was identified as one of the chemical constituents that may partly be responsible for the antiangiogenic activity of *M. citrifolia* fruits.

Conclusion: The present findings further support the use of *M. citrifolia* in cancer or other pathological conditions related to angiogenesis.

ISOLATION, CHARACTERISATION AND BIOLOGICAL EVALUATION OF NOVEL CYCLIC POLYAMIDES FROM *SALVADORA OLEOIDES DECNE.*

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Introduction: Salvadora oleoides commonly known as *pilu* or *wan is* used traditionally for treatment of fever and rheumatic pains while shoots of the plant are used as natural toothbrush. Literature reported significant hypoglycemic and hypolipidemic activities of ethanol extract of aerial parts of *Salvadora*.

Objectives: This study was conducted to isolate bioactive compounds from the methanol extract of the plant.

Materials and Methods: The plant, collected from Lahore, Pakistan was extracted with methanol. The compounds were isolated by repeated column chromatography and purified using cyclograph. Their structures were elucidated by IR spectroscopy, 1D and 2D NMR spectroscopy, mass spectrometry and elemental analysis. Compounds were biologically evaluated for cytotoxicity and antimicrobial activity by using MTT assay and broth micro dilution method, respectively.

Results: Purified compounds were found to be cyclic polyamides. A HRESIMS data of compound 1 exhibited a molecular ion peak $[M+Na]^+$ at 818.4370 and consistent with a molecular formula of $C_{35}H_{65}N_5O_{15}$. This compound is based on 5 similar monomers, each having the molecular formula $C_7H_{13}NO_3$ with m/z value of 159. Compound 2 was also constructed by 5 similar units, each unit having the formula $C_7H_{13}NO_2$ with m/z value of 143, while the molecular ion peak appeared at 738.4606 as $[M+Na]^+$ with the molecular

formula $C_{35}H_{65}N_5O_{10}$. All the analytical data supported the proposed structures of the compounds. Both compounds showed weak in vitro cytotoxicity activity when tested against breast cancer, liver cancer and colon cancer cell lines. However, good activity was observed against fungus, and both Gram positive and Gram negative bacteria.

Conclusion: This is the first report of natural occurrence of novel cyclic polyamides from a plant source. Both compounds exhibited antibacterial and antifungal activities.

BIOACTIVE COMPOUNDS FROM STEM BARK OF *ENTADA SPIRALIS* RIDL

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Introduction: Entada spiralis Ridl. (Leguminoceae) which is locally known as *beluru* or *sintok* is a liana or woody climber that grows wildly in Malaysia. The isolation and characterisation of the major chemical constituents from the bioactive extract of the stem bark of *E. spiralis* has been carried out. Our previous study revealed that the methanol extract was active against dermatophytes that causes skin infections. Strains of dermatophytes, *Trichophyton mentagrophytes* ATCC 9533, *Trichophyton tonsurans* ATCC 28942 and *Microsporum gypseum* ATCC 24102 were used.

Objectives: This study was to elucidate the structure of active constituents from the active fraction of methanol extract from the stem bark of *E. spiralis*.

Materials and Methods: The extract was further fractionated with different solvent gradient and repeated test on the dermatophytes were done. The structure elucidation of this compound was based on spectroscopic data (^{1}H and ^{13}C NMR, HMQC, HMBC and DEPT135).

Results: The most active fraction of the methanol extract was chloroform: methanol [6:4 (v/v)]. From this fraction, a white crystalline solid was isolated and its structure was proposed as a new triterpene derivative.

Conclusion: The current study provided important baseline information for the use of *E. spiralis* as well as its constituent for the treatment of skin infections associated with the studied microorganisms.

MANGOSTINS AND THEIR BIOACTIVITIES FROM *GARCINIA MALACCENSIS* HK.F

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Introduction: Guttiferae is a well-known family having a wide range of phytochemical constituents and bioactivities.

Objectives: To investigate phytochemicals and bioactivities of *Garcinia malaccensis*.

Materials and Methods: The isolated compounds were elucidated by spectroscopic methods. Antimicrobial activity was carried out by disc diffusion method. Antiproliferative activity was performed by tryphan blue exclusion and MTT assay. Antioxidant activity was conducted by DPPH radical scavenging.

Results: Phytochemicals investigation of the stem bark of *G. malaccensis* lead to isolation of α-mangostin, β-mangostin and a triterpenoid. The main priority of this study's activity was done on α-mangostin as the compound was found as a major constituent. Results showed that α-mangostin inhibits *Staphylococcus aureus*. The diameter of inhibition zone observed was 8 mm, minimum inhibition concentration (MIC) was 0.025 mg/mL and minimum bactericidal concentration (MBC) was 0.1 mg/mL. Dot-blot DPPH staining showed a positive antioxidant activity of α-mangostin. In FTC method, α-mangostin was proved to be a good lipid peroxidation inhibitor, whereas in DPPH free radical scavenging activity method, it has a very weak scavenging effects on free radicals. In the antiproliferative assay, α-mangostin exhibited activity against K562 (IC_{50} 20 µg/mL) and showed a weak activity against HSC3 and H1299 cell lines.

Conclusion: Three compounds have been isolated from *G. malaccensis*. α-mangostin showed antimicrobial, antoxidant and antiproliferative activities.

SYNTHESIS AND CYTOTOXIC ACTIVITIES OF 1,3,6-TRIOXYGENATED XANTHONE ANALOGUES TOWARDS MDA-MB-231 AND HELA CANCER CELL LINES

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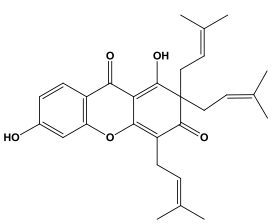
Introduction: Xanthones are heterocyclic compounds with the dibenzo-γ-pyrone framework which are found to be abundant in many higher plants from Gentianaceae, Guttiferae and Polygalaceae families. Many biological studies on the prenylated xanthones either synthetically or naturally, have found the group of compounds to show a broad range of cytotoxic activities towards various human cancer cell lines.

Objectives: To synthesise a series of 1,3,6-trioxygenated xanthone derivatives with typical and new chemical scaffold, and to establish a structure-bioactivity relationship study on the synthetic compounds towards MDA-MB-231 and HeLa cancer cell lines.

Materials and Methods: 1,3,6-trioxygenated xanthonic building block was obtained from the reaction of a mixture of 2,4-dihydroxybenzoic acid and phloroglucinol in Eaton's reagent. Prenylated xanthone derivatives were synthesised through the reaction of xanthonic block with prenyl bromide in alkaline medium. The cytotoxic assays were carried out using MDA-MB-231 and HeLa cancer cell lines via the colorimetric MTT method.

Results: A series of xanthone derivatives with typical and novel skeletons has been successfully synthesised and evaluated for their cytotoxic activities towards HeLa and MDA-MB-231 cancer cell lines. From the chemical synthesis, 1,6-dihydroxy-2,2,4-tris(3-methylbut-2-enyl)-2H-xanthene-3,9-dione (1) and 1,6-dihydroxy-2,2,4,5-tetra(3-methylbut-2-enyl)-2H-xanthene-3,9-dione (2), both with a novel skeleton of 2H-xanthene-3,9-dione have been reported for the first time along with 7 other typical and known xanthones. Preliminary biological screening on compound 1 and 2 had revealed these xanthones with a novel skeleton to elicit a higher inhibitory activity towards HeLa and MDA-MB-231 cancer cell lines with IC_{50} values in the range of 4 to 10 μM, in comparison with the typical xanthones tested.

Conclusion: This present study showed that prenylated xanthones with the 2H-xanthene-3,9-dione skeleton could be a potential prototype in the development of new anticancer drugs.



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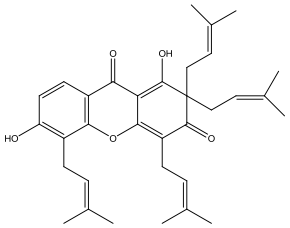

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EVALUATION OF ANTIOXIDANT POTENTIAL OF *FICUS DELTOIDEA* EXTRACTS AND THEIR PHYTOCHEMICAL SCREENING AND PRIMARY METABOLITES ANALYSIS

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Introduction: *Ficus deltoidea* Jack (Moraceae) is commonly known as *mas cotek* and is widely used in indigenous system of medicine and largely distributed in several countries of the South East Asia.

Objectives: This study was conducted to investigate the influence of primary metabolic contents for total proteins, total polysaccharides, glycosaponins, total flavonoids, polyphenols, and tannins which can interfere in the determination of antioxidant potential of methanol and water extracts of *F. deltoidea* leaves.

Materials and Methods: Methanol and water extracts of *F. deltoidea* were used. Antioxidant activity was determined by using in vitro models of free radical scavenging activity (DPPH), xanthine oxidase (XOD) and β-carotene using spectrophotometric methods.

Results: *F. deltoidea* methanol extract showed higher antioxidant activity in DPPH assay with EC_{50} of 1.229 mg/mL when compared to water extract with EC_{50} of 2.52 mg/mL. Both extracts displayed high antioxidant activity in xanthine/ xanthine oxidase system at 63.77% for methanol and 62.58% for water extract. The result of antioxidant activity by β-carotene assay showed medium inhibition for both extracts (45% for methanol and 40% for water extract). Antioxidant activity of the extracts is comparable to quercetin and butylated hydroxyanisole. Primary metabolic contents were evaluated for both extracts. Methanol and water extract showed high content of proteins while the content of glycosaponins was high in methanol extract. The content of total polyphenolics, flavonoids and tannins in water and methanol extracts were estimated. The results showed that methanol extract contain higher polyphenolics, flavonoids and tannin content than the water extract.

Conclusion: This present study showed that antioxidant activity of *F. deltoidea* methanol and water extracts may be considerably related to the contents of total flavonoids, polyphenols, and tannins.

FORMULATION AND EVALUATION OF TASTE MASKED SUMATRIPTAN SUCCINATE ORALLY DISINTEGRATING TABLETS PRODUCED BY FREEZE DRYING TECHNIQUE

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Introduction: Sumatriptan succinate is a highly selective 5-HT_1 receptor agonist used for the treatment of migraine. High solubility in water and bitter taste makes sumatriptan succinate a challenging drug candidate for the preparation of orally disintegrating tablets.

Objectives: The present research formulated taste masked orally disintegrating tablets of sumatriptan succinate using freeze drying technique.

Materials and Methods: The bitter taste of the drug was masked by coating it with Eudragit EPO polymer using spray drying technique. The prepared microspheres were evaluated for yield, particle size, entrapment efficiency and incompatibility between the drug and Eudragit EPO. Drug containing microspheres, different matrix forming agents (gelatin, hydroxypropyl methylcellulose-E3, polyvinyl pyrrolidine K-90, Cryogel and Instagel) in a concentration range of 1%–5% and other excipients were mixed to prepare fine aqueous suspension and then freeze dried to produce orally disintegrating tablets. The prepared tablets were evaluated for weight variation, drug content, water content, in vitro disintegration time and in vitro drug release. The optimised formulation was evaluated for taste, mouth feel and in vivo disintegration time using human volunteers and compared with uncoated orally disintegrating tablets.

Results: DSC results of spray dried powder did not show any incompatibility between drug and Eudragit EPO. The particle size and entrapment efficiency of the microspheres were 7.77 µm and 92.86%, respectively. All the tablet formulations disintegrated in vitro within 40–300 seconds. The optimised formulation released more than 90% of drug in 10 minutes in 0.01M HCl. In human volunteers, optimised formulation was found to have a good taste and mouth feel compared to uncoated orally disintegrating tablets. The tablets disintegrated in the oral cavity within 42 seconds.

Conclusion: The freeze dried orally disintegrating tablets were successfully prepared with desirable taste, mouth feel and rapid disintegration in the oral cavity.

COUMARINS FROM *MEIOGYNE VIRGATA*

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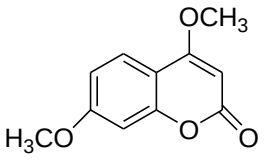
Introduction: The genus *Meiogyne* (Annonaceae) consists of about 24 species, widely distributed in IndoChina, Thailand, Peninsular Malaysia, Sumatra, Java, Borneo and the Philippines. Several plants of the genus *Meiogyne* have been used as folk medicine. In this paper we report the isolation of 5 coumarins; 4,7-dimethoxy-coumarin (1), 3,7-dimethoxy-coumarin (2), 3,8-dimethoxy-coumarin (3), 3,4-dihydroxy-7-methoxy-coumarin (4) and 3,4-dihydroxy-8-methoxy-coumarin (5) from the bark of *Meiogyne virgata*.

Objectives: To isolate, purify and elucidate the structure of the chemical constituents from the bark of *M. virgata*.

Materials and Methods: Dried and ground bark of *M. virgata* was defatted with petroleum ether overnight before being extracted with dichloromethane for 8 hours using Shoxhlet extractor. The dichloromethane extract was subjected to acid-base extraction before being subjected to vacuum liquid chromatography (VLC) eluted gradiently with Hex:EA and DCM:MeOH. The combined fractions were subjected to repeated PTLC using solvent system DCM:MeOH to give compounds 1–5.

Results: Phytochemical work on the bark of *M. virgata* from the family Annonaceae has resulted in the isolation of five coumarins. Various chromatographic techniques were employed for the isolation and structures were determined on the basis of spectroscopic analysis which include 1D-NMR (^{1}H, ^{13}C, DEPT), 2D-NMR (COSY, HMQC, HMBC), UV, IR and MS. Spectral data of known compounds were compared with literature values.

Conclusion: Previous studies on *M. virgata* reported presence of aporphines and oxoaporphines alkaloids. This is the first report of coumarins in *M. virgata.*



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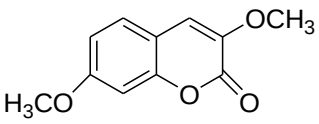

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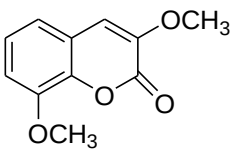

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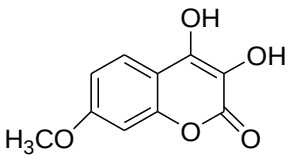

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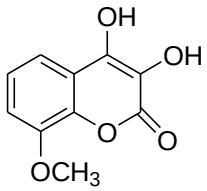

5

ANTIBACTERIAL ACTIVITY OF INDOLE DERIVATIVES ISOLATED FROM THE SOFT CORAL *SINULARIA FRONDOSA*

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Introduction: Malaysia has about 4006 km^2 of coral reef. The soft corals used in this study, *Sinularia frondosa*, are common in shallow coral reef habitats. In some areas, they can make up over 95% of the living animal cover. The lack of predation by fishes on the *Sinularia* spp. has been attributed to their production of chemical and structural defenses.

Objectives: The present research isolated, purified and characterised the chemical constituents of the soft coral and evaluated their antibacterial activities.

Materials and Methods: N-methyl-3,4,5-tribromoindole and N-methyl-3,5,6-tribromoindole have been isolated by column chromatography using hexane:dichloromethane (1:1) and preparative thin layer chromatography using hexane:diethyl ether (4:1). 3,5,6-tribromoindole was purified by column chromatography using CH_2Cl_2. The structures of all compounds were established by various spectroscopic techniques such as infrared, nuclear magnetic resonans (^{1}H-NMR, ^{13}C-NMR) and mass spectrometry.

Results: Three indole derivatives have been isolated, purified and characterised from *S. frondosa*. The compounds have been identified as N-methyl-3,4,5-tribromoindole, N-methyl-3,5,6-tribromoindole and 3,5,6-tribromoindole. N-methyl-3,4,5-tribromoindole and N-methyl-3,5,6-tribromoindole were obtained as a white crystalline needles with melting point of 116°C–118°C and 160°C–162°C, respectively. 3,5,6-tribromoindole was obtained as a pale brown crystalline needles with melting point of 106°C–108°C. Antibacterial screening on the pure compounds showed that N-methyl-3,4,5-tribromoindole and N-methyl-3,5,6-tribromoindole exhibited strong antibacterial activity towards Gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* and Gram positive bacteria *Bacillus subtilis* and *Staphylococcus aureus* at a concentration of 2.5 mg/disk. 3,5,6-tribromoindole showed no antibacterial activity at the similar concentration.

Conclusion: N-methyl-3,4,5-tribromoindole and N-methyl-3,5,6-tribromoindole showed antibacterial effect against Gram negative and Gram positive bacteria at a 2.5 mg/disk concentration.

CHEMICAL CONSTITUENTS FROM *NEONAUCLEA SP-1* (RUBIACEAE)

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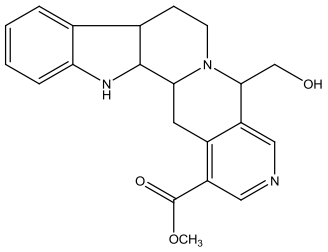
Introduction: *Neonauclea sp-1* locally known as *bangkal* belongs to Rubiaceae family. It is widely distributed in Sabah, Sarawak and Peninsular Malaysia, Malaysia and Sulawesi, Indonesia. *Neonauclea* species has been known to produce various chemical constituents such as indole alkaloids, glycosides, triterpenoids and saponins.

Objectives: The study was undertaken to isolate and elucidate the chemical constituents from the bark and leaves of *Neonauclea sp-1.*

Materials and Methods: Samples of *Neonauclea sp-1* were collected at the Reserve Forest Sg. Tekam, Jerantut, Pahang. A phytochemical study on the leaves of *Neonauclea sp-1* has been carried out by using n-hexane, dichloromethane and methanol to extract the crude.

Results: The crude extract of dichloromethane produced compound such as: Harmane, naucledine (2), 3α-dihydrocadambine, cadamine (1), cinamide, benzamide and blumenol A. The isolation and purification of the compounds were achieved by using column chromatography and PTLC techniques. The structural elucidation was performed by spectroscopic methods such as NMR, TOFLCMS, IR and UV.

Conclusion: Chemical structural study on the leaves and bark of this species has afforded indole alkaloids [Harmane, naucledine (2), 3α-dihydrocadambine and cadamine (1)], amide (cinamide and benzamide) and terpenoid (blumenol A). This is the first pytochemical study on the species of *Neonauclea sp-1.* Further investigation is focusing on bioactivity study of all the compounds isolated from this species.



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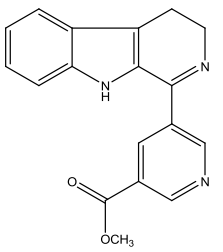

2

ALKALOIDS ISOLATED FROM THE BARK OF *LITSEA GRANDIS* AND *LITSEA LANCIFOLIA* (LAURACEAE)

SYAZREEN NADIA SULAIMAN, KHALIJAH AWANG, A. HAMID A. HADI AND MAT ROPI MUKHTAR
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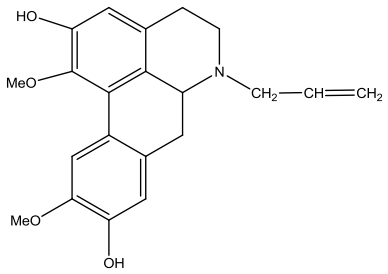
Introduction: *Litsea,* a genus belonging to the family Lauraceae, is widely distributed in tropical Asia, Africa and America. In Malaysia, its contribution is about 213 species, from 16 genera and it is known as *medang* or *tejur*.

Objectives: The study was undertaken to isolate alkaloid compounds from *Litsea grandis* and *Litsea lancifolia.*

Materials and Methods: *L. grandis* is a tropical tree that can be found in Hutan Simpan Bukit Berangi, Kedah which is locally named as *medang daun lebar* meanwhile *L. lancifolia* or locally known as *medang melukut* was collected in Hutan Simpan Tembat Ulu Terengganu, Terengganu. The isolation and purification of the compounds were achieved by using column chromatography and PTLC techniques.

Results: The dichloromethane extracts of *L. grandis* and *L. lancifolia* produced eight alkaloids namely laurotetanine, reticuline, *N*-methylisococlaurine, boldine, norboldine, actinodaphnine, pallidine and *N*-allyllaurolitsine. The structural elucidation was performed by spectral methods namely 1D and 2D NMR, UV, IR and LCMS-IT-TOF.

Conclusion: Chemical structural study on the leaves and bark of these species has afforded various type of alkaloids such as aporphine [laurotetanine, boldine, norboldine and N-allyllaurolitsine (1)], oxo-aporphine [actinodaphnine (2) and cassythicine], benzylisoquinoline (reticuline and N-methylisoquinoline) and morphine (pallidine). This is the first time alkaloids were isolated from *L. grandis* and *L. lancifolia.* Further investigation is focusing on bioactivity study of all the compounds isolated from these species.



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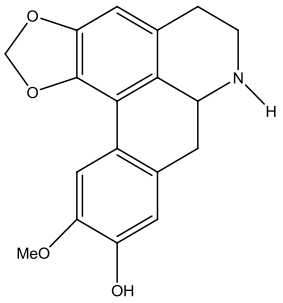

2

SYNTHESIS OF SUBSTITUTED CHALCONES

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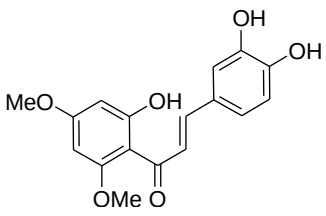
Introduction: Chalcones are naturally occurring flavonoids composed of two aromatic rings linked by a three carbon unit forming an α,β-unsaturated carbonyl group. They are pharmacologically significant because of their capability to exert antioxidant, antimicrobial and antiinflammatory activities. Thus, our study provided synthetic pathways of non-natural substituted chalcones.

Objectives: The study investigated the synthetic pathways of substituted chalcones. To accomplish this, the chalcones mainly hydroxylated, methoxylated and prenylated substituted were synthesised.

Materials and Methods: The initial step in the synthesis was to prepare various derivatives of key precursors on 2′,4′,6′-trihydroxyacetophenone and 3,4-dihydroxybenzaldehyde by prenylation, methylation and protection of the phenolic hydroxyl groups. The target chalcones were then synthesised via Claisen-Schmidt condensation under basic condition. Finally, the deprotection step was required to afford hydroxylated chalcones.

Results: The non-naturally occurring chalcones that had been successfully obtained were compounds 1, 2, 3, 4, 5, 6 and 7.

Conclusion: Our results displayed that several substituted chalcones which were mainly hydroxylated, methoxylated and prenylated were possibly synthesised.



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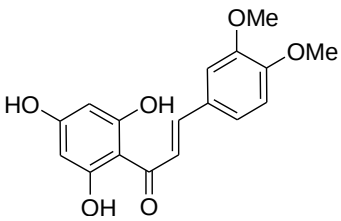

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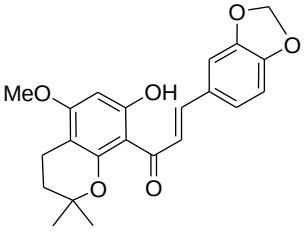

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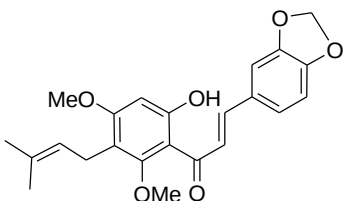

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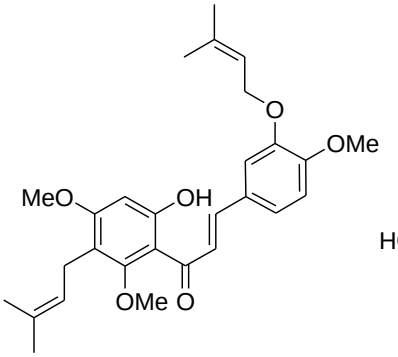

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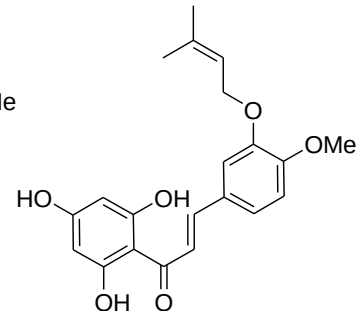

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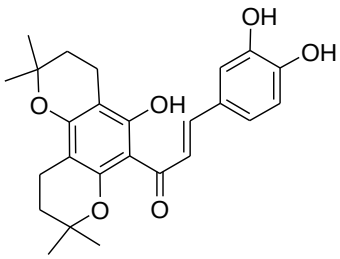

7

ACCELERATED SOLVENT EXTRACTION (ASE) OF *ORTHOSIPHON STAMINEUS* LEAVES AND HPLC ANALYSIS OF METHANOL EXTRACTS USING RAPID SEPARATION LIQUID CHROMATOGRAPHY (RSLC)

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Introduction: An approach comprising accelerated solvent extraction followed by quantitative, fast HPLC analysis has been adopted in quantification of methanol extracts of *Orthosiphon stamineus* leaves.

Objectives: To develop and optimise a new method of extraction and rapid HPLC analysis based on four marker compounds namely rosmarinic acid (RA), 3′-hydroxy-5,6,7,4′-tetramethoxyflavone (TMF), sinensetin (SEN) and eupatorin (EUP) in methanol extracts of the leaves of *O. stamineus*.

Materials and Methods: *O. stamineus* dried leaves were cultivated and collected from the Garden of Titi Tinggi, Perlis, Malaysia and processed at Centre for Herbal Standardization (CHEST), USM. The raw materials were grounded to a fine powder and extracted using methanol in a Dionex ASE 200 with 66 mL stainless-steel extraction cell. Various temperatures (40ºC, 60ºC, 80ºC, 100ºC and 150ºC) were used for this extraction. The methanol extract was subjected to the HPLC analysis using Dionex-Ultimate 3000 rapid separation liquid chromatography (RSLC) system. The developed HPLC method utilises gradient elution on an analytical Acclaim® PolarAdvantage II (PA2) column and UV detection at 340 nm.

Results: The recovery of the methanol crude extract were 1.87%, 3.027%, 4.823%, 2.424% and 9.541% with respect to the temperature of 40ºC, 60ºC, 80ºC, 100ºC and 150ºC respectively. Gradient elution method was used for the simultaneous assay of the authentic markers, which separated within a total time of less than 10 min. The standards were resolved and eluted at 0.797, 5.353, 6.617 and 8.423 min, with respect to RA, TMF, SEN and EUP respectively. All the marker compounds are present in the chromatographic profiles of the methanol extracts running at five different oven temperatures. The peaks of RA, TMF, SEN and EUP were confirmed by comparing the retention times of reference standards. Comparison with conventional HPLC was also carried out.

Conclusion: The ASE method is suitable for herbal application due to the low consumption of solvents, being environmental friendly and having a high yield of crude extract recovery. The RSLC method detects all reference markers in less than 10 min and this makes this method easier to use and is faster when compared to conventional HPLC method.

CHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF *PIPER CANINUM*

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[2]Department of Chemistry, Faculty of Science, Universiti Teknologi MARA, Kampus Samarahan, Jalan Meranek, 94300 Kota Samarahan, Sarawak

Introduction: *Piper caninum* is a member of the Piperaceae family. For years, the chemistry of the *Piper* species has been widely investigated and phytochemical studies from all over the world have resulted in the isolation of numerous biologically active compounds.

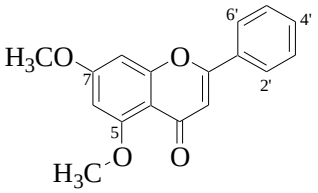
Objectives: The objectives of this study are to purify and identify the phytochemicals from *P. caninum*. The evaluation of the biological activities of the crude extracts and the characterised compounds will be screened for antimicrobial activities.

Materials and Methods: The plant sample was collected from Sarawak and extracted with hexane, ethyl acetate and methanol sequentially by Soxhlet extraction. Fractionation and

purification by chromatographic methods followed by recrystallisation gave five pure compounds. The structures of the pure compounds were elucidated by IR, UV, NMR and MS. Antimicrobial activities have been carried out on the crude extracts and pure compounds.

Results: Phytochemical studies of *P. caninum* yielded 5 pure compounds identified as 5,7-dimethoxyflavone (1), 4′,5,7-trimethoxyflavone (2), 5,7-dimethoxyflavanone (3), *N*-isobutyl-15-(3′,4′-methylenedioxyphenyl)-2*E*,4*E*,12*E*-pentadecatrienamide (4) and *N*-isobutyl-(2*E*,4*E*,14*Z*)-eicosatrienamide (5). Antimicrobial assay showed that compound 1 was active against *B. subtilis* and *E. coli* while compound 2 was effective against *B. subtilis*.

Conclusion: Phytochemical studies on the barks of *P. caninum* has yielded three flavonoids and two amides. Significant antimicrobial activities were shown by compound 1 against *B. subtilis* and *E. coli* whilst compound 2 was effective against *B. subtilis*.



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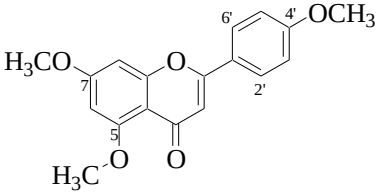

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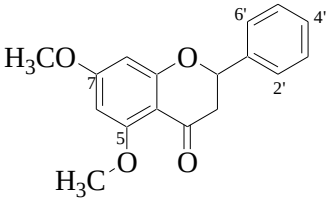

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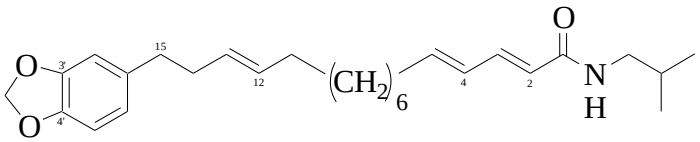

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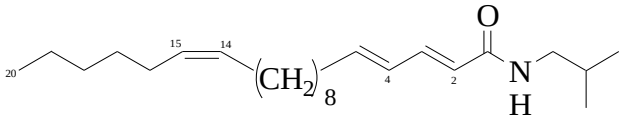

5

PHYSICOCHEMICAL CHARACTERISATION AND DISSOLUTION KINETICS OF ACECLOFENAC COPRECIPITATES IN PVP AND PVP/VA-64

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[2]Department of Life Sciences,
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57000 Kuala Lumpur, Malaysia

Introduction and Objectives: Aceclofenac (AF) is a poorly water-soluble, new generation non-steroidal antiinflammatory drug. The aim of the present work was to investigate the comparative effect of polyvinyl pyrrolidone (PVP) and polyvinyl pyrrolidone vinyl acetate (PVP/VA-64) on in vitro dissolution characteristics of aceclofenac.

Materials and Methods: AF-PVP and PVP/VA-64 coprecipitates were prepared in different ratios with slow evaporation of ethanolic (95% v/v) solutions of drug and carrier in a vacuum. The prepared formulations were characterised for % yield, drug content, average particle size, hygroscopic studies, wettability studies, scanning electron microscopy, IR spectral and DSC studies. Dissolution studies were performed in 0.1N HCl (pH 1.2) and phosphate buffer (pH 7.4). The release data were fitted to zero order, first order, matrix (Higuchi model), and Hixson-Crowell equations to ascertain the kinetic modeling of drug release.

Results: All the prepared coprecipitates exhibited appropriate yield, average particle size, drug content, wetting time and moisture content. Scanning electron microscopy indicated the amorphous nature of the drug in the prepared formulation. The carriers did not show any incompatibility when tested using FTIR and DSC. A higher release in both 0.1N HCl and phosphate buffer was observed as compared to pure drug and their corresponding physical mixtures. With perspective of the dissolution media, phosphate buffer showed higher dissolution as compared to 0.1N HCl. The highest improvement in dissolution was found with PVP as carrier. The in vitro release from all the formulations was best described by first order kinetics (R^2 = 0.9246 and 0.9444 in 0.1N HCl and phosphate buffer, respectively) followed by Higuchi release model (R^2 = 0.9704 and 0.9618 in 0.1N HCl and phosphate buffer, respectively) with better intestinal absorption, analgesic and antiinflammatory activity ($p < 0.05$). The intestinal absorption followed the first order kinetics (R^2 = 0.9852).

Conclusion: With enhanced solubility and dissolution, it is expected that aceclofenac in coprecipitates will demonstrate improved bioavailability.

FORMULATION AND EVALUATION OF CURCUMINOIDS AND QUERCETIN MICROCAPSULES

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Introduction: Curcuminoids and quercetin are two naturally occurring antioxidants that are constituents found in the human diet. However, these two compounds are poorly soluble in water at acidic and physiological pH, and are unstable towards oxidation, light, alkalinity, enzymes and heat.

Objectives: The aims of this study were to increase the solubility and stability of curcuminoids and quercetin using the phase separation-coacervation microencapsulation method.

Materials and Methods: Curcuminoids was microencapsulated with gelatin at a ratio of 1:1 (w/w) using hardening agent from 0.5% to 2%. The stirring speed and temperature of microencapsulation process were kept constant at 500 rpm and 50^{o}C, while the stirring time was varied from 1 to 3 hr. The optimised condition of microencapsulation of curcuminoids was applied to quercetin microencapsulation. The in vitro release, flow property and the staining power of the microcapsules were examined. In addition, the solubility and stability of the microcapsules were compared with the powders.

Results: The microencapsulation of curcuminoids showed that when the ratio of hardening agent to coacervated layer at 1:1 (v/v) with a stirring time of 2 hr, drug loading and entrapment efficiency were increased significantly. However, further increase in the amount of hardening agent and stirring time did not show any significant difference in the results. The encapsulated curcuminiods and quercetin exhibited better flow property, reduced the colour-staining and slower release rate than their powder forms. At the end of 8 hr, 100% of curcuminoids and quercetin powders were released, while only 70%–80% of curcuminoids and quercetin were released from the microcapsules. The encapsulated curcuminiods and quercetin were more soluble and stable than powders in 0.01M phosphate buffer (pH 7.4) containing 0.5% Tween 20.

Conclusion: The present study showed that microencapsulation played an important role in enhancing the solubility and stability of curcuminoids and quercetin.

DISSOLUTION ENHANCEMENT OF PIROXICAM BY SOLID DISPERSION AND INCLUSION COMPLEX TECHNIQUES

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Introduction: Piroxicam is a potent non-steroidal antiinflammatory drug with problems of variable oral bioavailability owing to poor water solubility. Thus, improvement of piroxicam dissolution from oral dosage form is critical for enhancing the oral bioavailability.

Objectives: The present study was to enhance the dissolution of piroxicam using solid dispersion and inclusion complex techniques.

Materials and Methods: Solid dispersions were prepared in different ratios with polyethylene glycol (PEG1500, PEG4000 and PEG6000) using hot melt method, and polyvinylpyrolidone (PVP-K29/31 and PVP-K90) using solvent evaporation method. Inclusion complex was also prepared in different ratios with hydroxypropyl β-cyclodextrin using grinding method. The dissolution profile of piroxicam formulations was compared with that of the pure drug.

Results: There was a statistically significant enhancement ($p < 0.05$) in the dissolution of the piroxicam formulations compared to the pure drug. The highest enhancement in the dissolution was found in the inclusion complex of piroxicam with hydroxypropyl β-cyclodextrin at a ratio of 1:5. Thus, further study was carried out to investigate the physicochemical characteristics of this inclusion complex formulation using differential scanning calorimetry (DSC), X-ray diffraction (XRD) and fourier transform infrared spectroscopy (FTIR). The data obtained from DSC and XRD results suggested that piroxicam was converted from crystalline to the amorphous form when it was complexed with hydroxypropyl β-cyclodextrin. The FTIR spectra revealed no chemical interaction between the drug and hydroxylpropyl β-cyclodextrin.
Conclusion: Complexation with hydroxypropyl β-cyclodextrin greatly enhanced the dissolution of piroxicam.

SIMULTANEOUS DETERMINATION OF SECONDARY METABOLITES FROM *C. ROSEUS* PLANT EXTRACTS BY REVERSE PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Introduction: *Catharanthus roseus* (Apocynaceae) is one of the most important and high value medicinal plants known for its anticancer alkaloids. Some of the isolated secondary metabolites are used in chemotherapy to treat various cancers. Even though several high performance liquid chromatography (HPLC) methods have been developed to quantify the active alkaloids in the plant, however the developed method can be used to quantify *C. roseus* plant extracts in totality.

Objectives: To develop and validate the reverse phase–HPLC (RP-HPLC) method for simultaneous determination of secondary metabolites namely alkaloids from *C. roseus* plant extracts.

Materials and Methods: The quantitative determination was conducted by RP-HPLC equipped with ultraviolet detector. Optimal separation was achieved by isocratic elution with mobile phase consisting of methanol:acetonitrile:ammonium acetate buffer (25 mM) with 0.1% triethylamine (15:45:40 v/v) on an Zorbax Eclipse plus C_{18} (250 mm x 4.6 mm; 5 µm) column. The standard markers (vindoline, vincristine, catharanthine and vinblastine) were identified by co-injection with reference standard along with retention time and quantified by external standard method at 297 nm.

Results: The precision of the method was confirmed by the relative standard deviation (R. S. D.), which was ≤ 2.68%. The recovery was in the range of 98.09% to 108.0%. The limits of detection (LOD) for 4 marker alkaloids were ≤ 0.20 µg mL^{-1}. Different parts of the plant extracts showed different content of alkaloids. Flower methanol was extract high in vinblastine content (1.5 mg/g), whilst the leaves methanol extract contains all four alkaloids in a good amount (vindoline is 5.1 mg/g, vincristine is 0.13 mg/g, catharanthine is 1.5 mg/g and vinblastine is 0.18 mg/g). Water extracts contains very low amount of marker alkaloids.

Conclusion: The HPLC method established is appropriate for the standardisation and quality assurance of *C. roseus* plant extracts.

ISOLATION OF TWO OLIGOSTILBENE FROM *SHOREA BRACTEOLATE*

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Introduction: The *Shorea* species is the largest subfamily of the Dipterocarpaceae family and is the source of resveratrol oligomers (oligostilbenoids), sesquiterpenes and triterpenes. *Shorea bracteolate* also called white meranti with the local name *meranti pa'ang* is widely distributed in Sumatera, Peninsular Malaysia, Indonesia and Singapore. The tree grows up to 50 m in height and the timber is of light hardwood.

Objectives: The study was undertaken to extract and isolate the chemical constituents from the stem bark of *Shorea* species namely *S. bracteolate* and to elucidate the structures of the chemical constituents isolated using modern spectroscopic methods.

Materials and Methods: The stem bark of *S. bracteolate* was dried and cut into small pieces and ground to about 1 mm mesh size. The sample was extracted with acetone and the extract was evaporated in vacuo at 40°C to yield a crude extract. The tannin free crude extract was fractionated using vacuum liquid chromatography (VLC) to give six fractions. Compound 1 (**ε**-viniferin) was obtained from fraction 2 [Hex:EtOAc (5:5)] and further purified using radial chromatography [$CHCl_3$:MeOH (9.5:0.5)]. Compound 2 (hopeaphenol) was obtained from fraction 5 [EtOAc:MeOH (9:1)] and further purified using radial chromatography [$CHCl_3$:acetone:MeOH (7:2:1)] .

Results: Compound 1 (**ε**-viniferin), a dark brown powder and compound 2 (hopeaphenol), a yellow dark powder was obtained from the stem bark of *S. bracteolate.* Structures of the isolated compounds were determined based on the analysis of the spectroscopic data, including NMR, UV, IR and compared with previous reported studies.

Conclusion: Two oligostilbene, **ε**-viniferin and hopeaphenol were isolated from the acetone extract of the stem bark of *S. bracteolate.*

PREPARATION OF LIPOSOME OF DICLOFENAC SODIUM WITH PALM OIL DERIVATIVES

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Introduction: Palm oil, a lipid extracted from the mesocarp of the fruits of the oil palm tree, *Elaeis guineensis*, consists of saturated and unsaturated fatty acids in a ratio near to unity and minor constituents such as vitamin E (tocopherol and tocotrienol), carotenoids, sterols and hydrocarbon. Furthermore, vitamin E in palm oil has been found to possess antioxidant properties. Besides that, some constituents of palm oil also offer some health advantages such as lowering of cholesterol levels, tumour suppressive activities, and also antiproliferating effect of cancer cells. Liposomes are spherical vesicles which are usually made of phosphatidylcholine and cholesterol to control the release rate of the drugs.

Objectives: In this study, the possibility of using palm oil derivatives to prepare liposomes of diclofenac sodium was studied. By encapsulating diclofenac sodium, liposomes can provide prolonged release and reduce myotoxicity when compared to free drug solution.

Materials and Methods: Different concentrations (% w/w) of palm oil lipid fractions and phosphatidylcholine with the same amount of cholesterol were formulated and prepared by reverse-phase evaporation method. Scanning electron microscope (SEM) was used to examine the formation and morphology of liposomes while the in vitro release of diclofenac sodium and entrapment efficiency were determined using ultra-centrifugation. Particle size and zeta potential of liposomes were determined by Zetasizer.

Results: Results showed that liposomes were formed. The particle sizes of liposomes were between 290 nm and 500 nm while the zeta potential of liposomes ranged from approximately –29.0mV to –50.0mV. Different entrapment efficiency and release pattern of diclofenac sodium were seen among formulations.

Conclusion: In conclusion, palm oil was found to be possible to be used in liposome preparation, with Formulation IV which contained 33.33% w/w of palm oil lipids and 33.33% w/w of phosphatidylcholine showing high entrapment efficiency, desired prolong release of diclofenac sodium and exhibiting similar release pattern with the standard formulation.

COMBINATION OF CHROMATOGRAPHY TECHNIQUES FOR THE ISOLATION OF MINOR BIOACTIVE METABOLITES FROM A MALAYSIAN ENDOPHYTIC FUNGI

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Introduction: Nature has been a source of medicines for treating various types of diseases in humans and animals. Endophytic *Aspergillus* HAB10R12 was isolated from *Garcinia scortechinii* (*kenondong krot @ kandis*), collected in a secondary forest in Kuala Pilah. It was shown to contain notably five cytotoxic peptides, for which a patent was applied. In order to further develop these compounds, larger quantities were separated. The five peptides, A-1 to A-5 showed excellent activity against human breast cancer and colonic carcinoma. Two of the peptides, A-3 and A-5 were 4000 times more active when compared with commercial anticancer drugs (fluorouracil, cisplatin and tamoxifen). Unfortunately, these peptides are produced in a very small quantity together with a number of non active compounds.

Objectives: The present study further developed the cytotoxic peptides in larger quantities for structural characterisation and anticancer studies.

Materials and Methods: For this purpose 100 20 cm Petri dishes were inoculated and fermented according to a standardised procedure, by batches of 10 plates each. Each batch was extracted based on our standard operation procedure. Each extract was then subjected to LC/MS/MS technology Agilent 1200 series system equipped with Agilent 6410 Triple Quad. The purification of those peptides was carried out using two successive orthogonal separation steps. The 1st one was a gel filtration process using preparative HPLC from the Japan analytical industry using Polystyren gel column (Polymeric GPC column i.d. 20 × 600 mm) medium pressure, using chloroform and tetrahydrofuran as a solvent system with 2.5 mL/min flow rate. The presence of the expected compounds in one of the fractions was confirmed by LC/MS/MS. The peptides were then fractionated onto an analytical column in HPLC Agilent 1200 series system equipped with a diode-array detector (DAD) and evaporative light scattering detector (ELSD). All analyses and separations were carried out in a reverse phase mode, using a Synergy 4u Hydro-RP 80Å column (150 × 4.6 mm, 4 µm particle size, Phenomenex®, USA with flow rate of 1 mL/min) with a guard column filled with the same material. The column temperature was maintained at 36°C. The monitoring of the separation is carried out by both a DAD and an ELSD (as these peptides are not much UV-active). A fraction collector connected to the LC system allowed the collection of the pure targeted peptides.

Results: Their identity was confirmed by LC/MS/MS and capillary NMR. The additional peptides were also collected and their structure elucidation is in process.

ANALYSIS OF VINCRISTINE AND VINBLASTINE IN THE EXTRACT OF *CATHARANTHUS ROSEUS* BY HPLC

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Introduction: HPLC analytical techniques are often employed to identify compounds for qualitative and quantitative analysis. This analytical approach is often used to analyse secondary metabolites present in *vinca* alkaloids.

Objectives: In this study, the amount of *vinca* alkaloids vinblastine (VBL) and vincristine (VCR) were determined by using a HPLC instrument equipped with a UV detector. The contents of both compounds in the prepared extract, were characterised in order to assess the significance of these alkaloids towards the observed anticancer activity of the plant species.

Materials and Methods: Methanol, acetonitrile and phosphate buffer solution (pH 7) were mixed to produce a mobile phase solution with their respective ratios of 45:45:10. A 10 mL solution was eluted through the column at a flow rate of 1 mL/min at 35°C. VCR and VBL were detected at wavelengths of 297 nm and 262 nm respectively. The method was validated according to USFDA and ICH guidelines.

Results: The method was found to be efficient and reproducible producing good linearity with r value ranging from 0.9999 to 1. Presence of VCR and VBL in the extract was validated by comparing their peaks with VCR and VBL standards. The first eluted alkaloid was VCR with a retention time (R_t) of 3.2. minutes followed by vindoline (R_t 3.56 minutes), VBL (R_t 3.89) and catharanthine (R_t 4.14). The result of the HPLC analysis demonstrated that the ethanol extract of *Catharanthus roseus* has a higher content of VBL and VCR compared to the water extract. The minimum detectable limit was 1.25 ug.

Conclusion: The ethanol extract of *C. roseus* may have a more potent anticancer activity than the water extract given the higher amount of VBL and VCR present in the ethanol extract.

PRE-CLINICAL / CLINICAL TRIALS

ANTIANGIOGENIC ACTIVITY OF *GYNURA SEGETUM* LEAF EXTRACTS AND ITS FRACTIONS

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Introduction: Gynura segetum is a popular medicinal plant in Indonesia and Malaysia, known to possess various medicinal properties especially for treatment of cancer, diabetes and hypertension.

Objectives: This study was carried out to evaluate the antiangiogenic effect of *G. segetum* leaf extracts and its fractions. The chemical compositions of the active extracts were also determined.

Materials and Methods: The antiangiogenic activity of *G. segetum* leaf extracts and its fractions was evaluated in vivo using the chick embryo chorioallantoic membrane (CAM) assay. Gas chromatography mass spectrometry (GCMS) analysis was carried out to identify the chemical compositions of the active extracts.

Results: The CAM treated with *G. segetum* leaf extracts and its fractions showed a significantly greater antiangiogenic effect when compared to the positive control suramin. Chemical analysis of the active extracts from the leaves of *G. segetum* yielded 9 known compounds: undecane (1), neophytadine (2), hexadecanoic acid, methyl ester (3), 9,12-octadecadienoic acid, methyl ester (4), 9,12,15-octadecatrienoic acid, methyl ester (5), phytol (6), tetradecanal (7), octadecanoic acid, methyl ester (8) and γ-sitosterol (9).

Conclusion: These findings suggested that the fatty acids and sterol compounds in the leaves of *G. segetum* might be responsible for the antiangiogenic activity and may be a potential supplemental source for cancer treatment.

HERB-DRUG INTERACTION STUDIES OF *EURYCOMA LONGIFOLIA* EXTRACT TAF-273 ON THE METABOLISM OF ROSIGLITAZONE, AN ANTIDIABETIC DRUG

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Introduction: Presently, many natural products recommended for treatment of erectile-dysfunction are easily available in the market and one of them is *Eurycoma longifolia*. The belief that natural products are safer than synthetic drugs has led to the dramatic growth of herbal medicine usage. However, like synthetic drugs, these preparations may also cause adverse effects and drug interactions, therefore may affect the therapeutic effect of allopathic medicine.

Objectives: The present study evaluated a standardised extract, TAF-273 of *Eurycoma longifolia* on the metabolism of an antidiabetic drug, rosiglitazone.

Materials and Methods: Male Sprague-Dawley (SD) rats were divided into two main groups: normal and diabetic-induced. Each group was further divided into three sub-groups; old, adult and young. By using the collagenase perfusion technique, isolated hepatocytes were prepared from these rats, a total of six rats (n = 6) per group and rosiglitazone N-demethylase assay was then determined by measuring the quantity of formaldehyde formed by using a microplate reader at 415 nm.

Results: A significant increase in formaldehyde concentration was observed in the old and adult normal rats and the old diabetic rats ($p < 0.05$) but there was no significant difference in adult and young diabetic male rats ($p > 0.05$) when compared to the control group, respectively. In the young, normal male group, a significant increase was only found at the highest concentration of TAF-273 tested (100 µg/mL). In general, TAF-273 displayed a higher increase in formaldehyde concentration in the old group for both normal and diabetic male rats.

Conclusion: A significant increase in formaldehyde concentration was observed on TAF-273 treatment in old normal and diabetic male rats ($p < 0.05$), suggesting that TAF-273 increased the phase 1 rosiglitazone metabolism in male rat hepatocytes.

NANOSTRUCTURED LIPID CARRIERS (NLC) WITH WHOLESOME PALM-BASED PHYTONUTRIENTS CONCENTRATES

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Introduction: Wholesome palm-based phytonutrients concentrates are naturally derived compounds from palm oil. The concentrates consist of carotenoids and tocols as well as other minor components which include sterols, squalene, ubiquinones, coenzyme Q_{10} and phospholipids. The production of wholesome palm-based phytonutrients is much more economical compared to individual phytonutrients.

Methods and Results: In vitro dermal irritation assay was used as a screening safety test for a new cosmetic ingredient which is important to protect consumer's safety. Wholesome palm-based phytonutrients did not exhibit any irritation and sensitisation. In vitro UV-blocking activity of wholesome palm-based phytonutrients was shown to absorb light at both UVA and UVB regions whereas Tocomin® 50% (commercial tocols) and α-tocopheryl acetate only absorbed UVB light. This shows that wholesome palm-based phytonutrients have broad spectrum UV-protection towards UVA as well as UVB. The antioxidant activity of wholesome palm-based phytonutrients, α-tocopheryl acetate and Tocomin® 50% were evaluated by ABTS assay. The inhibition of $ABTS^+$ radicals for wholesome palm-based phytonutrients was 53.3% which was comparable with Tocomin® 50% which was 69.43%. Inhibition of $ABTS^+$ radicals for wholesome palm-based phytonutrients was much higher compared with α-tocopheryl acetate which was only 8.88%. Transmission electron microscopy proved that the wholesome palm-based phytonutrients were encapsulated in oil droplets of NLC. The particle size analysis showed that the size of NLC with wholesome palm-based phytonutrients was constant throughout the 9 weeks whereas the particle size of NLC without phytonutrients increases from week 1 to week 9, which was 268.55 nm to 444 nm.

Conclusion: NLC with wholesome palm-based phytonutrients have better stability than NLC without phytonutrients possibly due to the presence of monoacylglycerol and diacylglycerol which also function as emulsifiers.

THE HYPERLIPIDAEMIA RISK FACTORS AMONG HIV-INFECTED PATIENT TREATED WITH HAART IN PENANG HOSPITAL

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Introduction: HAART has markedly improved the prognosis of people with HIV infection but frequently caused metabolic syndromes such as hyperlipidaemia. A previous study had identified factors that are associated to hyperlipidaemia such as male gender, older age, AIDS diagnosis upon starting HAART and PI based HAART while HIV/HCV co-infection has found to cause less risk of developing hyperlipidaemia.

Objectives: This study was undertaken to evaluate the hyperlipidaemia prevalence and its associated risk factors.

Methods: As of May 2009 to April 2010, all patients on HAART treatments who have not obtained fasting plasma lipid test were required to perform one fasting plasma lipid test.

Results: A total of 382 patients with a majority of Chinese male (59%) acquired the infection through heterosexual contact and stable on HAART for a mean period of 2.2 ± 2.2 years (range 0.2–12) were enrolled into this study. Hyperlipidaemia occurred in 24% of the patients with triglycerides and HDL-cholesterol significantly high in PI-based (75%, $p < 0.01$) and NNRTI-based HAART (68%, $p < 0.01$) respectively. Both gender showed an increased HDL-cholesterol but males predominantly had higher triglycerides (48%, $p < 0.01$) compared to females. HIV/HCV co-infected patients showed lower rates of hypertriglyceridaemia (10%, $p < 0.01$) with no significant findings with the other lipid profiles. In contrast, older age (>40 years) and AIDS diagnosis at HAART initiation were less likely factors to induce hyperlipidaemia.

Conclusion: Main contributing factors causing hyperlipidaemia are undetermined, thus an appropriate HAART based selection and precise education from multidisciplinary healthcare approach are requisites in order to minimise the occurrences of hyperlipidaemia among the HIV/HCV-infected patients treated with HAART.

IN VIVO HEPATOPROTECTIVE ACTIVITY OF ETHANOL EXTRACTS OF LEAVES AND FRUIT OF *PIPER SARMENTOSUM*

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Introduction: Piper sarmentosum Roxb. (Piperaceae) is well-known in tropical countries due to its culinary and medicinal properties. In our previous work, ethanol extracts of leaves and fruit of the plant have exhibited in vitro and in vivo antioxidant activity. The use of antioxidants is not only beneficial in pharmacotherapy of liver disorders but also plays a pivotal role to minimise drug-induced oxidative stress on hepatocytes during long-term therapy.

Objectives: The present study aimed to investigate hepatoprotective activity of ethanol extracts of both the parts of the plant in rats against CCl_4-induced hepatotoxicity.

Materials and Methods: Seven groups of Sprague Dawley rats, each comprising six animals were treated as: group I (CCl_4), group II (control), group III and IV (fruit extract 500 and 250 mg/kg, respectively), group V and VI (leaf extract 500 and 250 mg/kg, respectively) and group VII (vitamin E, 100 mg/kg). The extracts and vitamin E were administered orally for 14 days whilst equivalent amount of sample vehicle was administered to CCl_4 and control groups. Four hours after the last dose, a single dose of CCl_4 (1.5 mg/kg, 1:1 olive oil) was administered orally to animals of all the groups except control. After 24 hours, the blood was collected for biochemical analysis of liver function markers, and the animals were sacrificed to get the liver for histology exam.

Results: Comparison of liver function markers and histology of pretreated and CCl_4 groups indicated that both the extracts at the two dose levels had protected the liver from CCl_4–induced toxicity ($p < 0.05$).

Conclusion: The extracts of the plant have hepatoprotective effect, therefore, using the plant as a vegetable or in the form of extracts may be valuable to protect the liver from oxidative stress in hepatitis and diseases requiring long-term pharmacotherapy.

PHASE II STUDY WITH MOUNTHRINSE CONTAINING PROPOLIS FOR THREE MONTHS: COMPLIANCE, APPRECIATION AND ACCEPTABILITY OF THE PRODUCT

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Introduction: Dental plaque is considered as a key etiological factor associated with arising gingivitis. Its removal can be difficult in patients with lack of coordination or any other problems. Therefore, the use of mouthrinse in adjunct to toothbrushing for control of plaque and gingivitis might increase the benefits of controlling dental plaque.

Methods: A phase II study was used to evaluate the compliance, appreciation and acceptability of an alcohol-free mouthwash containing 5% green propolis in control of plaque and gingivitis for 3 months. Each subject, at the end of the study, answered a questionnaire about appreciation and acceptance of the mouthwash.

Results: Twenty one subjects completed the study, although most of them felt the taste of 5% MGP unpleasant. They were satisfied with the product, pointing positive changes in oral health after the treatment period. Moreover, the compliance was satisfactory (≥80%) with no statistically significant difference between the periods of rinsing in the morning and at night.

Conclusion: The product was approved by the patients and was effective in controlling plaque and gingivitis. However it is necessary to carry out a phase III study to confirm its effectiveness compared with an known mouthwash available in the market.

IN VITRO ANTIMICROBIAL ACTIVITY OF MEDICINAL PLANT EXTRACTS USED IN BRAZILIAN FOLK MEDICINE AGAINST PATHOGENIC MICROORGANISMS OF INTEREST TO DENTISTRY

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Objectives: To verify the antimicrobial activity of Brazilian medicinal plants extracts used in Brazilian folk medicine against oral pathogenic microorganisms.

Methods: Candida albicans (ATCC 18804), *Streptococcus mutans* (ATCC 70069), *Staphylococcus aureus* (ATCC 12692) and *Aggregatibacter actinomycetemcomitans* (ATCC 33384) were tested. Brazilian medicinal plant extracts of *Schinus terebinthifolius* (*aroeira*), *Croton campestris* (*velame*), *Lafoensia pacari* (*pacari*), *Centaurium erythraea* (*centáurea*), *Stryphnodendron adstringens* (*barbatimão*), and *Anacardium humile* (*cajuzinho-do-cerr*ado) were compared to standardised antimicrobial agents (*nystatin, chloramphenicol*). Ethanol, hexane and butane fractions from stem barks, rinds, leaves, and/or roots were extracted and tested. Antimicrobial diffusion agar test and MIC were performed according to CLSI. After 24 h of incubation at 37ºC, the diameter of inhibition zones and spectrophotometer reading were measured and compared. The results were reported as means ± standard deviation (M ± SD).

Results: With the exception of five extracts that showed no antimicrobial activity, all the extracts tested showed antimicrobial activity in different levels.

Conclusion: This study suggests that extracts from the plants tested, when associated with gel, mouthwash and toothpastes, could be an alternative therapeutic option for infectious conditions of the oral cavity, such as denture stomatitis, dental caries and periodontitis.

EFFECT OF *EURYCOMA LONGIFOLIA* JACK ON NUMBER OF PUPS IN FEMALE RATS

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Introduction: Eurycoma longifolia Jack, known locally as *Tongkat Ali* is popularly used as a herbal medicine for enhancing libido of man and male fertility through improvement on the sexual quality and sperm count.

Objectives: The present study investigated the effect of *E. longifolia* (Simaroubaceae) on the number of pups in female rats.

Materials and Methods: In one experiment, a group (n = 6) of normal adult female rats of 8–10 weeks old with regular oestrus cycle, was orally fed for 3 weeks with 50 mg/kg/day of TAF 273, a standardised extract of *E. longifolia* whilst, a control female rat group (n = 6) was orally fed with vehicle (distilled water). At the end of treatment, mating was performed for treated female rats with fertile male rats. In another experiment, postnatal handled female adult rats of 9–11 weeks old were separated from their dam for 1 min every day for 10 days postnatal. Animals were then divided into 2 groups; one group (n = 6) was given vehicle (distilled water) whereas, the other group (n = 6) was given TAF 273 at 50 mg/kg/day for another 2 weeks. Mating was then performed as previously described.

Results: For the normal animals treated with TAF 273 the pups number (9 ± 2.7) was not significantly different when compared to those of the control group (10 ± 2.8). In contrast the handled animals treated with vehicle, produced less pups (6.83 ± 0.98) but for the handled animals treated with TAF 273, the number of pups was significantly increased ($p < 0.05$; 9 ± 2.86) when compared to those treated with the vehicle.

Conclusion: E. longifolia may potentially improve the fertility of induced infertile female rats resulted from postnatal handling.

ANTIDIABETIC EFFICACY OF METHANOLIC EXTRACT OF THE LEAVES OF *TETRACERA* INDICA MERR. (DILLENIACEAE) IN ALLOXAN INDUCED DIABETIC RATS

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Introduction: Tetracera indica Merr., (*mempelas paya*) is used in Malaysia and other parts of South-Asia as a folk medicine to treat fever, flu, skin rashes, itching, piles, mouth ulcer, diabetes, and insects bites.

Objective: The study has been carried out to screen the antidiabetic efficacy of methanolic extract (MeOH) of the leaves of *T. indica* in vivo in alloxan induced diabetic and normal rats.

Materials and Methods: The study was conducted in an animal model in vivo and comparison was made with standard antidiabetic drug, glibenclamide.

Result: In folk remedies, different parts of this plant have been found to act effectively in the treatment of fever, flu, skin rashes, itching, piles, mouth ulcer, diabetes, and insects bites. Glucose levels in rats with hyperglycemia induced by alloxan (160 mg/kg b.w.) were determined after the oral administration of the methanolic extract of the leaves of *T. indica*. Two doses of the methanolic extract (250 and 500 mg/kg b.w.) were evaluated in normal, and alloxan induced diabetic rats. The methanolic extract exhibited significant antihyperglycemic activity in alloxan induced diabetic rats, however in normal rats no hypoglycemic activity was observed, when compared with the both +ve and –ve controlled groups. The antidiabetic activity was also found to be comparable to that of the effect produced by a standard antidiabetic drug, glibenclamide (250 mg/kg b.w.). LD_{50} of the methanolic extract was found to be more than 5000 mg/kg body weight and no significant lethal toxicity was observed within this range. Phytohemical screening has scrupulously revealed the abundant presence of terpenoids and flavonoids in the MeOH extract which might be responsible for its significant antihyperglycemic effect.

Conclusion: It is concluded that the leaves of *T. Indica* possess potential antidiabetic agents which significantly lowered the blood glucose level in the diabetic rats. Hence, *T. Indica* could be screened further with respect to finding out its potential towards the management of diabetes in Malaysia.

ANTIINFLAMMATORY AND ANTINOCICEPTIVE EFFECTS OF *MITRAGYNA SPECIOSA* AQUEOUS EXTRACT

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Introduction: *Mitragyna speciosa* has been used traditionally in Southeast Asia to reduce pain, as cough depressant and to treat hypertension. Many studies on the effects of this plant as an antiinflammatory and antinociceptive agent have been carried out originating from Thailand. However, few studies were reported on the *M. speciosa* extracts from Malaysia.

Objectives: The study was carried out to determine antiinflammatory and antinociceptive effects of *M. speciosa* aqueous extract in vivo and in vitro.

Materials and Methods: The antiinflammatory effect was evaluated using the carrageenan-induced paw edema in rats and the antinociceptive activity was determined using the writhing test in mice. Different concentrations of *M. speciosa* aqueous extract were administered to the animal orally. Acetylsalicylic acid (100 mg/kg, p.o) was used as a positive control for both tests. Xanthine oxidase inhibitory assay was carried out to determine the effect of this extract to reduce gout inflammation in vitro.

Results: Oral administration of *M. speciosa* aqueous extract (200 and 400 mg/kg) to the carrageenan-induced paw edema rats resulted in a significant reduction in paw volume compared to the control group. Plus, *M. speciosa* aqueous extract (400 mg/kg, p.o.) significantly lowered acetic acid-induced writhes in mice compared to the control group. However, *M. speciosa* aqueous extract was not as potent as allopurinol in inhibiting formation of uric acid in vitro.

Conclusion: The results suggested the presence of potent antiinflammatory and antinociceptive activities in the *M. speciosa* aqueous extract but, the antiinflammatory effects may not be due to its ability to inhibit xanthine oxidase.

IN VITRO SYNERGY STUDY OF *ANGIOPTERIS EVECTA* METHANOLIC EXTRACT WITH ANTITUBERCULOSIS DRUGS AGAINST *MYCOBACTERIUM TUBERCULOSIS*

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Introduction: Angiopteris evecta, locally known as *paku gajah* is commonly used by traditional healers in Malaysia to treat cough. In our preliminary study, methanol extract of *A. evecta* was observed to have antituberculosis activity against *Mycobacterium tuberculosis* H37Rv with a minimum inhibitory concentration (MIC) value of 400 µg/mL.

Objectives: To investigate the interaction of the methanolic extract of *A. evecta* with four first-line antituberculosis drugs: isoniazid, rifampicin, streptomycin, and ethambutol, against *M. tuberculosis* H37Rv.

Methods: A. evecta sample was collected from the Tropical Spice Garden, Pulau Pinang, identified, and deposited at the School of Biological Sciences Herbarium, Universiti Sains Malaysia. The leaves of the plant were extracted by maceration in 80% methanol. In vitro antituberculosis study was carried out using tetrazolium microplate assay. The individual MIC of each drug was determined prior to the interaction study which was carried out using the classical checkerboard method. The dilutions of *A. evecta* extract and each drug were prepared twice below to three times higher than their respective MICs. The fractional inhibitory concentration values were calculated to characterise the interactions between the drugs and extract.

Results: Combination of methanolic extract of *A. evecta* with rifampicin showed a synergism effect against *M. tuberculosis*. No interactions were observed for the combinations of the plant extract with streptomycin and isoniazid. However, *A. evecta* combined with ethambutol showed antagonism interaction against the bacteria.

Conclusion: The results indicate that *A. evecta* is a potential plant candidate to be further investigated for the development of new plant-derived antituberculosis agents.

EVALUATION OF CHOLINESTERASE INHIBITORY ACTIVITY OF *PHYLLANTHUS NIRURI*

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Introduction: Phyllanthus niruri is a widely used medicinal plant in the tropical region. It consists of a wide range of bioactive chemical constituents which may be useful for the prevention and treatment of various diseases. Our initial screening of a few Malaysian medicinal plants showed that the methanol extract of *P. niruri* exerted an anticholinesterase property and may be important as a potential medication for Alzheimer's disease.

Objectives: The present study was undertaken to evaluate the potential cholinesterase inhibitory activity of *P. niruri* and to identify its bioactive fractions using cholinesterase inhibitory-guided fractionation.

Materials and Methods: Acetylcholinesterase and butyrylcholinesterase inhibitory activities were investigated using a 96-well plate assay following Ellman's method and thin layer chromatography assay with Fast Blue B salt as a spraying reagent.

Results: P. niruri methanolic extract was found to have cholinesterase inhibitory activity with IC_{50} values of 134.09 µg/mL and 43.81 µg/mL for acetylcholinesterase and butyrylcholinesterase, respectively. Among its 5 fractions, fraction 2 showed the highest activity for acetylcholinesterase inhibition (IC_{50} of 18.44 µg/mL) followed by fraction 3 with an IC_{50} of 22.04 µg/mL. In contrast, for butyrylcholinesterase inhibition, fraction 3 showed the highest activity followed by fraction 2, with IC_{50} values of 6.37 µg/mL and 12.40 µg/mL respectively. Thin layer chromatography assay has shown a few inhibitory spots for fractions 2 and 3.

Conclusion: Methanolic extract of *P. niruri* was found to have potential anticholinesterase activity. Upon fractionation, the activity was found to be concentrated in fractions 2 and 3. These fractions are worth further investigation for new acetylcholinesterase and butyrylcholinesterase inhibitors.

ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACTS OF VARIOUS PARTS OF *MESUA FERREA*

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Introduction: Mesua ferrea, locally known as *penaga lilin*, is an evergreen iron wood tropical tree, belonging to the Guttiferae family. It has been used traditionally in Indian folk medicine for treating snake-bites and scorpion-stings, bleeding piles, leprosy and skin disorders.

Objectives: To investigate antimicrobial activities of the methanolic extracts of various parts of *M. ferrea* against a series of pathogenic microorganisms.

Materials and Methods: Antimicrobial properties of methanolic extracts of various parts of *M. ferrea* were investigated using agar disc diffusion assay and microdilution method.

Results: Leaves, fruit and bark extracts of *M. ferrea* displayed good antimicrobial activity, with zone of inhibition in the range of 4–10 mm. However, the antibacterial effects of these crude extracts were slightly less potent as compared to standard antibiotics. The MIC values for all three methanolic extracts of *M. ferrea* ranged from 0.012 mg/mL to 12.5 mg/mL. Of interest, the leaves and fruit extracts had MIC values against *Staphylococcus aureus* that were only 3 and 1.5 times less potent than the standard reference, vancomycin. Likewise, the leaves and fruit extracts displayed good antimicrobial efficacies of 2 times lesser than the standard antibiotic, gentamycin. The leaves and fruit extracts also showed good antimicrobial activities of 15 and 2 times lesser than the standard antifungal drug, micanazole. MBC values for active extracts of *M. ferrea* ranged from 0.012 mg/mL to 0.78 mg/mL.

Conclusion: Different parts of *M. ferrea* showed potential antimicrobial activity against *S. aureus*, which is comparable with clinically used antibiotics.

ANTIMICROBIAL ACTIVITY OF METHANOLIC EXTRACTS OF VARIOUS PARTS OF *IXORA COCCINEA*

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Introduction: Ixora coccinea L. (Rubiaceae) has been used traditionally for treating a variety of ailments and is also cultivated for ornamental purposes. The leaves are used to treat diarrhoea whereas the roots are used to treat hiccup, fever, sores, chronic ulcers, and skin diseases. The flowers have been used in the treatment of catarrhal bronchitis and dysentery.

Objectives: To investigate the antimicrobial activity of methanolic extracts of various parts of *I. coccinea*.

Materials and Methods: Preliminary evaluation on the antimicrobial activity of *I. coccinea* was carried out using agar disc diffusion method; whole minimum inhibitory concentration (MIC), minimal bacteriostatic/bacteriocidal (MBC) and minimum fungicidal concentration (MFC) was determined using the 96 well-plate microdilution method.

Results: All methanolic extracts of *I. coccinea* leaves, flowers and stems showed good inhibitory activity against all tested bacteria and fungi. The inhibition zones of the extracts were within the range of 6–12 mm. However, their antibacterial effects were slightly less potent as compared to standard antibiotics. All extracts had MIC values ranging from 0.78 mg/mL to 12.5 mg/mL. The stem extract showed MIC values that were 50.0 and 31.2 times lower than vancomycin and gentamycin respectively against *Staphylococcus aureus* and *Shigella dysentery*. Another interesting finding is that the stem extracts had MIC values against *Aspergillus niger* of only 31.2 times lower than the standard reference, micanazole. MBC values for active extracts of *I. coccinea* ranged from 0.78 mg/mL to 12.5 mg/mL.

Conclusion: The present study showed that the extracts of *I. coccinea* have potential antibacterial and antifungal activity.

REPRODUCTIVE TOXICITY AND TERATOLOGICAL STUDIES OF STANDARDISED *EURYCOMA LONGIFOLIA* EXTRACT ON RATS

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Introduction: *Eurycoma longifolia* Jack, a small tree of the Simaroubaceae family is well-documented for its various biological properties. Our previous studies have shown that a standardised extract of *E. longifolia* improved normal fertility and drug-induced infertility of both male and female rats. Hitherto, no studies of *E. longifolia* on the female reproductive system and foetus development have been recorded.

Objectives: The current study investigated the effects of a standardised *E. longifolia* extract on the reproductive system of the female rats and the teratological effect on the foetuses.

Materials and Methods: The reproductive toxicity studies of three doses (10, 25 and 100 mg/kg body weight) of the standardised *E. longifolia* extract on female rats were compared with that of the control. The indices of mating, fecundity and fertility, together with litter size, sex ratio and body weight of pups were recorded. The reproductive parameters of dams orally administered daily throughout the gestation period with the extract and the control including the average number of corpora lutea per dam, average number of implantation per dam, pre-implantation loss, post-implantation loss, total number of live foetuses and foetal weight were recorded. The external anomalies of foetuses such as ectrodactyly, scoliosis, umbilical hernia, kinky tail of the foetuses were also observed for teratological investigation. Approximately one-half of the total foetuses from each group were eviscerated and stained for skeletal examinations. The remaining foetuses were preserved in Bouin's solution for evaluation of visceral abnormalities.

Results: Dams administered with different doses of standardised *E. longifolia* extract (10, 25 and 100 mg/kg) showed no signs of toxicity such as tremor, eye or nose bleeding. Fertility index of the female rats treated with 25 and 100 mg/kg of standardised *E. longifolia* extract increased significantly ($p < 0.05$) compared to that of the control. The litter size of the *E. longifolia* treated groups was elevated significantly ($p < 0.01$) compared to that of the control. None of the foetuses from the treated dams showed morphologically anomalies. In general, the skeletons of all the foetuses from the standardised *E. longifolia* extract treated dams were normal in appearance.

Conclusion: The present study showed that the standardised *E. longifolia* extract significantly increased the litter size and female fertility index. The extract did not show any reproductive toxicity on the treated dams and the foetuses appeared normal from visceral and skeletal examinations.

EFFECT OF HARUAN AQUEOUS EXTRACTS IN WOUND CLOSURE OF NIH/3T3, HFF-1 AND JB6 CL 30-7B CELL LINES

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Introduction: Haruan fish is a very common and well-known type of fish that has been used to accelerate healing process after surgery. The fish content which is rich in amino acids and fatty acids were thought to promote the healing process.

Objectives: The study was undertaken to investigate the wound closure effect of haruan aqueous extract in keratinocyte and fibroblast cells which play the important role in the healing process.

Materials and Methods: The study was conducted using human foreskin fibroblast (HFF-1), mouse fibroblast (NIH/3T3) and mouse keratinocyte (JB6 CL 30-7B) cells. The experiment was performed in monolayer cells which then scratched to produce a wound. The cells were divided into 3 groups according to the treatments. Group 1, 2 and 3 were treated with 5%, 10% and 20% of haruan aqueous extract respectively in serum-free conditions. Each group was also treated with 5%, 10%, and 20% of sterile water respectively as control. Images were captured within 0 hour, 2.5 hours, 5.0 hours and 24.0 hours after wounding and treatment. All the experiments were run in triplicate and data was analysed using one way ANOVA followed by Duncan post hoc test with $p < 0.05$.

Results: Group 1 showed significantly higher percentage of closure after 2.5, 5.0 and 24.0 h of treatment compared to other groups for keratinocyte cells. As for the NIH/3T3 cells after 24 h treatment, a significant difference in the percentage of wound closure for group 3 were observed. The highest concentration of haruan extracts gave the smallest percentage of closure. On the other hand treatments on HFF-1 cells showed no significant difference between the control and treated groups.

Conclusion: Haruan aqueous extract could stimulate wound closure of keratinocyte cells at concentration of 5% and delayed the migration of NIH/3T3 cells at concentration of 20%.

IN VITRO DISSOLUTION AND EX VIVO ABSORPTION STUDIES OF FORMULATED STANDARDISED EXTRACT PNF5 OF *PHYLLANTHUS NIRURI* LINN

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Introduction: *Phyllanthus niruri* Linn (Euphorbiaceae) known as *dukong anak* in Malaysia has been reported to reduce high uric acid content in hyperuricemic-induced rats. The bioactive extract PNf5 of this plant has been identified to contain lignans, possessing antihyperuricemic activity comparable to that of clinically used antihyperuricemic drugs. The extract PNf5 has been standardised to contain a fixed concentration of the pharmacologically active lignans that were poor in aqueous solubility but high in lipid permeability. Thus, the lignans can be classified as Class II drugs of high membrane permeability but low in aqueous solubility under the Biopharmaceutics Classification System (BCS). In general, the lignans were found to have poor oral bioavailability.

Objectives: To develop and characterise an oral dosage form which can improve the bioavailability of the lignans significantly but is of sufficient simplicity to be viable for pharmaceutical production.

Materials and Methods: Gelucire® 44/14 was used as a dissolution and absorption enhancer. Gelucire® 44/14-PNf5 solid dispersion capsules were prepared for in vitro dissolution testing and ex vivo membrane penetration studies using the everted gut sac method. A reversed phase high performance liquid chromatographic (RPHPLC) system was used to analyse the concentrations of the active compounds.

Results: The dissolution test showed that > 70% of the bioactive phyllanthin from the Gelucire® 44/14-PNf5 solid dispersion capsules was released into the dissolution medium within 45 minutes. For the everted gut sac study, there was about 24-fold increase in absorption of phyllanthin from the Gelucire® 44/14-PNf5 solid dispersion formulation when compared to that of PNf5 alone.

Conclusion: This result indicated that the Gelucire®44/14-PNf5 solid dispersion formulation can improve in vitro dissolution and ex vivo absorption of the lignans. In vivo oral bioavailability of the formulation is thus worthy of further investigation.

ANTILEUKEMIC ACTIVITY OF *ALPINIA CONCHIGERA* AND *ALPINIA MUTICA*

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Introduction: Alpinia conchigera which is also known as *lengkuas ranting* and *Alpinia mutica* (syn: *Languas mutica*) are perennial herbs belonging to the family Zingiberaceae. *A. conchigera* has been used to treat rheumatism, arthritis and as a poultice after confinement while *A. mutica* has been cultivated as an ornamental and its rhizomes have been used as a stomachic.

Objectives: This research is undertaken to isolate the chemical constituents from rhizomes of *A. conchigera* and *A. mutica* and to carry out the cytotoxic test towards human leukemic cell lines.

Materials and Methods: A. conchigera was collected from Kelantan, Malaysia while *A. mutica* was collected from FRIM, Kepong. Both plant materials were extracted consecutively with hexane, chloroform, ethyl acetate and methanol using conventional soaking method. Extracts were then fractionated and purified using chromatographic methods while spectroscopic methods (IR, MS, NMR) were used to characterise the isolated compounds. Cytotoxic test was carried out using the MTT assay method.

Results: Isolation work on crude extracts of *A. conchigera* has yielded *p*-hydroxycinnamaldehyde, *trans*-*p*-coumaryl diacetate, *β*-sitosterol-3-*O*-*β*-D-glucopyranoside and 1'-acetoxychavicol acetate while 5,6-dehydrokawain, Flavokawin B, pinostrobin and pinocembrin have been obtained from the isolation work on hexane and chloroform extracts of *A. mutica*. All crude extracts were subjected to cytotoxic test against leukemic cancer cell lines (HL-60 or CEMss). The non-polar and semi-polar extracts from *A. conchigera* were active against HL-60 cell line, with IC_{50} values < 20 µg/mL while the crude extracts of *A. mutica* were very active against CEMss cell line with IC_{50} values < 10 µg/mL.

Conclusion: All crude extracts tested showed promising antileukemic activity.

ANTIOXIDANT AND ANTIDIABETIC ACTIVITIES OF ISOPTEROPODINE AND PTEROPODINE FROM MALAYSIAN *UNCARIA LANOSA* VAR. *FARREA*

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Introduction: Uncaria species are used for the treatment of a variety of conditions including treatment for wounds and ulcers, fevers, headaches, gastrointestinal illness, bacterial/fungal infections and diabetes. The dried hooks of some *Uncaria* species have been integral components in traditional oriental medicines, and generally have been used as spasmolytics, analgesics, and sedatives for symptoms associated with nervous disorders. In Malaysia, *Uncaria* species is also known as *gambir* or *akar kait-kait* or *akar hitam*. We have previously reported on the phytochemical study and biological activities of Malaysian *Uncaria longiflora* var *pteropoda*.

Objectives: The objective of the study is to evaluate the antioxidant and antidiabetic activity of alkaloids isolated from Malaysian *Uncaria lanosa* var *farrea*.

Materials and Methods: Isolation of compounds from the methanolic extract of *U. lanosa* var *farrea* employed various chromatographic techniques including open column and radial chromatography as well as preparative thin layer chromatography. The structure of compounds was elucidated on the basis of 1D and 2D NMR data. Measurement of antioxidant activities employed ferric thiocyanate (FTC) and thiobarbituric (TBA) methods while antidiabetic potential was evaluated by α-glucosidase inhibitory assay.

Results: Two major compound, ULs 1 (isopteropodine) and ULs 2 (pteropodine) were isolated from the stem extract of *U. lanosa* var *farrea* (stems). Both compounds exhibited strong antioxidant potential in the FTC and TBA assays. ULs 1 showed higher inhibition than ULs 2. However both compounds showed poor α-glucosidase inhibitory activities.

Conclusion: This study has found that isopteropodine and pteropodine possessed strong antioxidant property but have poor antidiabetic potential.

EFFECT OF *EURYCOMA LONGIFOLIA* JACK ON OVARIAN HISTOLOGY IN POLYCYSTIC OVARIAN IN FEMALE RATS

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Introduction: Eurycoma longifolia Jack, locally named *Tongkat Ali*, has been used as traditional herbal medicine to enhance male libido and improve male fertility. However, low libido and infertility are more common in women then in men.

Objectives: The present study investigated the effect of *E. longifolia* (Simaroubaceae) on the polycystic ovarian (PCO) histology of female rats induced by estradiol valerate (EV).

Materials and Methods: In one group (n = 12) of normal adult female rats of 8–10 weeks old with regular oestrus cycle, PCO was induced by single dose of EV at 2 mg/rat subcutaneously. After 1 month post-EV injection, the animals were divided into 2 groups; 1 group was given vehicle (distilled water) (n = 6) only as a positive control, whereas the other group (n = 6) was orally fed with a standardised extract TAF 273 of *E. longifolia* at 50 mg/kg/day for another month. A negative control group was given only the vehicle of EV and TAF 273. At the end of treatment, the animals were sacrificed and both ovaries were excised and cleaned from other adherent tissues. The ovaries were fixed in formalin-saline for further histological process. In another experiment, PCO was induced in immature female rats of 3 weeks old by treatment with testosterone (T) at 10 mg/kg/day subcutaneously for 3 weeks and was subsequently divided into 2 groups; 1 group (n = 6) was given vehicle (distilled water) alone and another group (n = 6) was given TAF 273 at 50 mg/kg/day for another 3 weeks. At end of treatment the animals were sacrificed and the ovaries excised and cleaned as previously described.

Results: The ovarian section of the animals treated with EV+TAF 273 showed less follicular cysts but more of healthy corpus lutea and follicles when compared to ovarian section of animals treated with EV alone. In the second experiment, ovarian histological section from T+TAF 273 revealed that less follicular cysts with more healthy follicles were observed in the treated group when compared to those given T alone.

Conclusion: The standardised extract TAF 273 of *E. longifolia* may be worthy for further investigation against polycystic ovarian syndrome.

EFFECT OF CONCOMITANT ADMINISTRATION OF PIPERINE ON THE BIOAVAILABILITY OF SULPIRIDE

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Introduction: Piperine is a compound obtained from black pepper and has been shown to have P-glycoprotein (P-gp) inhibitor activity. Furthermore it has also been proven to be an intestinal permeation enhancer. Sulpiride is an antipsychotic drug which has poor oral bioavailability (30%) owing to poor solubility, poor permeability and P-gp efflux. In order to overcome the solubility problem, it was formulated as a self microemulsifying drug delivery system (SMEDDS).

Objectives: The objective of the present work was to determine the effect of piperine on the bioavailability of sulpiride in SMEDDS and suspension formulations.

Materials and Methods: Twenty four rabbits were divided into four groups. The first group was dosed with sulpiride suspension; the second was with SMEDDS formulation orally, while the third and fourth group were fed with the suspension and SMEDDS formulation respectively half an hour after oral administration of piperine. Blood samples were collected through the marginal ear vein at predetermined time intervals. The drug content in the samples was determined using a validated HPLC method. The pharmacokinetic parameters were calculated and the relative bioavailability was also calculated and compared.

Results: There was a statistically significant difference ($p < 0.05$) in C_{max} and $AUC_{0-\infty}$ values between the four groups studied. However in case of T_{max} there was a statistically significant difference between all the groups except suspension with piperine dosed groups. The highest increase in the relative bioavailability was observed with the group that was concomitantly dosed with SMEDDS formulation and piperine.

Conclusion: Concomitant administration of piperine with sulpiride both in suspension and SMEDDS was able to enhance the bioavailability. The increment in the bioavailability of the drug present in SMEDDS was higher compared to suspension when concomitantly administered with piperine.

SYNERGISTIC ACTIVITY OF LIGNANS FROM *PHYLLANTHUS AMARUS* WITH VINCRISTINE ON HEPG2

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Introduction: Phyllanthus amarus has been used as a traditional medicine against various liver disorders including cancer. For the first time, the lignans isolated from *P. amarus* were examined alone and in combination with vincristine as therapeutic agents on human hepatocarcinoma cells.

Objectives: The investigation studied the effectiveness of *P. amarus* lignans alone or in combination with vincristine to synergistically improve the chemosensitivity and apoptotis of the hepatocarcinoma cells.

Materials and Methods: The isolated lignans and vincristine, alone and in combination, at 50% equitoxic molar ratios were treated on in vitro HepG2 cells for 48 h and 72 h. Thereafter, the cell viability was assessed using the MTT assay. The chemosensitivity of vincristine, the lignans and in combination were analysed and compared using the Chou-Talalay median-effect method.

Results: The effectiveness of combined treatment of vincristine and lignans were concentration and time-dependant. Combined treatment of vincristine and niranthin produced a synergistic effect of 58.6 ± 0.32% and 74.5 ± 0.85% reduction in IC_{50} values at 48 and 72 h, respectively. Nirtetralin alone showed only weak activity at IC_{75} and IC_{90} after 72 h treatment, but on combined treatment with vincristine, the reduction of IC_{50} values was 31.3 ± 3.57% and 50.6 ± 0.90% for 48 and 72 h, respectively. In contrast, no complete reversion of vincristine resistance was obtained from the co-treatment of phyllanthin, phyltetralin and hypophyllanthin, up to the IC_{90} inhibition levels. Phyllanthin, on the contrary, showed strong antagonistic effect on the proliferation of the HepG2 cells at both time points.

Conclusions: Our preliminary results suggested that a beneficial outcome of enhancing the chemosensitivity of the liver carcinoma could be derived by the co-administration of *P. amarus* lignans and vincristine. The present research may be worthy of further investigation to source for better therapeutic effectiveness.

ACUTE TOXICITY AND ANTIPROLIFERATIVE EFFECT OF MYRMECODIA PLATYTYREA METHANOLIC EXTRACT

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Introduction: Myrmecodia platytyrea or locally known as *sarang semut* is an epiphytic species native to Asia. This member of the Rubiaceae family can be found on many trees including the mangrove forest and was traditionally used in management of cancer, hyperuricaemia and coronary heart diseases.

Objectives: The present study was carried out to investigate the safety of *M. platytyrea* methanolic extract and the effects of the extract on human hepatoma cells.

Materials and Methods: The cytotoxicity effects of methanolic extract from the tuber of *M. platytyrea* were determined using the 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS) assay against the normal African green monkey kidney epithelial cells (Vero) and human hepatoma (HepG2) cell line. The acute toxicity of *M. platytyrea* methanolic extract (2 and 5 g/kg, p.o) were determined using male and female albino mice (n = 3, 25–35g).

Results: The methanolic extract of *M. platytyrea* inhibited the proliferation of HepG2 cells without affecting Vero cells. No death was reported after 24 h of oral administration of 2 and 5 g/kg of the extracts. Blood biochemistry analysis of male and female mice given methanolic extract of *M. platytyrea* (2 and 5 g/kg, p.o) showed no significant differences compared to control (normal saline).

Conclusion: M. platytyrea methanolic extract may possess potential inhibition of hepatocarcinogenic effect, which requires further in-depth study.

ACETYLCHOLINESTERASE AND BUTYRYLCHOLINESTERASE INHIBITORY ACTIVITIES OF *AILANTHUS MALABARICA* AND ITS FRACTIONS: A SOURCE FOR POTENTIAL ANTI-ALZHEIMER'S DRUGS

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Introduction: *Ailanthus malabarica* is a local medicinal plant traditionally used for the treatment of dysentery, dyspepsia, febrifuge and bronchitis. Preliminary anticholinesterase screening of some Malaysian plants with thin layer chromatography

(TLC) bioautographed with Ellman and Fast Blue B reagents revealed that the plant has potential anticholinesterase activity.

Objectives: The present study investigated the acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities of *A. malabarica* and its fractions, with the hope of finding novel chemical entities with anti-Alzheimer's activity.

Materials and Methods: The quantitative inhibitory activities were evaluated using a 96-well plate assay based on Ellman's method, while the qualitative inhibitory activities were evaluated by TLC bioautography with Ellman and Fast Blue B reagents.

Results: The methanolic extract of *A. malabarica* showed weak inhibitory activities against AChE and BuChE enzymes with IC_{50} values of 756.95 µg/mL and 617.41 µg/mL, respectively. Upon fractionation, the AChE inhibitory activity was found to be concentrated in fraction 4 with an IC_{50} value of 379.37 µg/mL whereas, the BuChE inhibitory activity was concentrated in fraction 5 with an IC_{50} value of 330.71 µg/mL. These results indicated that different chemical constituents were responsible for the observed inhibitory activities.

Conclusion: *A. malabarica* and its fractions have weak AChE and BuChE inhibitory activities.

INHIBITORY ACTIVITIES OF *ANDROGRAPHIS PANICULATA* AND ITS FRACTIONS ON ACETYLCHOLINESTERASE AND BUTYRLCHOLINESTERASE ENZYMES: A SOURCE FOR POTENTIAL ANTI-ALZHEIMER'S DRUGS

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Introduction: *Andrographis paniculata* (Burm. F.) Nees (Acanthaceae) is an evergreen shrub widely distributed in Asia. Some studies have shown that *A. paniculata* and its chemical constituents have effects on the central nervous system, such as sedative effect in the brain.

Objectives: The present study evaluated the potential inhibitory activities of *A. paniculata* and its fractions on acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) enzymes, with the hope of discovering potential novel chemical entities against Alzheimer's disease.

Materials and Methods: The extract and the fractions were tested for their AChE and BuChE inhibitory activities by in vitro spectrophotometric Ellman asssay and thin layer chromatography (TLC) bioautographic method with Ellman and Fast Blue B as reagent.

Results: The methanolic extract and its fractions F5, F4, F3, F2 and F1 showed weak inhibitory activities on AChE with IC_{50} values of 471.37 µg/mL, 473.72 µg/mL, 219.29 µg/mL, 570.28 µg/mL, 716.99 µg/mL and 3196.60 µg/mL, respectively. On the other hand, the methanolic extract and its fractions F5 and F4 displayed good inhibitory activities on BuChE with IC_{50} values of 191.03 µg/mL, 98.09 µg/mL and 56.12 µg/mL, respectively. In contrast, weak BuChE inhibitory activities were observed from fractions F3, F2 and F1 possessing IC_{50} values above 1000 µg/mL.

Conclusion: The methanolic extract of *A. paniculata* crude extract showed potential inhibitory activities on BuChE greater than on AChE. Upon bioactivity-guided fractionation, the anti-BuChE activities were concentrated in the non-polar fractions of F4 and F5.

CHEMICAL AND IN VITRO ANTIFUNGAL STUDIES ON THE ALKALOIDS OF SUDANESE *NICOTIANA RUSTICA*

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Introduction: Leaf powder of *Nicotiana rustica*, commercially grown in Sudan, is thoroughly, but lightly, moistened with aqueous alkali in a local method used to make a snuff-type tobacco preparation. This preparation is also used in folk medicine for the treatment of ear infections (otomycosis) in humans without reported side-effects.

Objectives: As alkaloids seemed to be involved in the claimed therapy, the main study objective was to test crude and purified preparations made from leaves of *N. rustica* for their in vitro activity against the main microorganisms responsible for otomycosis in Sudanese patients. Elucidating the nature of the active chemical ingredient was another target.

Materials and Methods: Snuff tobacco powder was obtained (untreated with alkali) from several retail shops in Sudan. The total alkaloid fraction (TAF) was prepared by wetting the leaf powder followed by extraction in chloroform. The TAF and its components, separated by TLC, were evaluated in vitro for their antifungal activity against *Aspergillus niger* using standard laboratory methods. Similar in vitro bioassays were also performed with an aqueous formulation containing 1.0% TAF (pH 6.2) subjected to different storage conditions.

Results: Fungal species associated with otomycosis in Sudanese patients were *A. niger* (60%), *A. flvavous* (30%) and *A. aculeatus* (10%). The TAF inhibited the growth of *A. niger* in vitro, in a dose-dependent manner. The MIC for TAF ranged between 100 and

200 µl/mL. The drug formulation containing 1.0% showed in vitro antifungal activity against *A. niger* exceeding that of the standard antifungal drug clotrimazole, when compared weight for weight. Antifungal activity of the formulation was not affected by storage at room temperature for at least 3 months. Samples of commercial snuff tobacco leaf powder obtained from different retail shops showed large variations in their constituent alkaloids. Two of the TLC separated alkaloids were antifungally active, one of which, according to initial spectroscopic examination, suggests itself as a nicotine derivative.

Conclusion: N. rustica contains potent antifungal alkaloids of potential use in the treatment of otomycosis (ear infection) in humans. Other aspects, including elucidation of the chemical structures of active ingredients and their toxicity remain to be clarified.

ACUTE AND SUBCHRONIC ORAL TOXICITY STUDIES OF 25% ETHANOL EXTRACT OF *GYNURA PROCUMBENS* IN SPRAGUE–DAWLEYS RATS

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Introduction: The leaves of *Gynura procumbens* (*sambung nyawa* in Malay) have been popularly used in folk medicine for treatment of diabetes. Glucose tolerance tests showed that the 25% ethanol extract of the leaves was the most effective in inhibiting the rise of blood glucose level of glucose loaded rats.

Objectives: The aim of this study was to evaluate the acute and subchronic toxicities of 25% ethanol extract of *G. procumbens* leaves extract.

Materials and Methods: Acute oral toxicity was studied using up and down procedure where 5 female rats were orally administered with single dose of 2000 mg/kg 25% ethanol extract and kept under observation for 14 days before being sacrificed for postmortem. Subchronic toxicity was evaluated by administering 250, 500 and 1000 mg/kg/day 25% ethanol extract to groups of rats (5 male and 5 female for each dose level) for 28 days. At the end of the study period, blood was withdrawn by cardiac puncture for haematological and clinical biochemical examinations before the rat was sacrificed for postmortem.

Results: There was no toxic signs and symptoms observed after single dose administration of the extract and the animal survived to the end of the experiment. Therefore the acute oral LD_{50} of *G. procumbens* 25% ethanol extract is higher than 2000 mg/kg. Daily oral administration up to 1000 mg/kg of 25% ethanol extract of *G. procumbens* leaves did not cause any significant changes in the general behaviour, body weight or gross appearance of the rats. Postmortem examination did not find any significant differences in the general condition, weight of the internal organs and haematological and clinical biochemistry values as compared to the control group.

Conclusion: Therefore, the NOAEL for 28 studies of the *G. procumbens* 25% ethanol extract is higher than 1000 mg/kg/day.

ANTIANGIOGENIC PROPERTIES OF MYRISTICA FRAGRANS ESSENTIAL OIL EXTRACT USING SUPERCRITICAL CARBON DIOXIDE

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Introduction: Angiogenesis is the growth of new blood vessels, which is crucial for tumour development. These blood vessels provide tumour cells with vital nutrients and oxygen to promote cancer cell survival and spreading of the disease. Blocking these blood vessels by antiangiogenic agents can stop tumour development and may also prevent the onset of the disease. Essential oils are natural products composed of many complex components, terpenes and non-terpenes. Monoterpenes have been shown to have chemopreventive and chemotherapeutic activities in tumour models, and thus may represent a new therapeutic agent to target cancer. The use of supercritical fluids has increased recently; it is a rapid, selective and convenient method of oil extraction.

Objectives: The aim of this study is to investigate the antiangiogenic activity of supercritical carbon dioxide (CO_2) extract of *Myristica fragrans* essential oil.

Materials and Methods: The supercritical CO_2 extraction of the essential oil was conducted between 40°C–50°C, with applied pressure of 27.6 and 34.5 MPa, and at an extraction time of 60 min. A 3-dimensional isolated tissue assay employing rat aorta was used to study the extent of angiogenesis inhibition of the compound.

Result: The result of this study showed potent antiangiogenic activity from supercritical CO_2 extract of *M. fragrans* obtained at 50°C under the pressure of 27.6 MPa. At a concentration of 125 ug/mL, the compound caused 100% inhibition of neovascularisation.

Conclusion: This finding suggests that the supercritical extract of *M. fragrans* essential oil has potent antiangiogenic activity and may be useful to treat cancer and also other diseases that are angiogenesis-dependent such as obesity, diabetis, blindness and rheumatoid arthritis.

ANTIMICROBIAL ACTIVITY OF *LACTOBACILLUS ACIDOPHILUS* GROWN IN SUBSTRATES DERIVED FROM NATURAL PRODUCTS

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Objectives: The present study investigated whether *Morinda citrifolia* (*noni*) and *Glycine max* (soya bean) could be used as a growth medium for *Lactobacillus acidophilus* to produce metabolites with antimicrobial activity.

Materials and Methods: Eight strains of *Lactobacillus acidophilus*, namely, FTDC 2804, FTDC 0785, FTDC 8592, FTDC 1295, FTDC 4793, FTDC 4462, FTDC 0582 and FTDC 2916, were cultivated in *M. citrifolia* and *G. max*. The bacteria metabolites were harvested and evaluated for antimicrobial activity against two pathogenic bacteria, *S. aureus* and *E. coli*.

Results: The metabolites of all *L. acidophilus* strains showed a significant bactericidal activity, as evidently indicated by the zone of inhibition in the culture plates when compared to control. There was a statistically significant difference in the zone of inhibition data for *S. aureus* and *E. coli* among the metabolites of the eight strains cultivated in the two growth media. Metabolites of *L. acidophilus* FTDC 4462 strain exhibited the highest zone of inhibition against *S. aureus* and *E. coli* in the 2 growth media. The metabolites of some *L. acidophilus* strains were more effective against *S. aureus*, while other strains were more effective against *E. coli*. On the other hand, the growth medium had no significant influence on the antimicrobial effect of metabolites of 7 strains except *L. acidophilus* FTDC 2916 against *E. coli*. As for *S. aureus,* the two growth media had no significant influence on the antimicrobial effect of metabolites of all the eight strains.

Conclusion: M. citrifolia (*noni*) and *G. max* (soya bean**)** could be used as the growth medium for *L. acidophilus* to produce metabolites with antimicrobial activity.

WATER-METHANOLIC EXTRACT OF *ORTHOSIPHON STAMINEUS* ALTERS ENDOTHELIUM-DEPENDENT VASOMOTOR RESPONSES OF AORTIC RINGS IN SPONTANEOUSLY HYPERTENSIVE RAT

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Introduction: Orthosiphon stamineus (OS) or locally known as *misai kucing* has been traditionally claimed to possess antihypertensive properties. Total peripheral resistance is an important factor in blood pressure regulation and the vascular reactivity concerning OS-mediated antihypertensive properties still remains elusive.

Objectives: The present study aims to investigate the mechanism(s) of endothelium-dependent vasomotor activities in spontaneously hypertensive rats (SHRs) treated with OS extract.

Materials and Methods: Eighteen male SHRs (250 g) were divided into three groups: negative control (vehicle only), positive control (losartan 10 mg/kg b.w.) and OS-treated group (50:50 water-methanol extract 1.0 g/kg b.w.). All animals received respective daily treatment orally for 14 days, after which the thoracic aortas were harvested and attached to force transducers in organ bath. Endothelium-dependent dose-response curve studies of phenylephrine, angiotensin II and acetylcholine (10^{-9} to 10^{-5} mol/L) in the absence or presence of L-NAME (100 µM), indomethacin (10 µM) and methylene blue (10 µM) were conducted.

Results: Significantly lower maximal tensions and higher relaxations in the treatment and positive control groups were observed as compared with the negative control groups.

Conclusion: These findings collectively suggest that the water-methanolic extract of OS induces vascular relaxation in SHR by means of endothelial-derived prostacyclin and nitric oxide pathways.

METHANOL EXTRACT OF *FICUS DELTOIDEA* LEAVES REDUCE CONTRACTION AND ENHANCE RELAXATION IN ISOLATED SPONTANEOUSLY HYPERTENSIVE RAT AORTIC RINGS

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Introduction: Hypertension has been associated with end-organ damage that may lead to stroke, heart and renal failure. *Ficus deltoidea* (FD) commonly known as *mas cotek* is traditionally claimed to possess antihypertensive effects.

Objectives: Since regulation of vascular resistance vessels have been implicated in hypertension, this preliminary study investigates the effects of the methanol extract of FD leaves on the vascular reactivity of spontaneously hypertensive rats (SHRs).

Materials and Methods: Eighteen SHRs were divided into three groups: negative control, positive control (losartan 10 mg/kg b.w.), FD methanol extract (1 g/kg b.w.) Having orally fed the animals daily with losartan and plant extract for 14 days, isolated thoracic aortic rings were harvested and subjected to endothelium-dependent contraction and relaxation studies using phenylephrine, angiotensin II and acetylcholine (10^{-9} to 10^{-5}mol/L) in the absence and presence of L-NAME (100 µM), indomethacin (10 µM) and methylene blue (10 µM).

Results: A significantly reduced vasoconstriction effect and potentiated vasodilator effect was observed in the FD extract treated groups.

Conclusion: Collectively, these findings suggest that the methanolic extract of FD alters vascular constriction and relaxation in SHRs through mechanisms involving endothelial-derived prostacyclin and nitric oxide pathways.

EVALUATION OF ANTIMALARIAL ACTIVITY OF SESQUITERPENE LACTONES OF WILD SUDANESE BASIL (*OCIMUM BASILICUM*) THROUGH THEIR IN VITRO INHIBITION OF DIHYDROFOLATE REDUCTASE (DHFR)

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Introduction: Basil (*Ocimum basilicum* L), which grows widely as a wild rainy season annual plant, is used, limitedly though, as one of the folk remedies of malaria in Sudan. Our study showed that 2 compounds of sesquiterpene lactones of ethanolic extract of basil out of 11 were inhibitory to dihydrofolate reductase (DHFR) in vitro assay.

Objectives: The study was undertaken to evaluate the potential antimalarial activity of sesquiterpene lactones separated compounds of ethanolic extract of basil by assaying their in vitro inhibition of DHFR.

Materials and Methods: Extraction method of sesquiterpene lactones from Sudanese basil was applied according to a previously used method. RPMI 1640 media (Gibco BRL, Sigma Co., USA) was used for the inhibition of *Plasmodium* parasite. The commercial DHFR enzyme (Sigma Co., USA) was used spectrophotometrically at absorbance of 340 nm (UV-3101PC, path light 1 cm, temperature 22°C) following the coupled conversion of NADPH to $NADP^+$.

Results: Crude ethanolic extracts of the wild basil leaves caused 100%, 80% and 50% inhibition of the growth of *Plasmodium falciparum*, at doses of 500, 50 and 5 µg/mL respectively when tested in vitro using RPMI 1640. Of the 11 compounds, eluted from TLC plates and tested, 2 inhibitory to DHFR (inhibitors of this enzyme deprive protozoa, as well as bacteria, of reduced folates, carriers of one-carbon fragments required for the biosynthesis of nucleic acids), resulting in specific activities of 25.0 and 11.0 µmole/min/mg protein.

Conclusion: The present study showed that the two isolated sesquiterpene lactones compounds were inhibitory to DHFR. Further work is underway.

INFLUENCE OF DAILY INGESTION OF *MORINDA CITRIFOLIA* JUICE ON BLOOD COAGULATION PROFILE, LIVER AND KIDNEY FUNCTIONS IN HUMAN VOLUNTEERS

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Introduction: Morinda citrifolia (Mc), commonly called *noni* is used in traditional medicines for treating many disease conditions, including diarrhoea, tuberculosis, asthma, arthritis, diabetes, hypertension, dysmenorrhoea, depression, drug addiction, AIDS, cancer and lupus. Mc constituents include phenolic compounds, organic acids and alkaloids. Our earlier studies with the fruit and leaf extracts of Mc hot water extracts have shown them to prolong blood coagulation.

Objectives: This study determines the effect of daily ingestion of a commercial Mc fruit juice on blood coagulation and blood profile in healthy human volunteers.

Materials and Methods: Thirty male volunteers (aged 18 to 30) ingested 30 mL of Mc fruit juice from a commercial source, daily for 24 weeks. Blood sample (10 mL) taken before the start of ingestion and on weeks 8, 16 and 24 were assessed for clotting time (CT), prothrombin time (PT), thombin time (TT), activated thromboplastin time (aPTT), liver enzymes [alanine transaminae (ALT), aspartate amino transferase (AST) and γ-glutamyl transpeptidase] electrolytes, uric acid and creatinine levels. Body weight, systolic and diastolic blood pressure of volunteers were monitored. Data (before and after treatment) was analysed using SPSS Version 15.0, and compared using ANOVA, paired t-test or the Wilcoxon signed rank test and the sign test where appropriate.

Results: Mc fruit juice had no significant effect on CT but increased PT ($p < 0.001$), which returned to baseline values with time. TT was prolonged ($p < 0.01$) only at 8 weeks, with no effect on aPTT. Potassium levels were raised ($p < 0.001$) on week 16. However AST ($p < 0.001$) and ALT ($p < 0.01$) were significantly raised over baseline levels, although still within normal range.

Conclusion: The commercial Mc fruit juice affects blood coagulation. The raised liver enzyme levels although within physiological range merits further investigation.

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EVALUATION OF WOUND HEALING IN FULL THICKNESS SKIN AUTOGRAFT WITH DIFFERENT CONCENTRATIONS OF TOPICAL *STICHOPUS SP1* (GAMAT) EXTRACT IN SPRAGUE DAWLEY RATS

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Objectives: A randomised control trial study was carried out to evaluate the wound healing process of skin autografts after the application of different concentrations of topical *Stichopus sp1* extract (gamat) on experimental wound beds.

Materials and Methods: Twenty four adult female Sprague Dawley strain rats were randomly divided into four groups; control (non-treated), low dose (5%), medium dose (10%) and high dose (20%) *Stichopus sp1*-treated groups. Dorsal full thickness skin sheets (2 cm x 2 cm) were harvested and were preserved for five days. Control base and topical test substances were uniformly applied onto the surgical wound beds at day zero and day five post-harvesting. All grafts were auto-transplanted at day five.

Results: Seven days post transplantation; macroscopic evaluation showed that there was no difference in term of graft adherence, graft colour and graft pliability between all *Stichopus sp1*-treated groups compared to the controls. Microscopically, there were no significant difference in term of inflammatory cells infiltration, proliferation of fibroblasts and new blood vessels between all *Stichopus sp1*-treated grafts compared to the controls ($p > 0.05$). *Stichopus sp1* treated full thickness skin grafts at any concentrations have poor wound healing response both macroscopically and microscopically and there are no differences between them.

Conclusion: The preparation of the topical substances used on experimental wounds may not be suitable for skin graft management applied topically on the graft wound beds.

CYTOTOXIC PRINCIPLES FROM ZINGIBERACEAE SPECIES

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Introduction: Species of the Zingiberaceae family have a long history in traditional medicine. Many have been traditionally utilised in the treatment for cancer. As part of our search for novel cytotoxic components from natural sources, several species of the ginger family have been investigated for their cytotoxic effect against 6 human carcinoma cell lines, namely MCF-7, KB, A549, Ca Ski, HCT 116, HT-29 and 1 non-cancer cell line (MRC-5). The ginger species investigated were *Curcuma mangga, Curcuma zedoaria, Curcuma aeruginosa, Curcuma inodora, Zingiber zerumbet, Zingiber officinale* variants *(jahe gajah* and *jahe emprit), Alpinia mutica,* and *Boesenbergia rotunda.*

Objectives: To determine the cytotoxic principles from several species of the Zingiberaceae family.

Materials and Methods: Cytotoxicity was determined by the neutral red cytotoxicity assay. Cytotoxicity of each sample is expressed as an IC_{50} value (the concentration of test compounds that cause 50% inhibition or cell death, averaged from 3 experiments). The cytotoxic components were obtained through bioassay-guided isolation.

Results: The cytotoxic principles identified were curcumin, (*E*)-labda-8(17),12-dien-15,16-dial and (*E*)-labda-8(17),12-dien-15-ol,16-al from *C. mangga*; alismol, dihydroalismol and dehydrocurdione from *C. zedoaria*; curzerenone from *C. aeruginosa*; zerumbone from *Z. zerumbet*; diacetoxy-6-gingerdiol, 6-shogaol, 8-shogaol and 10-shogaol from *Z. officianale* variants; Flavokawin B and pinostrobin chalcone from *A. mutica*; pinostrobin from *B. rotunda.* All these compounds demonstrated inhibitory activities in investigated cancer cell lines with IC_{50} values of less than 10.0 µg/mL.

Conclusion: The findings from this study provide scientific validation on the use of the selected Zingiberaceae species for the treatment of cancer in traditional medicine.

MONOGRAPH AND DOCUMENTATION

STUDIES ON STANDARDISED EXTRACTS OF *FICUS DELTOIDEA* FOR ANTIHYPERTENSIVE ACTIVITY

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Introduction: Ficus deltoidea is traditionally used in treating many diseases including high blood pressure to improve blood circulation, diabeties, gout, pneumonia, heart problems, diarrhoea and skin infection.

Objectives: To develop and optimise standardised extracts of *F. deltoidea* leaves and subjected for antihypertension properties by using angiotensin converting enzyme (ACE) assay.

Materials and Methods: the method for measuring ACE inhibitor activity was based on spectrophotometric determination using HHL as a substrate.

Results: F. deltoidea methanol and water extracts showed the inhibition of the ACE enzyme. Captopril, a widely used synthetic ACE inhibitor was used as a standard. ACE inhibitory activity of all the extracts was in the range of 11.39% to 73.27%. The methanol extracts exhibited higher inhibition as compared to the water extracts. A very good ACE inhibition of 73.27 ± 1.10% ($p < 0.05$) was obtained from the methanol extracts of the final concentration of 300 µg/mL.

Conclusion: The present study showed antihypertensive activities of the extracts were found to be comparable to captopril, a well known ACE inhibitor.

EVALUATION OF BIOACTIVE HERBAL PHYTOCHEMICAL FINGERPRINT PROFILE USING SPE-2D PLANAR CHROMATOGRAPHY TECHNIQUE

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Introduction: Various attempts in isolating active pure compound(s) from potential plant(s) by targeting a single compound have been practiced with the intention of developing a novel potent drug candidate. Yet, the possibilities of obtaining such novel substance(s) are low from such approach.

Objectives: In view of this consideration, fingerprinting analysis of the bioactive compounds as a whole formulation to yield a common fingerprint profile in first dimension is essential to enhance the drug discovery efficacy and provide a rapid understanding of the pharmacological profiles of the potential plants.

Materials Methods and: In this context, an attempt to establish SPE-two-dimensional planar chromatographic (HPTLC) fingerprint profiles of *Paraboea paniculata* as the model plant is demonstrated. The crude and SPE-fractionated extracts were screened at 50 µg/mL for cytotoxic activity against chronic myelogenous leukemic cell line, K-562 using MTT assay. Planar chromatography (HPTLC) combined with the digital scanning methodology gave a characteristic set of fingerprint profile of the cytotoxic fractions. The fingerprint profile(s) were developed with several optimised mobile profiles owing to the polarity nature of the active fractions, normal and reversed phase TLC stationary phase.

Results: The segregation of the active group of constituent(s) in both plant leave and rhizome parts of *P. paniculata* is clearly observed in these studies. Each active fraction(s) possess their distinct set of fingerprint pattern(s). The similar pattern of metabolite(s) of fraction V for both leave and rhizome indicate the presence of similar active group of constituent(s). The method was validated in terms of precision, robustness, specificity and stability.

Conclusions: The fingerprint profiles of *P. paniculata* were successfully documented, which can be employed as an alternative rapid identification method, and to interpret the bioactivities of similar species of plant(s) through pattern recognition, in the future.

POSTER PRESENTATIONS

COMMERCIALISATION, MARKETING AND REGULATORY AFFAIRS

PREPARATION AND EVALUATION OF A NOVEL SUSTAINED RELEASE MATRIX DOSAGE FORM OF THEOPHYLLINE USING NATURAL RETARDING MATERIALS

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Introduction: Sustained release dosage forms have been used to deliver therapeutic agents automatically at pre-determined rates over a long period of time. Release of the drug from these systems in a zero order fashion is considered a holy grail.

Objectives: The preparated and evaluated in vitro a novel sustained release theophylline matrix tablets constituting of a natural retarding agent in an attempt to achieve zero order release.

Materials and Methods: The initial part of study involved fabrication and preparation of mould. A perspex sheet of 6 mm thick was taken and holes of varying sizes were drilled in it. Holes of 3 mm and 4 mm in diameter were drilled in another sheet which was positioned so that the holes were concentric with those in the prior sheet. The entire assembly was firmly bolted. A central releasing hole (CRH) in the mould was made. Bees wax, cetostearyl alcohol and carnauba wax were first melted. Then PEG2000 and Tween 80 were melted and mixed well. Then finally theophylline was suspended in this molten base and stirred well until a homogeneous dispersion was obtained. The molten mass was poured into the mould and dispersed uniformly, and solidified to form a hard cylindrical matrix. The whole of the outer surface except the CRH was completely coated with hard paraffin. In vitro dissolution studies were carried out using basket type apparatus as per the pharmacopeias procedure.

Results: The matrix mould with 9 mm in diameter and 4 mm CRH and composition of 6% carnauba wax, 2% to 4% cetostearyl alcohol, 55% to 57% PEG2000 and a drug loading of 35% gave moulds that retained the shape intact. The drug release was found to be constant over a period of 12 hours indicating a zero order release.

Conclusion: A novel sustained release matrix mould of theophylline which released the drug in a zero order fashion was successfully prepared.

FORMULATION OF TABLETS FROM 70% ETHANOL LEAF EXTRACT OF *JUSTICIA GENDARUSSA* BURM.F. WITH LACTOSE AND CORNSTARCH AS DILUENTS

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Introduction: Gendarusin A found in 70% ethanol leaf extract of *Justicia gendarussa* Burm.f. has been proven to inhibit hyaluronidase activity that could prevent the penetration of spermatozoa into the ovum cell during the fertilisation process.

Objectives: To investigate the effect of lactose and cornstarch as diluents on tablet formulation of 70% ethanol leaf extract of *J. gendarussa*.

Materials and Methods: A 700 mg tablet containing gendarusin A in 300 mg of 70% ethanol leaf extract of *J. gendarussa*, Cab-O-Sil as adsorbent, primojel as disintegrant and a combination of lactose and cornstarch as diluents were prepared. The percentage of lactose and cornstarch was different in five formulas. The tablet formula containing only lactose as the diluent was employed as a standard. All the tablets were formulated by the wet granulation technique. The granule properties such as flowability, moisture content,

fineness and compactibility were determined. The tablet properties including hardness, friability and disintegration time were also evaluated in each formula.

Results: The formula containing lactose as the only diluent showed the highest tablet hardness and longest disintegration time. The presence of the cornstarch in the tablet formula decreased the hardness and disintegration time.

Conclusion: The study indicated that increasing the concentration of cornstarch in lactose resulted in the decrease of hardness and disintegration time of tablets.

ANTIOXIDANT AND CLUSTER ANALYSIS OF VARIOUS DURIAN CULTIVARS IN PULAU PINANG, MALAYSIA

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Introduction: Durio zibethinus or locally known as durian is one of most highly prized fruit in South East Asia.

Objectives: The main objectives of this study were to (i) screen antioxidant activity of different durian cultivars and (ii) compile the morphological variation and analyse the inter cultivar relationships using cluster analysis.

Materials and Methods: In this study, 35 durian cultivars were collected from Pulau Pinang, Malaysia. The macro and micro-morphological intraspecific variation of durian cultivars were observed and inserted in data matrix for construction of dendogram. Meanwhile the pulp of durian cultivar was subsequently extracted using three types of solvents; petroleum ether, ethyl acetate and methanol. Ethyl acetate and methanol extracts were then tested for their total phenolic content (TPC) and antioxidant activities by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging and ferric reducing antioxidant power (FRAP) assays.

Results: The result from the dendogram showed that *tangkai panjang* and *susu* have the highest of percent similarity (90.01%). While, the result from total phenolic and antioxidant activities indicated that *khun poh* has the highest result as compared to other cultivars. There are significant ($p < 0.05$) correlation between TPC and DPPH ($r = 0.727$), TPC and FRAP ($r = 0.894$) and lastly between DPPH and FRAP ($r = 0.844$).

Conclusion: In conclusion, the bioactivities of durian cultivars were high especially in methanol extract. The positive correlation between antioxidant activities and total phenolic content indicate that total phenolic content were the main contributor to the overall antioxidant activity.

RAW MATERIAL AND POST HARVESTING

CHEMICAL CONSTITUENTS OF *CALLICARPA PENTANDRA* (LAMIACEAE)

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Introduction: Callicarpa or beautyberry is a genus of shrubs and small trees of the family of Lamiaceae which can be found in the warmer part of Asia, Australia, the Pasific and in America. *Callicarpa pentandra* Roxb. is an erect shrub to small or medium-sized tree that can grow up to 25 m tall. This plant is used to treat toothache. A liquid prepared from *C. pentandra* is drunk for colds in folk medicine. For our initial investigation, bioassay-guided fractionation has been carried out to isolate the bioactive constituents responsible for the inhibition of acetycholinesterase enzyme of *C. pentandra* and the results showed acetylcholinesterase inhibitory effect only for hexane, dichloromethane and ethyl acetate fractions of leaves, stems and bark of *C. pentandra.*

Objectives: The study was done to isolate bioactive compounds from the leaves and barks of *C. pentandra*.

Materials and Methods: The air-ground leaves of *C. pentandra* (6.45 kg) were macerated in MeOH for 72 hours and the extraction process was repeated 3 times. The solvent was evaporated off under reduced pressure to yield 150 kg of crude extract. Then the crude MeOH (150 kg) extract was subjected to fractionation using liquid-liquid partition chromatography into hexane, dichloromethane, ethyl acetate and butanol fractions. The ethyl acetate fraction (9.6 g) was subjected to normal phase column chromatography and successively eluted with $CHCl_3$ followed by $CHCl_3$ enriched with increasing percentages of MeOH to yield 33 subfractions (F1-F33). Further separation and purification of the subfractions yielded 7 compounds namely Cp-LEA 1 (10.0 mg), Cp-LEA 2 (4.0 mg), Cp-LEA 3 (18.6 mg), Cp-LEA 4 (9.0 mg), Cp-LEA 5 (23.0 mg), Cp-LEA 6 (19.1 mg) and Cp-LEA 7 (10.4 mg).

Results and Conclusion: Seven compounds (Cp-LEA 1–7) have been isolated from the ethyl acetate fraction of the leaves of *C. pentandra.* The structure elucidation process of pure compounds is in progress.

OPTIMISATION OF ACCELERATED SOLVENT EXTRACTION (ASE) FOR THE *ORTHOSIPHON STAMINEUS* LEAVES

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Introduction: Orthosiphon stamineus or locally known *misai kucing* has been consumed as tea infusions in Malaysia in order to improve human health and to treat diseases such as kidney problem, bladder inflammation, gout and diabetes. *O. stamineus* has been reported to be rich in polyphenols (flavonoids and phenolic acid) and other active components such as diterpenes (terpenoids) and sterols. There is a dire need of green chemistry related methods in the field of extraction technology of herbal products. Accelerated solvent extraction (ASE) is an environmentally friendly-technique.

Objectives: To optimise the extraction procedure for *O. stamineus* leaves using ASE technique by application of various parameters (temperature, solvents, pressure, time and cycles).

Materials and Methods: O. stamineus dried leaves were extracted using Dionex ASE 200 with 66 mL stainless-steel extraction cells. Two different solvents were used, namely methanol and methanol - water (1:1). A system pressure of 1700 psi, an oven heat-up of 15 mins at 5 different temperatures (40°C, 60°C, 80°C, 100°C and 150°C). Two cycles per extraction were used for each solvent extraction.

Results: The result showed vast variations in the extractive values of ASE with respect to the different temperatures. The percentage yield of methanol extracts for 40°C, 60°C, 80°C, 100°C and 150°C were 1.87%, 3.03%, 4.82%, 2.42% and 9.54% respectively, while for methanol-water extracts were 3.24%, 4.68%, 6.70%, 6.78% and 20.2%, respectively. Comparative studies of yields with conventional method of extractions will be presented.

Conclusion: ASE, a relatively new technique for herbal extraction, is useful in saving solvent consumption compared to conventional method such as soxhlet and maceration. This technique is also environmental friendly and easy to handle. Vast potential awaits this technique in herbal application where environment friendly methods in the extraction technology are much needed.

MONITORING ANTICANCER COMPOUNDS OF *ORTHOSIPHON STAMINEUS* LEAVES FROM PERLIS HERBAL FARM

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Introduction: Orthosiphon stamineus Benth (Lamiaceae), is one of the most useful traditional medicinal herbs cultivated in South East Asia, particularly in Malaysia and Indonesia, and is popularly known as *misai kucing*. This herb gains wide and rapid acceptance for many ailments due to its established traditional use. In order to maintain consistent supply of quality raw materials for processing, *O. stamineus* was cultivated and harvested as per guidelines of Good Agriculture Practices (GAP).

Objectives: To evaluate and investigate the anticancer compounds of *O. stamineus* leaves from Perlis Herbal Farm.

Materials and Methods: A total of eight batches of *O. stamineus* leaves harvested from open and shaded areas at different months were collected separately, with and without flowers. Leaves were air dried, powdered and processed for the high performance liquid chromatography (HPLC) analysis. The reversed phase -HPLC (RP-HPLC) method was used to determine quantitatively anticancer compounds using an Agilent Technologies Series 1100 system equipped with degasser, an auto sampler, a column heater, quaternary pump and UV detector. Column (Nucleosil C_{18}, 250 mm x 4.6 mm, 5 µm i.d.) was maintained at 25°C and samples (20 µl) were eluted by an isocratic mobile phase.

Results: Results were good when *O. stamineus* were cultivated in an open area as compared to those under the shade. Secondly, *O. stamineus* harvested with flowers possessed the highest rosmarinic acid and sinensetin contents in the month of August, at 8.46% and 1.06% respectively. Orthosiphol A (diterpene) was reported highest, 1.06%, in September in the absence of flowers. Betulinic acid was not found in any batch.

Conclusion: In order to maintain consistent supply of quality raw materials with the required amounts of active compounds, it is necessary to cultivate and harvest this plant under strict controlled agronomical conditions in compliance with GAP.

ANALYSIS OF GAHARU OILS FROM ARTIFICIALLY INDUCED *AQUILARIA* SPECIES

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Introduction: Agarwood or *gaharu* is the result of a defence process of the karas (*Aquilaria*) tree against injury. High quality gaharu wood chips are sold directly to the Middle East and Japan as they are more favourable for religious purposes and as incense. The low quality grades are distilled to produce essential oils which are highly valued as perfumery ingredient. Currently, the suppliers of gaharu chips and oil are from Indonesia, Malaysia, India, Thailand and Vietnam where species such as *Aquilaria malaccensis, A. crassna* and *A. filaria* are being traded. All *Aquilaria* species are currently listed in Appendix II of the convention on trade in endangered species of wild fauna and flora (CITES) which means the trade of gaharu and its products is controlled and monitored by the relevant agencies for each producing country. In future the market will depend for supply from *Aquilaria* plantations in order to minimise the depletion of forest species. Thailand, Laos, Vietnam and Malaysia have already started planting trees in acreage. They have also developed their own inoculation technology and have already started using them on their plantation on a large scale.

Objectives: In an ongoing research on the chemical profiling of some locally produced gaharu from artificially induced karas trees, gaharu samples from different sources were analysed by GC, GC-MS and HS-SPME-GCMS.

Materials and Methods: Identification of the chemical components was based on comparison of calculated retention indices and mass spectral data with literature values and also comparison with commercial gaharu oils.

Results: The main chemical compounds of interest to be detected were α-bulnesene, β-agarofuran, α-agarofuran, agarospirol, 10-epi-γ-eudesmol, γ-eudesmol, valerianol and hexadecanoic acid. Distillation of all samples produced either resinous oil or semi solid substances with slight gaharu scent. GC and GCMS chromatographic profiles indicated the complexity of the essential oil components where most of the oils tested showed variation in terms of chemical components. Factors that may influence the chemical profiles of the samples could include the quality of the gaharu wood samples, how long gaharu is formed on the infected tree, distillation methods and the different inducement techniques used.

QUALITATIVE DETERMINATION OF POTENTIAL LYCORINE ALKALOID IN *HYMENOCALLIS LITTORALIS* (*MELONG KECIL*)

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Introduction: Hymenocallis littoralis is a bulbous and herbaceous plant from the family of Amaryllidaceae. In Malaysia, there are claims that the stems of *H. littoralis* which is commonly known as *melong kecil* is used to treat swollen joints and broken bones. Our preliminary study showed that the extract of *H. littoralis* contained a potential antineoplastic compound namely lycorine.

Objectives: The study was undertaken to determine the presence of lycorine in *H. littoralis* extract at different growth stages namely juvenile and after flowering.

Materials and Methods: The experiment was carried out by TLC analysis using aluminium plates and developed in chloroform-methanol (3:1) mobile phase system. The intensity of lycorine spot was observed under short ultraviolet spectrophotometer.

Results: H. littoralis extracts showed the presence of lycorine at both growth stages, juvenile and flowering. The highest lycorine content was found at juvenile stage in young leaves extracts followed by old leaves, bulbs and roots. Meanwhile, stems extract was found to contain the least amount of lycorine. In contrast, for the flowering stage, lycorine was to be the highest in roots followed by flowers, old leaves, young leaves, flower stalks, stems and the lowest in bulbs.

Conclusion: The present study showed that lycorine was present during the whole stages of juvenile and flowering of *H. littoralis* which was highest in young leaves and lowest in stems at juvenile stage, and highest in roots and lowest in bulbs at flowering stage.

CHEMISTRY OF NATURAL PRODUCTS INCLUDING QUALITY CONTROL, STANDARDISATION AND GOOD MANUFACTURING PRATICE (GMP)

ANTITUBERCULOSIS FLAVANOIDS FROM THE ROOT OF *SESBANIA GRANDIFLORA* (L.) PERS.

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Introduction: All parts of *Sesbania grandiflora* (L.) Pers. (Leguminosae) have been used empirically as a traditional remedy in folk medicine to treat various diseases such as hepatitis, parasites, and different kinds of infections as well as inflammation of skin and mucous membranes. Previous study revealed that *S. grandiflora* leaves could also afford a significant protective effect against erythromycin estolate-induced hepatotoxicity.

Objectives: This study aimed to isolate the chemical constituents from the root of *S. grandiflora* and to evaluate the antituberculosis activity of isolated compounds against *M. tuberculosis* H37Rv strain.

Materials and Methods: Dried and powdered roots of *S. grandiflora* were extracted exhaustively with methanol. The isolation of chemical components were conducted by repeated column chromatography of methanol extract, while the purified compounds were obtained by using preparative TLC. The structure elucidation of isolated compounds were determined on the basis of spectroscopic data (IR, MS, 1D, 2D NMR) and by comparison of spectral data with those reported earlier for the compounds. The purified compounds were tested for antituberculosis activity by using MTT assay against *M. tuberculosis* H37Rv strain.

Results: Three isoflavanoids, 7,4′-dihydroxy-2′-methoxyisoflavan (1), 3-hydroxy-9-methoxypterocarpan (2), and 7-hydroxy-2′,4′-dimethoxyisoflavan (3) were isolated from *S. grandiflora* root. The results exhibited that all isolated compounds inhibited the growth of *M. tuberculosis* H37Rv. Compounds 1–3 showed similar moderate activity with MIC values of 0.184, 0.185, and 0.175 μM respectively against *M. tuberculosis* H37Rv.

Conclusion: This is the first report on the natural occurrence of isoflavonoids in this plant. This study also indicates that *S. grandiflora* root could be a potential source of new antituberculosis agents.

FLAVONES FROM THE STEM BARK OF *POLYALTHIA CAULIFLORA* VAR. *CAULIFLORA*

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Introduction: Polyalthia cauliflora var. *cauliflora*, is one of the species from the *Polyalthia* genus (order Magnoliales, family Annonaceae), distributed widely in undisturbed forest up to at 1670 m altitude. This species can be found in Thailand, Peninsular Malaysia, Sumatra and Borneo.

Objectives: The intention of this research is to investigate the chemical constituents and to identify the structure of the compounds isolated from the crude methanolic extract of the stem bark of *P. cauliflora* var. *cauliflora*.

Materials and Methods: The non-alkaloial fraction obtained from acid base extraction was further investigated. The non-alkaloidal fraction was subjected to silica gel chromatography and eluted with hexane containing increasing amount of dichloromethane, ethyl acetate and methanol to yield 52 fractions. These fractions were subjected to extensive chromatographic techniques such as radial, preparative thin layer and glass column chromatography.

Results: The isolation and purification of non-alkaloidal extract from the stem bark of *P. cauliflora* var. *cauliflora* yielded 3 known flavones; 3,7-dimethoxy-5-hydroxyflavone, tectochrysin and 6,8-dimethoxy-5-hydroxyflavone which had never been isolated from this family. The structural elucidation of these compounds was accomplished by various spectroscopic methods including nuclear magnetic resonance (1D and 2D), IR, UV-Vis as well as mass spectroscopy.

Conclusion: Phytochemical study of *P. cauliflora* var. *cauliflora* has afforded 3 known flavone; 3,7-dimethoxy-5-hydroxyflavone, tectochrysin and 6,8-dimethoxy-5-hydroxyflavone.

THERMAL ANALYSIS OF BINARY MIXTURES OF VIRGIN COCONUT OIL AND SUNFLOWER OIL USING DIFFERENTIAL SCANNING CALORIMETRY (DSC)

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Introduction: Virgin coconut oil (VCO) is simply the coconut oil obtained without high heat and chemical treatments. The oil is rich in medium chain fatty acids, which has proven to be beneficial to health. Because of its nutritional value, VCO could be adulterated with oils of less value. Thus, an effective method to monitor adulteration in VCO is needed.

Objectives: This study was undertaken to investigate the cooling and heating thermograms of binary blends of VCO and sunflower oil for authentication purpose.

Materials and Methods: VCO was mixed with sunflower oil from 2% to 40% (wt/wt). A Perkin-Elmer differential scanning calorimeter (DSC) was used to perform the thermal analysis, which included heating and cooling thermogram. The fatty acid analysis of the binary mixtures were further analysed by gas chromatography to complement the data obtained from DSC.

Results: Introduction of polyunsaturated fatty acids from sunflower oil resulted in increase of long chain fatty acids particularly oleic, linoleic and linolenic fatty acids in the binary mixture. Heating thermogram of VCO adulterated with sunflower oil showed an adulteration peak appearing at the lower temperature region starting at the 10% adulteration level. Regression analysis using stepwise multiple linear regressions was used to predict the percentage of adulteration with coefficient of determination (R^2) of 0.9390. No adulteration peak was observed in the cooling curve of VCO adulterated sample.

Conclusion: The present study indicated that heating thermogram was better than cooling thermogram at predicting the chemical changes in binary mixtures of VCO and sunflower oil. In general, the DSC is suitable to monitor the thermal changes taking place in binary mixtures of VCO and sunflower oil.

INCREASED SOLUBILITY OF ARTEMISININ THROUGH COMPLEXATION WITH β-CYCLODEXTRIN

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Introduction: Malaria is still one of the major health problems in many areas of the world. The development of drug resistances (chloroquine, pyrimethamine) resulte in the suggestion to WHO policy to promote the use of artemisinin, an active principle isolated from the Chinese medical plant, *Artemisia annua* L.

Objectives: The aim of this work was to improve the solubility of artemisinin, poorly water-soluble anti malarial drug, through the complexation with β-cyclodextrin (βCD).

Materials and Methods: Phase and equilibrium solubility studies of artemisinin and βCD were carried out in water. Artemisinin-βCD complexes were manufactured by three different methods (slurry, kneading and spray drying). Characterisation of artemisinin raw material and of the complexes was carried out by solid state analysis (DSC and X-ray) and spectroscopic techniques (SEM and optical microscopy).

Results: The phase solubility profile of the complex in water at room temperature was classified as A_L type, indicating the formation of a 1:1 stoichiometric inclusion complex. Artemisinin was crystalline and thermal studies suggested that its crystallinity in the spray-dried microparticles decreased until 70% of the original value. In the case of the artemisinin-βCD complexes prepared by slurry and kneading methods, the artemisinin crystallinity decreased only 15% and 35%, respectively. The solubility in water of the artemisinin-βCD spray-dried microparticles increased about six-fold compared to the artemisinin raw material.

Conclusion: The present work showed the increase of artemisinin solubility through complexation with βCD.

OPTIMISATION OF LEMONGRASS OIL TEAT DIP USING THE MIXTURE EXPERIMENTAL DESIGN

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Introduction: Lemongrass (*Cymbopogon citratus* DC.) oil teat dip is developed as an alternative medicine to prevent bovine mastitis. The initial preliminary experiments showed that the amount of lemongrass oil (LG), span 80 and sodium dodecylbenzenesulfonate (SDBS) appeared to affect antimicrobial activity of LG teat dip formulations.

Objectives: The aim of this study was to develop a LG teat dip using the experimental mixture design.

Materials and Methods: Formulation optimisation of three factors of 2 concentration levels including LG, span 80 and SDBS in teat dip formulations was carried out using mixture design. MIC for *Staphylococcus aureus* (Y_1) MBC for *S. aureus* (Y_2) and *Escherichia coli* (Y_3), and *E. coli* (Y_4) were evaluated using microdilution method. Design-Expert® software was used to model the shape of the surfaces.

Results: The second-order polynomial regression equation (quadratic) was fitted for predicting the MIC and MBC for *S. aureus* and *E. coli.* In general, analysis of variance (ANOVA) showed high coefficients of determination values (R^2) in the range of 0.939–0.993 for these response surface models. The results also indicated that the interaction terms of LG and span 80, LG and SDBS had significant negative effect on MIC for *S. aureus* ($p < 0.05$), while the interaction effect between span 80 and SDBS were positively related to MBC for *S. aureus*. Moreover, it was found that the interaction between LG and span 80, LG and SDBS had a significant negative effect on MIC and MBC for *E. coli* ($p < 0.05$).

Conclusion: The present study showed that increasing the mixture between LG and span 80, LG and SDBS lowered the MIC for both tested strains and MBC for *E. coli.* Whereas, decreasing the mixture between span 80 and SDBS lowered the MBC for *S. aureus.*

ISOLATION AND PURIFICATION OF ANTITUMOUR-PROMOTING COMPOUNDS FROM *HOMALOCLADIUM PLATYCLADUM*

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Introduction: Medicinal plants have been used in the ethnopharmacological treatment of various diseases, including cancer. Cancer is a global burden disease, and is one of the leading causes of death in the world. Over the years, many ideal cancer treatments such as chemotherapy and surgery had been developed with total elimination in mind. However, carcinogenesis is a complicated multi-stage process that most conventional approaches for treatment do not only significantly decrease cancer mortality but also cause serious side effects. Hence, the development and discovery of chemopreventive agents are emphasised.

Objectives: This study was aimed to isolate and elucidate the active antitumour-promoting compounds from the roots of *Homalocladium platycladum*.

Materials and Methods: In the study, the roots of *H. platycladum* were subjected to bioassay-guided fractionation to isolate and purify the active antitumour-promoting compound(s), and the antitumour-promoting activity was evaluated using an in vitro EBV-EA induction assay.

Results: The crude methanol extract exhibited the highest inhibition of 92.08%, and showed moderate toxicity with cell viability of 53.64%. Further isolation of the methanol extract was done using silica gel packed glass column and high performance liquid chromatography. After repeated column chromatography, Fraction III and IV exhibited the highest inhibition of 80.68% and 83.18%, respectively. Both fraction III and IV were subjected to gas chromatography-mass spectroscopy analysis. Fraction III displayed a mixture of xanthen, triisopropylacetophenone, anthracenedione and decalin. Fraction IV contained anthracenedione with similar inhibition of 92%.

Conclusion: Preliminary studies have indicated that *H. platycladum* may contain potent antitumour-promoting compounds which may delay, stop, or reverse the tumour promoting stages in carcinogenesis.

A COMPARATIVE STUDY ON THE CONTENT OF SIX DIFFERENT BRANDS OF *GINKGO BILOBA* AVAILABLE IN THE MARKET

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Introduction: Ginkgo biloba-containing brands are one of the top 10 commercial products within the growing market for herbal remedies. Extracts from the leaves of *G. biloba* are among the most widely used phytotherapeutics. It has been shown to possess beneficial effects in the treatment of cerebrovascular insufficiency and peripheral circulatory problems. In the consumer's interest, these brands should feature a certain quality and should be transparent in quality claims.

Objectives: The study was performed to assess the discrepancies in the composition of six different brands of *G. biloba* available in the market.

Materials and Methods: In this investigation, a variety of *G. biloba* products in the Malaysian market was studied with respect to pharmaceutical quality such as the quantity of bioactive contents and heavy metal analysis using atomic absorption spectroscopy (AAS). The quantification of the marker compounds of *G. biloba* was also performed using high performance liquid chromatography (HPLC).

Results: A variation in the percentage of total proteins, glycosaponins and polysaccharides were observed in all the tested brands. Heavy metal analysis indicated an acceptable amount of lead (Pb) present in all the tested brands. The result obtained was compared against the label claim on the products and the pharmaceutical quality of the brands was evaluated.

Conclusion: Most products displayed insufficient information on the label claims. The study revealed the inconsistency in the composition of six different brands of *G. biloba* products in the market.

DETERMINATION OF HEAVY METAL CONTENTS IN VARIOUS REGISTERED DIETARY SUPPLEMENTS AVAILABLE IN MALAYSIA BY ATOMIC ABSORPTION SPECTROMETRY

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Introduction: In Malaysia dietary supplements are rampantly consumed for general health and well-being and therefore, available in community pharmacies, health food stores, night markets, grocery stores and is even obtained via internet. However, a gap in the

level of Malaysian consumer's awareness regarding the toxicity of dietary supplements is a major issue which needs to be addressed by the analysis of their constituents.

Objectives: To evaluate and analyse the heavy metal contents such as lead (Pb), cadmium (Cd), arsenic (As) and mercury (Hg) in registered dietary supplements available in community pharmacies in Malaysia and registered with NPCB Malaysia.

Materials and Methods: This study was focused on *Eurycoma longifolia* (*Tongkat Ali*), glucosamine, *Allium sativum* (garlic) and *Zingiber officinale* (ginger) products. A total of 20 registered single ingredient dosage form dietary supplements from different brands were purchased from community pharmacies located in Pulau Pinang. The samples were then evaluated and subjected to analysis by atomic absorption spectrometry.

Results: The study showed that variable amounts of Pb have been quantified; 0.29–0.47 ppm (*Tongkat Ali*), 0.19–0.27 ppm (glucosamine), 0.26–0.74 ppm (ginger) and was absent in garlic. While for Cd, the amounts were 0.02–0.24 ppm (*Tongkat Ali*), 0.03–0.22 ppm (glucosamine), 0.01–0.38 ppm (garlic) and 0.06 ppm (ginger). No traces of As and Hg were found in all four product samples. The regression linear coefficients (r^2) for Pb, Cd, As and Hg standards were 0.9990, 0.9958, 0.9960 and 0.9989, respectively.

Conclusion: The amount of Pb, Cd, As and Hg in all the products were found within the limits as per the requirement stated by NPCB. Thus, in order to ensure the safety and quality of dietary supplements, the analysis of heavy metals must be carried out on a routine basis using simple and robust atomic absorption spectrometry (AAS) techniques.

A NEW XANTHONE FROM *GARCINIA NITIDA*

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Introduction: Plants from the genus *Garcinia* are widely distributed in Malaysia and are known to have special economic importance. The fruit of many *Garcinia* species such as *G. mangostana, G. xanthochymus* and *G. multiflora* are edible while *G. picrorhiza*, is used for its medicinal properties. Plants from the genus *Garcinia* have been reported to be rich in xanthones and triterpenoids. In our continuing interest on Malaysian *Garcinia* plants, we carried out detail chemical studies on the stem bark and root extracts of *G. nitida*.

Objectives: The present study extracted and isolated the chemical constituents of *G. nitida* and elucidated their structures using 1D and 2D NMR spectroscopy.

Materials and Methods: G. nitida was collected from the Semenggok mixed dipterocarp forest, Sarawak, Malaysia. The fine ground stem bark and roots of *G. nitida* were extracted with hexane, ethyl acetate and methanol. The dry crude extracts were purified by column chromatography using hexane, hexane/chloroform, chloroform/ethyl acetate and chloroform/methanol as eluting solvents. The crude extracts were also purified by preparative thin layer and Sephadex LH-20 chromatography.

Results: One new xanthone, 1,6-dihydroxy-5-methoxy-6,6-dimethylpyrano[2′,3′:2,3]-xanthone (1) was isolated and identified. Besides that, 4 known xanthones, inophyllin B (2), 3-isomangostin (3) rubraxanthone (4), osajaxanthone (5) and one common triterpenoid, stigmasterol (6) were also identified. The structures of these compounds were established using mainly 1D and 2D NMR spectroscopy.

Conclusion: The present findings constituted additional evidence to the occurrence of xanthones in the genus *Garcinia*. Thus, the presence of xanthones, particularly compound 1 could be of chemotaxonomic significance to the genus *Garcinia* in particular, and also to the family Clusiaceae.

PROXIMATE, TOTAL PHENOLIC, TOTAL FLAVONOIDS AND ANTIOXIDANT CAPACITY OF BILIMBI (*AVERRHOA BILIMBI*) AND STAR FRUIT (*AVERRHOA CARAMBOLA*)

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Introduction: Bilimbi (AB) and star fruit (AC) are commonly consumed and widely cultivated in Southeast Asia. Traditionally, AB has been widely used for the treatment of cough and cold. AC is distinguishable from AB through its attractive star shape. It was reported to be a good source of antioxidant.

Objectives: To determine and compare proximate composition, antioxidant capacity, total phenolic content, total flavonoids and antioxidant vitamins in AB and AC.

Materials and Methods: Freeze-dried samples were analysed for moisture and ash contents by standard AOAC method, Clegg Anthrone method for total available carbohydrate, Kjeldahl method for total protein, Soxhlet method for total fat and total dietary fibre assay kit was used for total dietary fibre. Total phenolic content and total flavonoids were quantified by Folin-Ciocalteu and $AlCl_3$ method respectively. Antioxidant activity was

assessed with β-carotene bleaching and DPPH radical scavenging method whereas Vitamin A, C and E were evaluated using HPLC.

Results: AB was found to contain higher moisture, ash, carbohydrate, protein, fat and dietary fibre content compared to AC. Total phenolic content was higher in AC although AB yielded more total flavonoids. Besides, vitamin A, C and E contents of AB were also higher than AC. Antioxidant and scavenging activity of AC were significantly ($p < 0.05$) higher than AB. These results suggested that AC was a potent natural antioxidant food and that contribution of phenolic compounds to its antioxidant capacity was greater than that of antioxidant vitamins.

Conclusion: High antioxidant and scavenging activity were observed in AC although AB contained significantly higher antioxidant vitamins. The free radicals neutralising and scavenging effect of AC was attributed to its high total phenolic content. Thus, this study showed that AC was a potent natural antioxidant food.

1,3,5-TRIOXYGENATED XANTHONE DERIVATIVES: SYNTHESIS AND GROWTH INHIBITORY ACTIVITY ON MDA-MB-231 AND HELA CANCER CELL LINES

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Introduction: Prenylated xanthones are found to be chemically unique as this group of compounds was reported to exhibit a wide range of interesting biological activities. Synthetic approaches are rationally developed to provide a rapid and efficient access to various prenylated xanthone derivatives for biological activities studies.

Objectives: To synthesise a series of prenylated xanthones and to evaluate the cytotoxic activities of the synthetic xanthones towards MDA-MB-231 and HeLa cancer cell lines.

Materials and Methods: Prenylated xanthones were obtained from reaction of 1,3,5-trihydroxyxanthone with prenyl bromide in alkaline medium. Cytotoxic activities of synthetic xanthones were investigated towards MDA-MB-231 and HeLa cancer cell lines.

Results: A total of 6 prenylated xanthones were synthesised from the xanthonic building block 1,3,5-trihydroxyxanthone, namely 1,3,5-trihydroxy-2,4,7-tris(3-methylbut-2-enyl)xanthone (1), 1,3,5-trihydroxy-2,4-bis(3-methylbut-2-enyl)xanthone (2), 1,5-dihydroxy-2,4-bis(3-methylbut-2-enyl)-3-(3-methylbut-2-enyloxy)xanthone (3), 1-hydroxy-

3,5-bis(3-methylbut-2-enyloxy)xanthone (4), 1,3,5-trihydroxy-4-(3-methyl-but-2-enyl)xanthone (5) and 1,3,5-trihydroxy-2-(3-methylbut-2-enyl)xanthone (6). Compound 1 was found to give the highest inhibitory activity (IC_{50} = 24.6 μM) towards MDA-MB-231 cell line. Meanwhile, HeLa cell line was found most susceptible to compound 6 (IC_{50} = 22.0 μM). Stucture-activity relationship (SAR) study revealed the importance of free hydroxyl group in the xanthonic nucleus to elicit inhibitory activity towards the two cancer cell lines. Prenylation by substitution to the aromatic protons at position 2 and 7 in the xanthone nucleus was found to increase significantly the cytotoxic activities of the derivatives in comparison with the xanthonic building block. However, prenylation by etherification to the free hydroxyl group at position 3 and 5 had led to the total loss of cytotoxic activity as indicated by compounds 3 and 4.

Conclusion: This study showed that the presence of free hydroxyl groups in xanthonic nucleus was important to evoke significant inhibitory activity towards MDA-MB-231 and HeLa cancer cell lines, and the cytotoxic activity was found to be further enhanced with the presence of prenyl groups at position 2 and 7 in the xanthone nucleus.

FACTOR AFFECTING TEXTURE PROFILES OF *MAK-MAO* HAIR CONDITIONER

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Introduction: Mak-mao (*Antedesma valutinosum* Blume.) is an indigenous berry fruit of Thailand which mainly contains anthocyanin. Anthocyanin has been reported of having antioxidant activity and hair growth stimulating effect. Preliminary screening of *Mak-mao* conditioner formulations found that conditioning effect need to be improved.

Objectives: The aim of this study was to investigate factors affecting the texture profiles of hair conditioning creams containing *Mak-mao* extract.

Materials and Methods: Response surface analysis carried out by mixture design with 3 factors which were lexamine S-13, silicone and Abil®Quat was investigated. The texture of each formulation was measured using texture analyser (TA.XT Plus).

Results: In this study the formula containing lexamine S-13, silicone and Abil®Quat in the ratio of 1:1:1 exhibited the highest hardness, compressibility, adhesiveness and cohesiveness. Among the texture parameter values, adhesiveness appeared to be correlated with pH of the samples (r = .433) while compressibility and cohesiveness were significantly correlated with viscosity of samples with Pearson correlation of .541 and .629, respectively. Analysis of variance for mixture model revealed that there was no significant interaction among the factors.

Conclusion: Adhesiveness of conditioner texture might explain the conditioning efficiency of product to hair shaft. Apart from texture profiles, the ratio among the factors may also affect hair manageability as well as prevention of hair damage. Therefore, sensory evaluation of these conditioners on hair tresses would need to be carried out in future studies.

FORMULATION DEVELOPMENT OF GOAT'S MILK SHAMPOO

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Introduction: Current shampoo formulations are designed to not only remove surface grease and dirt from the hair shaft and scalp but also improve hair and scalp quality and also hair manageability. Goat's milk is likely to enhance hair care and avoid damaging hair due to skin's and hair's substantivity and hydrating properties. However, goat's milk may affect the foaming characteristics of a shampoo which plays an important role in its acceptability.

Objectives: To investigate factors affecting foaming properties of goat's milk shampoo formulations in comparison with a benchmark product.

Materials and Methods: In this study, 4 factors with 2 levels of fractional factorial design of goat's milk, Gafquat, Ultrasil DW-18, and Emal 207 were used primarily for screening. Foam properties which were flash foam and foam drainage in tap and hard water were investigated using the Ross miles method with some modification.

Results: Of the 15 formula tested, sample of standard order 7 which was a plain shampoo base with a high level of Emal 207 exhibited the highest volume of flash foam and foam drainage in hard and tap water. All of the samples possessed less flash foam and foam drainage compared to the benchmark product. Hard water did not significantly reduce the volume of flash foam. Adding Gafquat and Ultrasil DW18 into the shampoo base with low level of Emal 207 significantly reduced foam stability. All factors did not significantly affect flash foam in hard water but Ultrasil DW-18 significantly increased flash foam in tap water but significantly increased foam stability in hard water. Moreover, interaction between Gafquat and milk significantly increased foam stability in hard water whereas interaction between Gafquat and silicone significantly reduced foam stability in hard water. However, interaction between milk and Gafquat, and Ultrasil DW18 and Emal 207 significantly increased foam stability in tap water.

Conclusion: Goat's milk, Gafquat and Emal 207 alone did not significantly affect flash foam and foam stability in hard and tap water. However, adding Ultrasil DW-18 in shampoo may significantly reduce foam stability in hard water.

FORMULATION OF GREEN TEA EXTRACT GEL

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Introduction: Green tea, *Camellia sinensis* L. (Theaceae) contains substances having antioxidant activity. The antioxidant effect was a result of phenolic compounds such as (-) epigallocatechin gallate, (-) epicatechin, (-) epigallocatechin, (-) epicatechin gallate, (-) gallocatechin gallate and catechin. The compounds provided photoprotection and reducted skin deterioration.

Objectives: The objective of this study was to develop a topical gel containing green tea extract.

Materials and Methods: Green tea leaf was extracted with methanol at 60°C and tested by Ferric reducing antioxidant power (FRAP) assay. Physical characteristics of the product were investigated including viscosity, pH, colour, and odour at accelerated condition by hot-cold cycles in comparison with a benchmark antiaging gel. Preference and acceptance of the products were also tested in 80 female volunteers.

Results: Effective concentration providing 50% FRAP activity (EC_{50}) of the extract was 1.30 mg/mL. The extract was incorporated in a gel base at the concentration of 0.13% w/w. Gel base consisted of Carbopol® 940, propylene glycol, triethanolamine, alcohol, methylparaben, propylparaben and deionised water. The pH and viscosity of both products slightly decreased while the colour and smell of the products did not change. For sensory evaluation, there was no statistically significant difference at 95% confidence interval between volunteers who overall liked the benchmark antiaging gel and the developed product. The overall preference from the majority of volunteers towards the developed product showed a score of 6.96 ± 1.16 on a 9-point hedonic scale of the acceptance test.

Conclusion: The present study showed that the developed gel having antioxidant activity seemed to be stable compared to the benchmark antiaging gel. However, it may be better to remove the green colour of the methanol extract so as to improve the appearance of the developed gel.

ALKALOIDS OF *XYLOPIA FERRUGINEA*

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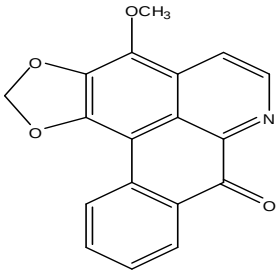
Introduction: Previous phytochemical study on the stem bark of *Xylopia ferruginea* (Annonaceae) has yielded two aporphinoid alkaloids namely liriodenine and atheroline.

Objectives: The objectives of this study are to isolate, purify and elucidate alkaloid constituents from the methanol extract from the stems bark of *X. ferruginea.*

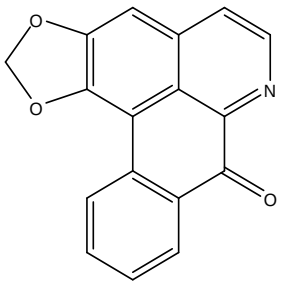
Materials and Methods: Acid-base extraction was carried out to yield a crude alkaloid extract. Fractionation and purification of alkaloid constituents were done using vacuum liquid, column, radial and preparative thin layer chromatography. Structural elucidations of the alkaloids were carried out using various spectroscopy techniques such as nuclear magnetic resonance, ultraviolet-visible, infrared, mass spectroscopy and comparison with available literature.

Results: Phytochemical investigation on the alkaloidal extract of *X. ferruginea* of the Annonaceae family has led to the isolation of three aporphinoid alkaloids and is identified as liriodenine, atherospermidine and lanuginosine.

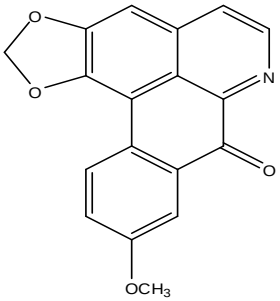
Conclusion: Plants from Annonanceae are known to contain a lot of alkaloids. The discovery of three alkaloids from *X. ferruginea* together with two previously reported have proven that this plant is rich with alkaloids.



Atheroline



Liriodenine



Lanuginosine

ANTIOXIDANT, ANTIBACTERIAL ACTIVITIES AND STRUCTURAL IDENTIFICATION OF α-VINIFERIN FROM DIPTEROCARPUS VERRUCOSUS (DIPTEROCARPACEAE)

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Introduction: Dipterocarpus verrucosus known locally as *keruing merah* is a species of tree in the family Dipterocarpaceae, endemic to Brunei, Indonesia (Kalimantan, Sumatra) and Malaysia (Peninsular Malaysia, Sabah, Sarawak) .

Objectives: The objectives of this study are to isolate, purify, characterise and evaluate the antioxidant and antibacterial activities of resveratrol oligomer from *D. verrocosus.*

Materials and Methods: The stems bark of *D. verrucosus* which were collected from Jengka, Pahang were extracted in acetone followed by methanol by cold immersion method. The acetone extract was fractionated by vacuum liquid chromatography and afforded six fractions (DVA-DVG). The structure of the compound was confirmed by ^{1}H and ^{13}C NMR analyses and comparison with previous data. The antioxidant activity was evaluated by DPPH, TPC, FTC and TBA methods. Meanwhile for antibacterial test, it was screened against *Escherihcia coli, Klebsiella pneumoniae*, *Bacillus subtilis, Staphylococcus aureus, Salmonella paratyphi* and *Pseudomonas aeruginosa* using disc diffusion method.

Results and Discussion: Further purification of the fraction DVC by column chromatography has led to the isolation of a resveratrol trimer, α-viniferin. The DPPH test indicated that α-viniferin showed no radical scavenging activity. In the TPC test, it gave 340 mg/g equivalent of gallic acid, while in FTC it displayed moderate inhibition (77.77%) when compared to vitamin E (95.63%). In the TBA test, α-viniferin displayed strong antioxidant activity as good as vitamin E with percent inhibition of 83.23% and 86.47%, respectively. α-viniferin showed moderate activity against 3 bacteria with inhibition zones of 17 mm against *E. coli,* 8.8 mm against *S. aurues* and 8.5 mm against *P. aeruginosa.*

Conclusion: α-viniferin, a resveratrol trimer from *D. verrucosus* indicated moderate activity towards antibacterial and antioxidant values. However this report is very important for the chemotaxonomy of Dipterocarpaceae family since no report was found on the chemical or biological study of this species.

ALKALOIDAL COMPOUNDS ISOLATED FROM THE LEAVES OF *BEILSCHMIEDIA KUNSTLERI* GAMBLE (LAURACEAE)

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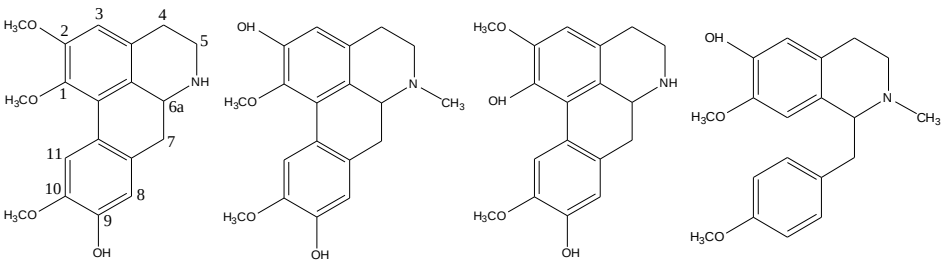
Introduction: The Lauraceae family contains about 55 genera and over 2000 species world-wide. In Malaysia, it contributies about 213 species, from 16 genera and is locally known as *medang* or *tejur*. *Beilschmiedia* is a genus of trees in the family Lauraceae. The genus *Beilschmiedia* comprises about 200 species, widely distributed in the intertropical region.

Objectives: This study was undertaken in order to isolate the alkaloidal compounds from *Beilschmiedia kunstleri* Gamble.

Materials and Methods: B. kunstleri was collected from Temau Sungai Tekam Reserve Forest. The dried and ground leaves of *B. kunstleri* was extracted first with hexane followed by dichloromethane. The dichloromethane extract was evaporated to 500 mL followed by an acid-base extraction to give the crude alkaloid. The alkaloids were separated using column chromatography over silica gel using CH_2Cl_2 gradually enriched with methanol as solvent. The structural elucidation of the alkaloid has been carried out using spectroscopic techniques such as UV, IR, MS , ^{1}H- and ^{13}C-NMR and 2D-NMR.

Results: The dichloromethane extracts of *B. kunstleri* gave three tetrahydroaporphines; laurotetanine (1), boldine (2) and norboldine (3), and one benzylisoquinoline, *N*-methylisococlaurine (4).

Conclusion: Chemical structural study on the leaves of this species has afforded various types of alkaloids such as tetrahydroaporphines and benzylisoquinolines. This is the first time these type of alkaloids were isolated from *B. kunstleri.* Further investigation on the isolation of other type of alkaloids and bioactivity study of the isolated alkaloids will be carried out.



1 2 3 4

A RESVERATROL TRIMER FROM *SHOREA MACROPTERA*

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Introduction: Shorea macroptera locally known as *meranti melantai* comes from the Dipterocarpacea family. Distribution of this species are in Peninsular Malaysia, Borneo and Sumatera.

Objectives: This study was undertaken to determine the chemical constituents from *S. macroptera* especially resveratrol oligomers.

Materials and Methods: Acetone extract of stem bark from *S. macroptera* was concentrated using rotary vacuum evaporator and afforded 250 g crude extract. The crude extract then was partitioned with diethyl ether and the diethyl ether extract was concentrated and subjected to vacuum liquid chromatography (VLC) and eluted with hexane-ethyl acetate. Seven fractions were obtained. Fraction 4 (8 g) was subjected to further VLC, and eluted with chloroform-methanol. Fraction 4-4 on repeated chromatographic purification using radial chromatographic method and eluting with chloroform-ethyl acetate-acetone, gave compound 1. The structure was determined using spectroscopic methods.

Results: A dark brown powder (136.5 mg) was obtained. The ^{1}H-NMR and ^{13}C-NMR data of the compound was compared with available literature and based on close similarity was determined to be Davidiol A, a resveratrol trimer.

Conclusion: Davidiol A, a resveratrol trimer was isolated from the stem bark of *S. macroptera*.

BIOACTIVE CONTENT ANALYSIS OF *EURYCOMA LONGIFOLIA* (*TONGKAT ALI*) PRODUCTS AND THEIR COMPARISON WITH UNFORMULATED STANDARDISED FREEZE DRIED EXTRACTS

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Introduction: Eurycoma longifolia or *Tongkat Ali* is a traditional Malaysian herb associated with medicinal benefits and well known as an energy booster and more importantly for aphrodisiac properties. More than 200 products in various pharmaceutical dosage forms containing *E. longifolia* either in a single or in combined preparations are available in the market.

Objectives: To analyse the bioactive content of *E. longifolia* (*Tongkat Ali*) registered products available in local pharmacies in or around Pulau Pinang were compared with unformulated standardised freeze dried extracts obtained from the industry.

Materials and Methods: Six different products containing *E. longifolia* root as a single herb were randomly chosen from the different pharmacies in Penang and standardised freeze dried extracts were obtained from industrial source. Eurycomanone marker was analysed by high performance liquid chromatography (HPLC) while total proteins, total polysaccharides, and glycosaponins were evaluated by gravimetric analysis.

Results: The study showed that Eurycomanone contents were in the range of 0.15–0.5% for market products while unformulated standardised freeze dried extracts were in the range of 0.53%–0.93%. Total proteins, total polysaccharides and glycosaponins evaluated by gravimetric analysis were in the range of 0.94%–6.26%, 1.78%–80.26%, and 4.17%–13.95% respectively in case of market products. While for unformulated standardised *E. longifolia* freeze dried extracts were 25.30%–29.49%, 37.87%–56.34% and 36.90%–45.25%, respectively.

Conclusion: There were vast variations observed between market products and standardised *E. longifolia* freeze dried samples. Lack of standardisation may be the reason for variation in market products.

MICROBIAL LIMIT TEST (MLT) FOR SELECTED HERBAL PRODUCTS IN THE COMMUNITY PHARMACY

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Introduction: The use of the herbal supplements is expanding rapidly and can easily be found in pharmacies around the world. As the demand for herbal supplements is increasing, therefore, consumer's safety should be the prime concern regarding microbial contamination.

Objectives: To perform the MLT for microbial contamination in various types of herbal supplements marketed in the community pharmacies around Pulau Pinang, Malaysia.

Materials and Methods: The study involves five different herbal supplements, *Eurycoma longifolia* (*Tongkat Ali*), *Echinacea purpurea*, *Ginkgo biloba*, *Centella asiatica* (*pegaga*) and *Labisia pumila* (*Kacip Fatimah*). The samples were subjected to the microbial limit test as per the United States Pharmacopoeia method.

Results: The results from all samples showed growth after 24 hours of incubation for the preparatory testing. For the total aerobic microbial count, results showed either not

detected or less than 10 CFU/mL (<10) except for M-Herbs, Gold Box (*Tongkat Ali*), and Orifera (*Kacip Fatima*) which showed contamination lower than 1100 CFU/mL. The microorganism detected was identified as *Bacillus sp.* through gram staining. For the total combined mold and yeast, all samples showed results of <10 CFU/mL and negative results for *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Salmonella sp* and *Escherichia coli.*

Conclusion: The study revealed the microbial safety aspect of the herbal supplements. Overall, there is no serious microbial contamination in all herbal supplements and the contamination is still within the acceptable limit.

A COMPARATIVE STUDY ON THE BIOACTIVE AND HEAVY METAL CONTENT OF SIX DIFFERENT BRANDS OF *GINKGO BILOBA* AVAILABLE IN THE MARKET

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Introduction: Ginkgo biloba-containing brands are one of the top 10 commercial products within the growing market for herbal remedies. Extracts from the leaves of *G. biloba* are among the most widely used phytotherapeutics. It has been shown to possess beneficial effects in the treatment of cerebrovascular insufficiency and peripheral circulatory problems. In the consumer's interest, these brands should feature a certain quality and should be transparent in quality claims.

Objectives: To assess the bioactive and heavy metals contents of six different brands of *G. biloba* available in the market.

Materials and Methods: Six samples of *G. biloba* products in the Malaysian market were analysed for the percentage of bioactive content using established gravimetric methods. Heavy metals content such as cadmium (Cd), mercury (Hg), lead (Pb) and arsenic (As) contents were also determined using atomic absorption spectroscopy (AAS).

Results: A variation in the percentage of total proteins ranging from 6%–21% was obtained for the analysed samples; while the content of total glycosaponins and polysaccharide in all the tested brands differed by about 9%–75% and 0.4%–69%, respectively. Heavy metal analysis indicated an acceptable amount of Pb present in all the tested brands, which is ranging from 0.09–0.13 ppm. On the other hand, no detectable amount of Cd, Hg and As were found in most of the samples. The results obtained for each brand was compared and the pharmaceutical quality of the product was evaluated.

Conclusion: Based on the gravimetric and heavy metal analyses, the study reveals the inconsistency of the bioactive and heavy metal contents of six different brands of *G. biloba* products in the market.

PHYTOCHEMISTRY AND BIOLOGICAL ACTIVITIES OF *PIPER VESTINUM*

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Introduction: Piper spp. are commonly used in treating various ailments and also as natural insecticides. Many species have been shown to possess antimicrobial, antifungal, antioxidant, insecticidal, allelopathic and antitumour activities. *Piper vestinum* is used by local communities in Sarawak to treat diarrhoea, gastric ailments, skin disease, paralytic and arthritic disorders and also as natural insecticides.

Objectives: This study was undertaken to investigate the chemical constituents and also to evaluate the biological activities of the crude extracts, essential oils and purified compounds of *P. vestinum*.

Materials and Methods: P. vestinum was collected from Limbang, Sarawak. The fresh samples were used for essential oils extraction. The oil was extracted using hydrodistillation methods. The dried leaves and stems were extracted separately using methanol. The methanol crude extract was concentrated and partitioned using solvent of increasing polarity. The purification process was carried out using column and preparative thin layer chromatography techniques. The structures of the purified compounds were elucidated based on spectroscopic data and comparison to published information.

Results: The essential oils of *P. vestinum* were rich in caryophyllene, myrcene, linalool and eugenol. Seven pure compounds identified as isoasarone, 2-(4'-methoxyphenyl)-3-methyl-5-propenylbenzofuran, 2,3-dihydro-2-(4-hydrophenyl)-3-methyl-5-propenylbenzofuran, methyl 3-geranyl-4-hydroxybenzoate**,** and 3,4-dihydroxyallylbenzene**,** piperine and pipernonaline were isolated from *P. vestinum*. The pure compounds especially isoasarone showed strong biological activity against *Aedes aegypti* and pseudogates of termites *Schedorhinotermes sarawakensis*. All other compounds including the essential oils also showed antibacterial and antifungal activities against several selected microorganisms.

Conclusion: Secondary metabolites of *P. vestinum* have been characterised. The isolated compounds and essential oils showed interesting biological activities and can be further studied in order to develop natural insecticides.

ANTIOXIDANT ACTIVITIES AND PHENOLIC CONTENTS OF *OENANTHE JAVANICA* (BLUME) DC. (WATER DROPWORT) IN DIFFERENT TEA PROCESSING METHODS

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Introduction: Oenanthe javanica (O.J) is reputed to have various medicinal properties, including hepatoprotective, antidiabetic, antimutagenic, antioxidant, neuroprotective, alcohol detoxication and antifibrotic activities. With a distinctive aroma and flavor, this perennial herb has been subjected to different tea processing methods to produce non-fermented/aerated green tea (GT), semi-fermented oolong tea (OT) and fermented black tea (BT).

Objectives: The main aim of this study is to investigate the antioxidant activity and total phenolic content of this herb after undergoing different processing and infusion in two solvent system (80% methanol and distilled water).

Materials and Methods: Antioxidant activity and total phenolic content were investigated by using DPPH and Folin-Ciocalteu's phenol assays.

Results: Preliminary screening results showed that the DPPH radical scavenging effects of all O.J hot water extracts were higher than 80% methanol extracts, supporting the traditional use of hot water in tea infusion. Among them, GT exhibited marked DPPH radical quenching effects with the lowest EC_{50} values in hot water extract (EC_{50} = 244.28 µg/mL) and 80% methanol extract (EC_{50} = 262.26 µg/mL) with high total phenolic content at 53.64 ± 0.71 µg GAE/mg extract and 84.46 ± 1.53 µg GAE/mg extract, respectively. Meanwhile OT and BT exhibited a moderate DPPH radical quenching ability in both hot water and 80% methanol extracts with EC_{50} ranging between 776–2145 µg/mL and total phenolic contents ranging between 17–81 µg GAE/mg extract.

Conclusion: These finding clearly reflected that the GT was the most efficient method to enhance the antioxidant capacity of O.J, by retarding the oxidation of polyphenol oxidase enzyme in O.J leaf.

FTIR AND MICROBIAL LIMIT TEST (MLT) ANALYSIS ON ENCAPSULATED EVENING PRIMROSE AND GARLIC OIL PRODUCTS

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Introduction: The usage of herbal products has expanded, including the sale of over the counter (OTC) dietary supplements. Evening primrose oil (EPO) capsule and garlic oil capsule are customary supplements used by consumers to maintain good health.

Objectives: To ensure authentication and safety of different EPO and garlic oil branded products by finger print profiling study with Fourier transform infrared spectroscopy (FTIR) and principal component analysis (PCA), and qualitative and quantitative estimations of specific viable microorganisms.

Materials and Methods: Six brands of EPO and six brands of garlic oil were randomly purchased from the USM pharmacy. All the products were profiled for fingerprints using FTIR and further processed using PCA. Microbial limit tests which include preparatory testing, total aerobic microbial count, total combined molds and yeast count and test for *Salmonella sp.*, *Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa* were carried out using the United States Pharmacopoeia (USP) method.

Results: FTIR spectroscopy coupled with principal component and discriminant analysis were able to authenticate and discriminate the various products. In the MLT, all products gave positive results in the preparatory tests which indicate that all 12 products do not inhibit the multiplication of microorganisms under test conditions. As for the total aerobic microbial count, no colony was found in all samples. Total combined molds and yeast count for all products are found to be <10 CFU/mL and all products were free from *Salmonella sp.*, *E. coli, S. aureus* and *P. aeruginosa*.

Conclusion: FTIR and PCA applied to a range of product samples revealed clustering according to product quality. Microorganism contaminations were either not found in the products or were within acceptable limits.

EFFECT OF EXTERNAL PHASE pH ON THE PHYSICAL PROPERTIES AND IN-VITRO RELEASE OF KETOPROFEN TOPICAL NANOEMULSION

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Introduction: Ketoprofen is a non-steroidal antiinflammatory drug and has been widely used for the treatment of rheumatoid arthritis and related diseases.

Objectives: The aim of this study was to formulate and characterise ketoprofen nanoemulsion for topical application in three different pH buffers as the external phase.

Materials and Methods: In the initial part of the experiment, pseudo-ternary phase diagrams with or without drug were constructed using the chosen oil (palm oil) and surfactant (Tween 80 and Span 20, HLB = 13.7) by water titration method to determine the emulsion area. Formulation containing 2.5% ketoprofen, 24% palm oil, 37% surfactant (Tween 80: 30% and Span 20: 7%) and 36% water was chosen from the emulsion area. In the next experiment, water was replaced with phosphate buffers to study the effect of the buffer's pH on the nanoemulsion droplet size, viscosity and transfer of drug through artificial cellulose membrane. Droplet size of the three preparations was measured using photon correlation spectroscopy. The viscosity was determined using a rheometer. The release rate was evaluated using Franz diffusion cells.

Results: The average droplet size for all the 3 nanoemulsions was in the range of 46–150 nm. The intrinsic viscosity of formulation with pH 7.4, 6.0 and 4.0 as external phase was 20, 19 and 17 Pa.s respectively. There was no significant effect of the external phase pH on the droplet size and viscosity of the nanoemulsions. However, the transfer of drug through the cellulose membrane was affected by the external phase pH of the nanoemulsions. Among the 3 formulations the one with phosphate buffer pH 7.4 produced the fastest transfer of drug through the membrane.

Conclusion: The external phase pH of the nanoemulsion affected the transfer of ketoprofen through cellulose membrane but not the viscosity and droplet size.

ANTIOXIDANT ACTIVITY OF PLANT EXTRACTS CONTAINING PHENOLIC COMPOUNDS

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Introduction: Phenolic compounds are commonly found in both edible and non edible plants, and they have been reported to have multiple biological effects, including antioxidant activity. Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials rich in phenolic compounds are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. Flavonoids and other phenolic compounds have been suggested to play a preventive role in the development of cancer and heart disease. Potential sources of antioxidant compounds are vegetables, fruits, leaves, oilseeds, cereal crops, barks and roots, spices and herbs and crude plant drugs.

Objectives: The aim of this study was to screen a large number of plant material extracts of Finnish origin with respect to their total phenolic content and antioxidant activity in order to find new potential sources of natural antioxidants.

Materials and Methods: The 92 different plant materials were either purchased from a market place or collected from nature. The materials included berries, fruits, vegetables, cereals, herbs, plant sprouts, and seeds as well as tree leaves and bark.

Results: On the basis of this study, the most potent Finnish plant sources for natural phenolic antioxidants are berries and apples, certain medicinal plants and vegetable peels, and different tree materials. Further work is under way to confirm the antioxidative effect of these promising plant extracts by using other types of lipid models and to characterise the active phenolic antioxidants.

Conclusion: The total phenolic content measured by the Folin-Ciocalteu's procedure does not give a full picture of the quantity or quality of the phenolic constituents in the extracts. Efficiency of antioxidants depends strongly on the oxidation conditions and lipid substrate.

PHARMACOGNOSICAL AND PHYSICOCHEMICAL CHARACTERISTICS OF MALAYSIAN *ALLIUM FISTULOSUM* BULB

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Introduction: Allium fistulosum (Liliaceae), also known as *kucai*, is used by the local people in Malaysia to treat cold and abdominal disorders as well as to drain pus from sores, boil and abscesses.

Objectives: This recent study aimed to generate various parameters of pharmacopoeial standards of this species such as macroscopic characteristics, loss on drying, total ash, extractive value and thin layer chromatography profiles.

Materials and Methods: Most of the methods were based on World Health Organization (WHO) guidelines for quality control methods for medicinal plant materials.

Results: Powdered leaves of the plant showed high moisture content which could be represented by loss on drying (20.63% ± 0.2). Water soluble extractive value (28.17% ± 4.2) of the plant was approximately three times higher than ethanol extractive value (9.9% ± 0.2). Determination of total ash value gave the result of 16.57% ± 0.0033. TLC of 3 different extracts which were hexane, chloroform and ethanolic extracts, using chloroform as mobile phase and silica gel as stationary phase showed 5–7 clear spots that were detected under daylight and UV light (254 and 365 nm).

Conclusion: The results of the study could be useful in setting some parameters for identification and preparation of a monograph of the plant.

SELDI-TOF PROFILING OF PROTEINS/PEPTIDES FROM THE LEAVES EXTRACTS OF *FICUS DELTOIDEA*

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Introduction: Ficus deltoidea is one of the well known herbs in Malay traditional medicine. Decoction of the plant has been used as a remedy to treat various ailments, especially in womb recovering after giving birth. It is known locally as *mas cotek*. Pharmacological studies of this plant suggested that the aqueous extract contains pharmacologically active constituents. In the present study, protein profile of *F. deltoidea* leaves aqueous extract from different variety was investigated using proteomic techniques.

Objectives: To profile *F. deltoidea* leaves aqueous extract using proteomics techniques.

Materials and Methods: Three varieties that were used and compared in this study were small type leaves (SL), medium type leaves (ML) and big type leaves (BL). Protein profile of the extracts was first resolved using SDS-PAGE, followed by visualisation using silver staining. Detailed analysis of the SDS gels shows that there were high and low molecular weights of proteins present in the plant extracts. However, low molecular weight proteins were not well resolved in SDS-PAGE. Therefore, profiling of *F. deltoidea* leaves extracts was divided into 2 parts which consists of protein profiling using 2-D gel electrophoresis separation for higher MW, and profiling of low MW proteins using SELDI-TOF. This report focused on the latter (less than 10 kDa), while the former will be discussed in a future report.

Results: EDM analysis of the 3 varieties showed a total of 14 clusters. Comparing across these 3 varieties, 5 peaks (m/z 7640, 7477, 5351, 4814, and 4232) showed significant difference in their intensities. The peak of m/z 7640 is prominent in ML and BL. The peaks of m/z 5351 and 4232 showed prominence in both SL and ML, whereas peak at m/z 4814 was prominent in ML. Peak at m/z 7478 which is present in all 3 varieties showed difference in intensity, where it is highly expressed in ML, followed by SL and BL.

Conclusion: These results suggested that the peak m/z 4814 might be a characteristic peak for ML. The observation of only 2 peaks (m/z 7477 and 7640) in the mass spectrum of BL differentiate itself from ML, while the presence of m/z 7477, 5351 and 4232 represent the profile for SL.

A STUDY ON THE CONTENT OF THREE DIFFERENT BRANDS OF REGISTERED *ECHINACEA PURPUREA* HERBAL SUPPLEMENTS MARKETED IN THE COMMUNITY PHARMACIES

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Introduction: Nowadays, herbal supplements are widely used as health food to increase stamina and energy and as preventive cures of different ailments. *Echinacea purpurea* herbal supplements have been used mainly to prevent common cold and flu. All herbal supplements marketed in the community pharmacy should fulfill the health food requirements such as the quality and safety to ensure safety for consumers as per label claim.

Objectives: To compare the quality and safety of different brands of *E. purpurea* products available in the local pharmacies.

Materials and Methods: Three brands of registered *E. purpurea* products were randomly chosen and the bioactive content such as total protein, total polysaccharide and glycosaponins contents were determined. Atomic absorption spectroscopy (AAS) were

used to analyse the heavy metals [cadmium (Cd), mercury (Hg), lead (Pb) and arsenic (As)] found in the products.

Results: The total polysaccharides, total proteins and glycosaponins were in the range of 0%–1.06%, 4.52%–15.44% and 5.28%–21.57%, respectively. Heavy metal contents by AAS showed that Cd, Hg, Pb and As levels in all the products were within the acceptable limits.

Conclusion: Various brands showed variations in the levels of bioactive compounds and heavy metal content, thus revealing the inconsistent quality and safety of the registered products.

COMPREHENSIVE STUDY ON ANTIOXIDANT PROPERTY OF *BETA VULGARIS* (BEETROOT)

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Introduction: Beta vulgaris has been commonly consumed and traditionally used for various medicinal purposes.

Objectives: The present study scientifically evaluates the antioxidant potential of the plant using various assays.

Materials and Methods: The root part of *B. vulgaris* were collected and extracted using methanol and then fractionated with hexane, ethyl acetate and water. In another extraction process, the fresh root of *B. vulgaris* is juiced and made into an extract. Antioxidant activity of the juice extract, crude methanolic and fractionated extracts (hexane, ethyl acetate and water) of the plants was evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay, reducing power assay, β-carotene bleaching assay, metal chelating assay, superoxide dismutase (SOD) activity assay and lipid peroxidation assay. The standards used were ascorbic acid and BHA. Isolation of compounds from ethyl acetate extract and juice extract was carried out using high performance liquid chromatography (HPLC) method.

Results and Discussion: The ethyl acetate extract exhibited the highest DPPH scavenging potential (IC_{50} = 0.310 mg/mL), followed by the methanol, juice extract, water and hexane extracts. For reducing power assay, the ethyl acetate fraction again showed the highest antioxidant activity with the highest potential of reduction in converting ferricyanide complex to the ferrous form. This was followed by BHA, water, methanol, hexane and juice extracts. In the *β*-carotene bleaching assay, the ethyl acetate extract again showed the highest antioxidant activity followed by BHA, juice extract, methanol, hexane and water extract. In the metal chelating assay, methanol extract showed the highest ability

(IC_{50} = 1.12 mg/mL) to chelate ferrous ions. This is followed by hexane, ethyl acetate and juice and water extract and the results were compared to EDTA. In the SOD activity assay, highest antioxidant activity was exhibited by the methanol extract, followed by BHA, ethyl acetate, water, juice and hexane extract. Finally, for lipid peroxidation assay, the inhibition rate of lipid peroxidation was highest for juice extract followed by hexane, methanol, water, BHA, ascorbic acid and ethyl acetate extract. Compounds isolated from the crude extracts had distinct activity in DPPH assay with IC_{50} values of < 0.8 mg/mL.

Conclusion: Beetroot has high antioxidant potential as investigated by various assays. The beetroot exhibited even better antioxidant activities in comparison to the standards (BHA) used in the reducing power assay, β-carotene bleaching assay and the SOD assay. Further research on isolation and identification of compounds are ongoing.

STABILITY STUDIES ON THE LEAF EXTRACTS OF *FICUS DELTOIDEA* (JACK)

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Introduction: Stability is defined as the capacity of the product to remain constant within specifications established to ensure its identity, strength, quality and purity. International Conference of Harmonization (ICH) have issued guidelines for the purpose of stability testing to provide evidence on how the quality of drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light, and enables recommended storage conditions, retest periods and shelf lives to be established.

Objectives: To investigate the stability of the extracts of three types of *Ficus deltoidea* (FD) leaf by accelerated stability studies.

Materials and Methods: The stability of the reference markers (vitexin and isovitexin) in methanol and water extracts of three types of FD leaf labeled as FDLNA, FDLSPLS (FD var. *terengganuensis*), FDLTKS (FD var. *angustifolia*) and FDLNHT (FD var. *deltoidea*) was evaluated using FTIR and HPLC. These extracts were stored at 4 different temperature and relative humidity (RH) (30 ± 2°C/65 ± 5% RH, 40 ± 2°C/75 ± 5% RH, 50 ± 2°C/85 ± 5% RH and 60 ± 2°C/85 ± 5% RH) for over 6 months. The shelf life and the recommended storage conditions of the extracts were established.

Results: Data analysis showed decrease in concentration and shelf life of the reference markers with the increase of storage temperature. The degradation of the reference marker followed the first-order reaction that indicated logarithmic transformation of the drug characteristic following a linear function of time.

Conclusion: Results of the present study suggested that FD herbal leaf extracts preparation should be stored at temperature 25 ± 2°C/65 ± 5% RH. From the study, the highest estimated shelf life of vitexin was 1.51 months for FDLNHT methanol extracts, whereas the highest shelf life for isovitexin was estimated as 14.07 months for FDLSPLS methanol extracts.

PRECLINICAL INCLUDING BIOLOGICAL ACTIVITY AND CLINICAL TRIALS OF NATURAL PRODUCTS

HYPERLIPIDAEMIA PREVALENCE AMONG HIV-INFECTED PATIENTS RECEIVING HAART IN PENANG HOSPITAL

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Introduction: HAART has dramatically improved the prognosis of people with HIV infection and increased patients' quality of life. However it is frequently associated with metabolic syndromes such as hyperlipidaemia, which can lead to cardiovascular diseases and hence increase the mortality rate.

Objectives: A cross-sectional study had been conducted in Penang Hospital to evaluate the hyperlipidaemia prevalence in different groups of HAART and gender.

Materials and Methods: From January to June 2010, all patients on HAART treatments (NNRTI-based, PI-based and PI+NNRTI) which have been initiated since 1995 to 2010 and have not obtained fasting plasma lipid test were required to perform one fasting plasma lipid test.

Results: A total of 232 patients stable on HAART for a mean period of 1.8 ± 2.1 years (range 0.2–12.0) were enrolled into this study. The majority of them were Chinese male (54%) aged ≥ 40 years. Higher HDL ($p < 0.01$) was seen in NNRTI based HAART ($p < 0.01$) while triglycerides seemed to be high in PI and PI+NNRTI based HAART ($p < 0.01$). This finding was in line with previous studies. LDL and total cholesterol level was similar between both genders but triglycerides and HDL cholesterol were significantly higher in male ($p < 0.05$) and female ($p < 0.05$) respectively.

Conclusion: NNRTI based HAART could remain as a first line therapy in treating HIV-1 infected patients in Malaysia while male patients on HAART should always be advised on healthy lifestyle practices such as low fat diet intake and smoking cessation in order to obtain a good lipid control and therefore preventing them from getting cardiovascular diseases.

CLINICAL PRELIMINARY EVIDENCE OF THE EFFICACY OF A MOUTHWASH CONTAINING 5% PROPOLIS FOR THE CONTROL OF PLAQUE AND GINGIVITIS: PHASE II STUDY

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Objectives: To obtain preliminary evidence about the efficacy of an alcohol-free mouthwash containing 5% green propolis (MGP 5%) on plaque and gingivitis control.

Methods: A clinical trial phase II study with 25 subjects who had a minimum of 20 sound, natural teeth; a mean plaque index (PI) of at least 1.5; a mean gingival index (GI) of at least 1.0. They were instructed to rinse with 10 mL of their assigned mouthwash for a minute, immediately after brushing in the morning and at night. Participants were required not to use other mouthwashes throughout the study.

Results: The PI and GI were significantly reduced when compared to the first examination, reduced at 24% and 40% respectively ($p < 0.05$). The changes found in hard and soft oral tissue were not different from the currently used mouthwashes.

Conclusion: The MGP 5% showed evidence of its efficacy in reducing PI and GI, with the necessity of doing a double-blind randomised clinical trial to validate such effectiveness.

EFFECT OF *TABERNAEMONTANA DIVARICATA* (CREPE JASMINE) FLOWER METHANOLIC EXTRACT ON PYLORUS LIGATED RATS

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Introduction: Tabernaemontana divaricata Linn. belonging to the Apocynaceae family is traditionally used by people in many parts of the world to treat various disorders like abdominal tumours, arthralgia, asthma, diarrhoea, epilepsy, eye infections, fever, fractures, headache, inflammation, leprosy, mania, oedema, paralysis, piles, rabies, rheumatic pain, skin diseases, ulceration and vomiting. It is also used as antihelmintic, antihypertensive, aphrodisiac, diuretic, emmenagogue, hair growth promoter, purgative, remedy against poisons and tonic for brain, liver and spleen. *T. divaricata* is rich in phytochemicals, contains at least 66 indole alkaloids, terpenoids, flavonoids, steroids, phenolic acids, phenyl propanoids, enzymes, etc.

Objectives: The study was undertaken to screen antiulcer potential of *T. divaricata* flower methanolic extract in rats.

Materials and Methods: The antiulcer property was evaluated using a physical method i.e., pylorus ligation induced gastric ulceration in albino Wistar rats. Rats were divided into 3 groups: group-1 (control) receiving distilled water orally, group-2 (standard) treated with omeprazole (8 mg/kg, p.o) and group-3 (test) treated with *T. divaricata* flower methanolic extract (500 mg/kg, p.o.) with 6 animal per group. Five parameters namely pH, volume of gastric juice, free and total acidity and ulcer score were determined.

Results: The methanolic extract of *T. divaricata* flowers significantly decreased volume of gastric juice, free and total acidity and ulcer index. An increase in pH and gastric protection (79.53%) was observed when compared to the control. The gastric protection for omeprazole was found to be 89.84%. This suggests that *T. divaricata* possess remarkable antiulcer effect.

Conclusion: The methanolic extract of *T. divaricata* flowers exhibited significant antiulcer activity at a dose of 500 mg/kg. The effect could be due to the phytoconstituents like alkaloids, flavonoids, terpenoids and phenolic compounds present in it. Some of the above mentioned groups of chemical constituents are reported to have gastroprotective effects in animal studies. Further studies are required to confirm antiulcer property of this medicinal plant. The underlying mechanism and responsible chemical constituents are to be elucidated.

INHIBITION OF HCL INDUCED GASTRIC ULCER IN RATS PRE-TREATED WITH *FICUS DELTOIDEA* ETHANOL EXTRACT

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Introduction: Mas cotek or *Ficus deltoidea* (FD) (family: Moraceae) is known in the Malay community for its medicinal value. Observation on practitioners with continuing use of *mas cotek* showed it could help in the treatment of diseases such as hypertension, diabetes, pneumonia and also shrink the uterus and ease blood circulation for women recently given birth. Several studies have been carried out to evaluate the properties of *F. deltoidea* such as antidiabetic, antinociceptive, and antiinflammatory by using various extracts.

Objectives: This study was conducted to evaluate gastric antiulcer effect of FD ethanol extract on rats that have been induced with 0.6M HCl to produce ulcers.

Materials and Methods: Six groups consisting of six rats with each group given different treatments for seven consecutive days. These groups consist of: I = positive control (omeprazole), II = negative control (1% Tween 80), III = 50 mg/kg body weight FDEE, IV = 100 mg/kg FDEE, V = 200 mg/kg FDEE and VI = 400 mg/kg FDEE. Each treatment

was given orally for seven consecutive days. On the 7th day, 1 hour after the last treatment, they were given orally, 0.6M HCl to induce gastric ulcers. After 3 hours, each rat was sacrificed and the stomach was taken out. The surface area of the ulcer was measured**.** Data was analysed using one-way ANOVA, followed by Dunnett's Test. Results were considered significant if $p < 0.05$.

Results: Significant reduction of the surface area of the ulcers was observed in 200 mg/kg/body weight FDEE (82.68%) concentration followed by 400 mg/kg FDEE 76.44% while 100 mg/kg FDEE were 75.56% and 50 mg/kg FDEE were 55.11%. All inhibiting effects obtained were compared with omeprazole, 30 mg/kg (73.02%).

Conclusion: FD ethanol extracts has the property to reduce surface area of stomach ulcer lesion in rats.

THE EFFECT OF GOAT MILK LOTIONS EMULSIFIERS ON SKIN CHARACTERISTICS

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Introduction: Goat milk can be used as a source of natural moisturiser with emollient properties in cosmetic skin care due to the small size of fat globules and similarity of milk fat components in goat's milk to skin lipids. Lotion, an emulsion dosage form, is a popular skin care cosmetic. Its physical properties depend on the ingredients in the formula. Surfactants may play an important role on skin feel as well as skin characteristics.

Objectives: The aim of this study was to investigate the effect of emulsifiers on characteristics of skin nourishing lotion containing goat milk.

Materials and Methods: Goat milk lotions were formulated using 3 different types of emulsifier i.e. triethanolamine stearate, cetomacrogol 1000 and cocamidopropyl betaine. After the samples were applied on panel forearms, skin feel was evaluated using questionnaires. The skin pH, moisturisation and pigmentation were measured using skin pH meter, Corneometer and Mexameter MX 18 respectively.

Results: It was found that types of emulsifiers did not significantly affect skin feel. However, both triethanolamine stearate and cetomacrogol 1000 in the lotion base significantly increased skin pH. Both cetomacrogol 1000 and cocamidopropyl betaine possessed moisturising effect. Only cetomacrogol 1000 significantly increased skin moisturisation and decreased skin pigmentation. In addition, all formulations containing goat milk significantly improved skin moisturisation.

Conclusion: This study demonstrated that apart from the moisturising effect of goat milk alone, selection of emulsifiers plays an important role for skin characteristics particularly moisturising effect and skin pH.

PHYTOCHEMICAL INVESTIGATION OF BIOACTIVE CONSTITUENTS FROM THE EXTRACT OF *KACIP FATIMAH* (*LABISIA PUMILA)*

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Introduction: Kacip Fatimah (*Labisia pumila*) is a herb belonging to the family Myrsinaceae and is used by Malay women for induction and facilitation of childbirth in traditional folk medicine. Other traditional uses include for the treatment of dysentery, rheumatism and as an antiflatulence. This species has also been commonly used in the local herbal and pharmaceutical industries.

Objectives: The present study was conducted to isolate and characterise the compounds in the aqueous MeOH extract from the leaves of *L. pumila* and to investigate the cytotoxic activity of the extract and compounds against of MCF-7 breast cancer cells.

Materials and Methods: The dried leaves of *L. pumila* were grounded and extracted with hexane, $CHCl_3$ and EtOAc and then isolated and purified by silica gel, Sephadex LH-20 column and preparative RP C18 HPLC. Structural elucidation of the purified compounds was performed with ^{1}H and ^{13}C NMR, and mass spectrometry.

Results: The investigation of the hexane extract prepared from the leaves of *L. pumila* led to the isolation and identification of one new and three known alkylphenols. Five flavonoid glycosides and three phenolic compounds were isolated from the ethyl acetate extract, and their breast cancer cytotoxic activity were tested. The hexane extract and the two alkylphenols showed antitumor activity against MCF-7.

Conclusion: One new compound with 11 known compounds were isolated from the leaves of *L. pumila.* The hexane extract and two alkyl phenols possessed potential cytotoxic activities.

GROWTH INHIBITORY EFFECT OF *PIPER BETLE* EXTRACT ON ORAL *CANDIDA*

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Introduction: Piper betle is a tropical plant belonging to the pepper family. The leaves of *P. betle* have been reported to possess antimicrobial properties, thus making it widely used as traditional remedy to treat infectious diseases including those occurring in the oral cavity.

Objectives: The study was undertaken to evaluate the antifungal activities of *P. betle* against oral *Candida* based on their growth profiles produced following treatment with the extract of *P. betle*.

Materials and Methods: Crude aqueous extract of *P. betle* leaves was prepared and stored at 4°C. Seven strains of oral *Candida* species used in the study were purchased from the American Type Culture Collection (ATCC), USA. The strains were *C. albicans* ATCC 14053, *C. dubliniensis* ATCC MYA-2975, *C. glabrata* ATCC 90030, *C. parapsilosis* ATCC 22019, *C. krusei* ATCC14243, *C. lusitaniae* ATCC64125 and *C. tropicalis* ATCC 13803. The growth of the *Candida* was monitored periodically and the changes in the growth turbidity were recorded. Growth curves of each of the strains were plotted and the generation time was determined. The readings obtained were compared to those obtained following treatment of the strains with the *P. betle* extract.

Results: At concentration of 12.5 mg/mL, which represents the minimal inhibitory concentration (MIC) value of *P. betle* extract, it was found that *P. betle* was able to highly suppress the growth of all oral *Candida* as indicated by the extended lag phase. These suggest fungistatic effect of the extract whereby cells become dormant for a period of time before being able to multiply. In addition, *P. betle*-treated *Candida* also exhibited reduction in the generation time which involves the time taken for the cells to double. *P. betle* extract has caused the growth generation time to reduce as much as 80.7% for *C. dubliniensis*, followed by *C. parapsilosis* (45.6%), *C. albicans* (35.8%), *C. glabrata* (30.1%), *C. tropicalis* (17.43%), *C. lusitaniae* (12.95%) and *C. krusei* (3.14%). In addition, the cells were only able to be in the log phase for a shorter period of time and ended with less population produced. The presence of *P. betle* aqueous extract is thought to possibly interfere with the normal biological functions and suppress the growth generation time of *Candida* species.

Conclusion: P. betle exhibited fungistatic activity towards oral *Candida*. The extended lag phase and reduced generation time indicates the ability of *P. betle* extract to interfere with the normal growth activities of the candidal cells that leads to the inhibition of growth.

CORRELATION BETWEEN ANTIOXIDANT ACTIVITY AND TYROSINASE INHIBITION OF CLOVE OIL

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Introduction: Clove oil is a plant extract which was reported of having an antioxidant activity via free radical scavenging and lipid peroxidation inhibition. According to melanogenesis process, tyrosinase activity and tyrosine oxidation can play an important role in producing melanin. However, whitening effect of clove oil might take place due to a correlation between tyrosinase inhibition and antioxidant activity.

Objectives: To examine the correlation among free radical scavenging activity, lipid peroxidation inhibition and mushroom tyrosinase inhibition of clove oil.

Materials and Methods: Two methods of antioxidant activity including DPPH test and TBARs assay were compared with mushroom tyrosinase inhibition. Correlation between antioxidant activity of the 2 methods and their regression analysis were performed using SPSS version 17.0.

Results: Free radical scavenging activity was significantly correlated with lipid peroxidation inhibition (r = 0.890) and tyrosinase inhibition (r = 0.693). Linear regression analysis revealed that lipid peroxidation inhibition was more correlated to tyrosinase inhibition than free radical scavenging activity. Lipid peroxidation inhibition could significantly predict tyrosinase inhibition of clove oil ($p < 0.001$) with approximately 72.7%.

Conclusion: Clove oil may not only play a role as an antiaging agent but also as a whitening agent via the antioxidant activity. Moreover, it also acts as a melanin inhibitor via tyrosinase enzyme inhibition. Lipid peroxidation inhibition may be used as a preliminary indicator for tyrosinase inhibition. Clinical study on antiaging and whitening activities of clove oil would be further investigated.

NITRIC OXIDE INHIBITION ACTIVITY OF SELECTED SOLANACEAE

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Introduction: Nitric oxide (NO) plays an important role in many physiological and pathological processes such as blood pressure and host defence mechanisms. However, overproduction of NO will lead to many disorders such as diabetes, atherosclerosis,

inflammatory diseases, and cancer. *Solanum melongena* (*terung telunjuk*) and *Solanum macrocarpon* (*terung bulat*) are used traditionally to treat inflammation.

Objectives: This study was taken to investigate the ability of *S. melongena* and *S. macrocarpon* to inhibit NO production.

Materials and Methods: S. melongena and S. macrocarpon were extracted in ethanol, hexane and ethyl acetate. The extracts were then evaluated using Griess Assay for the effects on NO production in lipolysaccharide (LPS) stimulated RAW 264.7 cells. Cell viability was evaluated using 3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide (MTT) assay to rule out cytotoxic effects of the extracts.

Results: The extracts were evaluated at 3.13 µg/mL, 6.25 µg/mL, 12.5 µg/mL, 25 µg/mL, 50 µg/mL, 100 µg/mL and 200 µg/mL. Results indicated that all extracts inhibited NO production in a dose-dependent manner. Viability of the cells treated with extracts at 200 µg/mL was found to be more than 60%. Hexane and ethyl acetate extract of *S. melongena* and *S. macrocarpon* showed moderate NO inhibition in LPS-stimulated cells, with IC_{50} values (concentration of drug required for 50% of NO inhibition) of 52.15 µg/mL, 47.05 µg/mL, 54.45 µg/mL and 44.78 µg/mL, respectively. Results also showed that ethanol extract of *S. melongena* and *S. macrocarpon* weakly inhibited NO production in LPS-stimulated cells with IC_{50} value of 86.78 µg/mL, and 111 µg/mL, respectively. LPS-stimulated RAW 264.7 cells not treated with any plant extracts served as negative controls.

Conclusion: S. melongena and *S. macrocarpon* may be potential sources of NO inhibitors and can be further developed as an antiinflammatory agent.

STRUCTURE-ACTIVITY RELATIONSHIP OF 9,10-ANTHRAQUINONE ANALOGUES AND ITS ANTIPLASMODIAL ACTIVITY

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Introduction: Anthraquinones isolated from the roots of *Rennellia elliptica* Korth. demonstrated interesting antiplasmodial activity. The activity however, varied depending on substitution pattern of the anthraquinone skeleton. This paper reports preliminary structure activity relationship of a series of 9,10-anthraquinones and its antiplasmodial activity.

Objectives: This study was conducted with the aim to determine the structure-activity relationship of anthraquinone analogues based on substitution patterns of anthraquinones isolated from *R. elliptica*.

Materials and Methods: The natural anthraquinones were isolated from the root extract of *R. elliptica*. The analogues were synthesised through Friedel-Craft reaction between phthalic anhydride and various benzene derivatives in the presence of eutectic mixture of aluminium chloride and sodium chloride. The antiplasmodial activity was determined through the inhibition of the compounds against *Plasmodium falciparum* (3D7) growth in vitro.

Results: Combination of methyl and hydroxyl substituents at different positions on the anthraquinone skeleton caused strong antiplasmodial activities. The *ortho*-arranged substituents at 2, 3 positions exhibited strongest activity with IC_{50} value of 0.08 µg/mL followed by the compound with subtituents at 1, 2 positions. The *para*-arranged (1, 4) and *meta*-arranged (1, 3) substituted antharquinones showed less potent activity. On the other hand, the presence of additional methyl substituent at C-6 or C-7, reverse the order of activity with the strongest inhibition shown by the 1,3-disubstituted anthraquinones. Other combination of substituents such as formyl and hydroxyl, and methoxy and hydroxyl showed significant variation in antiplasmodial activity. The presence of substituents at 2, 3 position regardless of the type of substituents promotes antiplasmodial activities whereas the combination of three substituents at C-1 and C-2 at ring A and at C-6 or C-7 at ring C also promotes antiplasmodial activity.

Conclusion: Structural differences due to different substitution pattern affects antiplasmodial activity of 9,10-anthraquinones. 9,10-anthraquinones with substitutions at C-2 and C-3 at ring A promote antiplasmodial activities.

ANTIMICROBIAL ACTIVITY OF *DENDROBIUM CRUMENATUM* (PIGEON ORCHID)

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Introduction: Dendrobium crumenatum locally known as 'pigeon orchid' belongs to the family Orchidaceae. There are 74 species of *Dendrobium* plants found in China and about 30 species of them are used in traditional or folk medicine as antipyretic, in eye remedies, as an immunoregulator and also as an antiaging agent.

Objectives: The present study was undertaken to investigate the potential antimicrobial activity of methanolic extract of various parts of *D. crumenatum* (i.e; leaf, stem, root and pseudo bulb).

Materials and Methods: The antimicrobial activity was investigated using disc diffusion assay and microdilution test for determination of minimum inhibitory concentration (MIC), and minimal bactericidal/fungicidal concentration (MBC/MFC).

Results: The methanolic extracts of stem, root and pseudo bulb were found to exhibit comparable antimicrobial activity to that of the standard antibiotics against all tested microorganisms with inhibition zone in range of 7 to 10 mm except on *Bacillus cereus* and *Candida albicans*. The antimicrobial activity of leaf extract against all tested microorganisms was generally low. Subsequent MIC determination found that the stem extract of *D. crumenatum* had good antimicrobial activity against *Staphylococcus aureus, Klebsiella pneumonia* and *Enterobacter aerogenes* with MIC values of 0.39 mg/mL, 0.195 mg/mL and 0.195 mg/mL, respectively. On the other hand, root and stem were found to be active against *Streptococcus pneumonia, Shigella dysentriae* and *Saccharomyces cerevisae* with MIC values of 0.78 mg/mL compared to 0.00312 mg/mL, 0.025 mg/mL and 0.0125 mg/mL for amoxcillin, chloramphenicol and kanamycin respectively. Subsequent evaluations showed that the stem and root had MBC/MFC values in the range of 0.78 mg/mL to 6.25 mg/mL on *S. aureus, E. aerogenes, K. pneumonia* and *S. cerevisae.*

Conclusion: The present study showed that *D. crumenatum* exhibited potential antimicrobial activity which could be due to the presence of alkaloid and flavanoid compounds.

WOUND HEALING EFFECT OF HARUAN AND FUSIDIC ACID AEROSOL FOR INCISION AND BURN WOUNDS

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Introduction: Aerosol containing haruan extract is a combination between a good dressing for dermal wounds and a source of nutrition to the wound site.

Objectives: The aim of this study is to investigate the effects of aerosol containing haruan extract and fusidic acid in the healing of incision and burn wounds.

Materials and Methods: Incision wounds were created by a 6 cm long full-thickness incision on the back of rats with a scalpel blade. Wounds were closed with equally spaced interrupted catguts stitch. Degree of healing was observed by determining the tensile strength at the 3rd, 6th, 9th and 12th day after wounding and treatment. Burn wounds were created using a metal rod (1.5 cm diameter) heated to 95 ± 3°C in boiling water and exposed for 10 sec to the back of rats. Wound sizes were determined using wound tracing method and percentages of wounds closure were calculated.

Results: The tensile strength of the healed wound by the group treated with formula E2 which contained Haruan extract, was higher than the formula base of aerosol and formula G1 which contained Haruan extract and fusidic acid on each day of observation. Group treated with formula E2 reached 82% wound closure on day-10 and 98% wound closure on day-15, whereas formula base of aerosol and formula G1 reached 82% and 98% closure only on day-12 and on day-18 respectively.

Conclusion: The aerosol formula containing haruan extract could promote the healing process. The addition of fusidic acid in aerosol interfered with the acceleration of healing which turned to delay the healing process.

HEPATOPROTECTIVE POTENTIAL OF HYDRO-ALCOHOLIC EXTRACT OF *MOMORDICA CYMBALARIA* HOOK. F. ON THIOACETAMIDE INDUCED HEPATIC DAMAGE IN RATS

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Introduction: Momordica cymabalaria Hook. F. (Cucurbitaceae) is found in the south Indian states of India as a weed. The different parts of the plant is used for blood, nervous, liver and internal urogenital disorders, piles, abortion, open boils and whooping cough. The phytochemical profile of the plant reveals the presence of alkoloids, tannins, aminoacids, proteins, phenols, Vitamin C, β-carotene and carbohydrates.

Objectives: The study was aimed at assessing the in vivo antioxidant and hepatoprotective activity of hydro-alcoholic extract of fruits of *M. cymabalaria* (HEMC) against thioacetamide (100 mg/kg, sc) induced hepatic damage in albino rats.

Methods: The in vivo antioxidant activity was determined by estimating the tissue levels of GSH and lipid peroxidation. The degree of hepatoprotection was assessed by estimating levels of biochemical markers like SGPT, SGOT, ALP, bilirubin (total and direct), cholesterol and HDL. 200, 400 and 600mg/kg were used to assess the protective property in thioacetamide model of hepatotoxicity in rats.

Results: The HEMC produced significant effect by decreasing the activity or level of serum enzymes, bilirubin, cholesterol, HDL and tissue lipid peroxidation, while it significantly increased the levels of tissue GSH in a dose dependent manner. The effects of extract were compared with standard silymarin at 100 mg/kg dose.

Conclusion: These results suggested that hydro-alcoholic extract of *M. cymabalaria* fruits possess hepatoprotective activity against thioacetamide induced hepatic damage and significant antioxidant activity in rats.

SCREENING FOR PHOTO-CYTOTOXIC AND CYTOTOXIC COMPOUNDS FROM MALAYSIAN PLANTS

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Objectives: Thirty one plant extracts from 15 different species were screened for in vitro photo-cytotoxicity and cytotoxicity to search for new photosensitisers and cytotoxic compounds against cancer.

Materials and Methods: All of the extracts were screened at 20 µg/mL using MTT cell viability test against human leukemia cell line HL60, with and without exposure to 9.6 J/cm^2 of a broad spectrum light.

Results: Eight of the extracts were photo-cytotoxic by virtue of being able to reduce the cell viability by at least two-fold in the irradiated plate compared to the unirradiated control. Additionally, 2 of the extracts were able to reduce the cell viability by more than 50% even when unexposed to light, and were therefore deemed cytotoxic. All of the eight photo-cytotoxic extracts were from the leaves of different species of plants, and were analysed by LC/MS to attain their UV-vis absorbance profiles and nominal masses. Comparison of the UV-vis data as well as their nominal masses with the known photosensitisers indicated that the extracts contain known photosensitisers which are based on the cyclic tetrapyrrole structure. On the other hand, the cytotoxic extracts were from the roots and branches of the same species, *Brucea javanica*. Fractions of the *Brucea* extracts were further subjected to cell viability test and LC/MS analysis to identify the cytotoxic compounds. Further detailed examination of the photo-cytotoxic and cytotoxic extracts will be necessary to identify novel compounds that may be exploited for cancer treatment.

EVALUATION OF TOXICITY AND TOTAL SERUM TESTOSTERONE LEVEL ON *DERRIS TRIFOLIATA* LEAF EXTRACTS

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Objectives: Studies were conducted to evaluate toxicity level of *Derris trifoliata* leaf extracts using *Artemia salina* and total serum testosterone level in male rats.

Materials and Methods: D. trifoliata leaves were taken from Sabah Borneo with voucher specimens SAN149216 for taxonomical identity. Dried leaves were extracted successively with 100% ethanol (ED), 50% ethanol:water (EWD) and water (WD). The crude extracts were subjected to brine shrimp *(A. salina)* lethality test (BSLT) for toxicity evaluation. The survivors' larvae were counted to determine the LC_{50} using the Spearment-Karber analysis. Determination of total testosterone was done using treated male rats for four days, after which blood samples were taken for analysis.

Results: Toxicity results showed that all of the extracts were non-toxic according to 'The Globally Harmonized System (GHS) for Hazard Classification and Communication'. The highest total serum testosterone level of 150.10 pg/mL was obtained using 1000 mg/kg WD, followed by 2000 mg/kg EWD, 1000 mg/kg EWD and 2000 mg/kg ED at 142.27 pg/mL, 135.73 pg/mL and 101.47 pg/mL, respectively. All the results above showed significant $p < 0.05$ value when compared to negative control (NC).

Conclusion: Based on the results, it was suggested that *D. trifoliata* leaf extracts have the potential to increase the total serum testosterone level. Studies on the aphrodisiac activity of these crude extracts will be carried out in the future.

ANTIINFLAMMATORY AND CYTOTOXICITY EVALUATION OF METHANOL EXTRACTS OF SOME MALAYSIAN MEDICINAL PLANTS

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Introduction: Medicinal plants are widely used traditionally, for the treatment and prevention of various diseases such as fever, pain, as insect repellent and also tonic.

Objectives: The aim of this study is to evaluate the antiinflammatory and cytotoxic activities of 16 methanol extracts from different parts of 7 plant species.

Materials and Methods: The antiinflammatory activity was measured using hyaluronidase, lipoxygenase and xanthine oxidase inhibitory assay while cytotoxic activity was tested on human liver embryonic (WRL-68) and African green monkey kidney (Vero) cell lines. Sulforhodamine (SRB) protein staining was used to measure the number of cell viability after 72h exposure to the various extracts.

Results: The leaf extract of *Homalomena sagittifolia* (*keladi kemoyang*) showed the highest inhibition effect in hyaluronidase inhibitory assay (92.48%) with a 50% inhibition of cell viability (IC_{50}) value of 112.19 and 95.86 µg/mL in kidney and liver cell lines, respectively. In lipoxygenase inhibitory assay, *Mesua ferrea* (*penaga lilin*) showed the highest level of inhibition with 86.13%. All extracts gave low percentages of inhibition except *Brucea javanica* (*lada pahit*) in xanthine oxidase inhibitory assay. However, in cytotoxicity test *B. javanica* and *M. ferrea* showed toxic effect with an IC_{50} value in the range of 2.94 to 38.44 µg/mL. All other extracts showed lower antiinflammatory activities with lower toxic effect in both cell lines. Three extracts did not exhibit any IC_{50} value even at the highest concentration tested (625 µg/mL).

Conclusion: This study showed that the leaf part of *H. sagittifolia* could be further explored for potential usage as remedies for inflammation treatment.

EXPERIMENTAL EVALUATION OF *HIBISCUS SYRIACUS* FLOWERS FOR WOUND HEALING ACTIVITY IN RATS

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Introduction: Wound poses problems in clinical practice and has extensively been investigated. Perhaps there is no subject in medicine that has more literature than does wound healing. *Hibiscus syriacus* L. (Malvaceae) is a well known plant in the Ayurvedic system of medicine. A decoction of the flowers is used as diuretic, for the treatment of eye and stomach disorders as well as for itchiness and other skin diseases.

Objectives: The aim of the present study was to investigate the in vitro wound healing activity of *H. syriacus* flower extract on different wound models in rats.

Methods: Ointment formulations containing 5% and 10% methanol extract of *H. syriacus* flowers (5% and 10% w/w extract in simple ointment base) were evaluated for wound healing potential in excision and incision wound models in rats.

Results: Both concentrations of the methanol extract ointment showed significant responses in both the wound types tested when compared with the control group. The effect produced by the extract ointment, in terms of wound contracting ability, wound closure time, regeneration of tissues at wound site, tensile strength of the wound and histopathological characteristics were comparable to those of a standard drug nitrofurazone ointment.

Conclusion: It is concluded that the *H. syriacus* flowers extract has wound healing properties and thereby justifies its use in folklore medicine in India.

THE EFFECT OF DAUN KESUM (*PERSICARIA MINOR*) ON LIPID PEROXIDATION AND ANTIOXIDANT STATUS IN MICE LIVER

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Introduction: Persicaria minor (also known as *Polygonum minus*, family Polygonaceae), also known as *daun kesum* by the Malays, is a Malaysian herb frequently used in cooking.

Studies have shown that *P. minor* has one of the highest phenolic content and antioxidant capacity amongst several local herbs. Therefore, *P. minor* supplementation might be useful in the prevention of diseases in which free radicals are implicated.

Objectives: The study was undertaken to investigate the effect of aqueous extract of *P. minor* on lipid peroxidation and the antioxidant status in mice liver.

Materials and Methods: Different doses of aqueous extract of the leaves of *P. minor* (100, 500 and 1000 mg/kg body weight) were administered orally to mice for 14 days. After 14 days, the mice were killed and their livers harvested. The level of hepatic lipid peroxidation was determined by measuring the amount of malondialdehyde (MDA), whereas the antioxidant status was determined by measuring the activities of superoxide dismutase (SOD), catalase (KAT), glutathione peroxidase (GPx) and glutathione reductase (GR) as well as the level of reduced glutathione (GSH).

Results: It was found that the level of MDA in mice treated with 100, 500 and 1000 mg/kg *P. minor* decreased significantly compared to mice in the control group. The activity of SOD increased significantly in mice treated with 100, 500 and 1000 mg/kg *P. minor* compared to the control group. There was no significant difference in the activities of KAT and GR as well as in the level of GSH in the livers of *P. minor* treated mice. The activity of GPx showed significant reduction in all *P. minor* treated groups.

Conclusion: The aqueous extract of *P. minor* might be able to protect mice livers against damage caused by oxidative stress mainly through the inhibition of the lipid peroxidation process.

ANTIOXIDANT AND CYTOTOXIC ACTIVITY OF MANGROVE PLANTS, *BARRINGTONIA CONOIDEA* AND *ELAEOCARPUS FLORIBUNDUS*

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Introduction: Barringtonia conoidea and *Elaeocarpus floribundus* are mangrove plants that belong to the family Lecythidaceae and Elaeocarpaceae, respectively. These two plants have been used as traditional medicine to treat inflamed gums, high blood pressure, eye inflammation and asthma. Our preliminary researches on these two plants have successfully isolated some of their triterpenoids constituents.

Objectives: Studies were carried out to investigate the antioxidant and cytotoxic activity of the crude extracts of stem bark and leaves of *B. conoidea* and *E. floribundus*.

Materials and Methods: The air-dried finely ground samples were extracted by the conventional soaking method using several solvents with different polarity successively. The obtained crude extracts were then screened for antioxidant and cytotoxic activity using DPPH free radical scavenging method and MTT assay against leukemic cancer cell line (CEM-SS).

Results: The ethyl acetate extract of the leaves and methanol extract of stem bark of *E. floribundus* showed strong antioxidant activity with IC_{50} values of 0.097 and 1.922 µg/mL, respectively. The methanol extracts of leaves and stem bark of *B. conoidea* exhibited moderate antioxidant activity with IC_{50} values of 97.95 and 87.52 µg/mL, respectively. As for cytotoxic activity, the chloroform extract of the leaves of *E. floribundus* showed the strongest cytotoxic activity with IC_{50} of 2.556 µg/mL. Meanwhile, the extracts of *B. conoidea* exhibited moderate activity with IC_{50} values of > 50 µg/mL.

Conclusion: The extracts of *E. floribundus* which showed potent antioxidant property has a potent cytotoxic activity, whereas the extracts of *B. conoidea* which exhibited moderate antioxidant property has a moderate cytotoxic activity.

CYTOTOXIC ACTIVITY OF *AGLAIA OLIGOPHYLLA* ISOLATES TOWARDS LEUKEMIC CANCER CELL LINES (CEM-SS)

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Introduction: Aglaia oligophylla (Meliaceae) is a large tree growing up to 25 m high. The genus *Aglaia* itself has been pronounced to have strong insecticidal activity and previous studies also revealed its strong cytotoxic activity. Rocaglamide, a bioactive component that can be found in most *Aglaia* species has been proven to have strong anticancer and insecticidal activities. Species of *A. oligophylla* was comparatively less studied and few publications are available.

Objectives: Study was conducted to investigate the cytotoxic activity of stem bark and trunk of *A. oligophylla* towards leukemic cancer cell lines (CEM-SS).

Materials and Methods: Several extracts and chemical constituents isolated from various chromatography techniques were tested for cytotoxicity activity towards CEM-SS cancer cell lines via MTT assays and their IC_{50} were calculated.

Results: All extracts from stem bark of *A. oligophylla* showed strong activity towards CEM-SS cell lines whereby methanol extract was prominently active with IC_{50} of 2.54 µg/mL, while petroleum ether extract, chloroform extract and ethyl acetate extract showed IC_{50} of 9.23 µg/mL, 10 µg/mL and 7.0 µg/mL respectively. Meanwhile, all extracts from trunk of

A. oligophylla showed no activity towards CEM-SS cell lines with IC_{50} > 30 µg/mL. Silvaglin A, a new compound to the species isolated from the trunk of *A. oligophylla* also showed no activity towards CEM-SS cell line with IC_{50} > 30 µg/mL.

Conclusion: All extracts of the stem bark of *A. oligophylla* showed strong activity towards CEM-SS cell line especially the methanol extract while all extracts from the trunk of *A. oligophylla* showed no activity.

CYTOTOXIC CONSTITUENTS FROM *CURCUMA MANGGA*

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Introduction: Curcuma mangga is locally known as *mango turmeric* or *mango ginger* and is commonly grown in Thailand, Peninsular Malaysia, Bengal, North Eastern India and Java. For medicinal purposes, the rhizomes are used as a stomachic and for chest pains, gastric ulcer, fever and general debility. It is also used in postpartum care, specifically to aid womb healing. Diarylheptanoids, diterpenes, phenylpropanoid and coumarin with anticancer, antiinflammatory and antioxidant properties have been reported from this species.

Objectives: The study was carried out to investigate the chemical constituents from rhizomes of *C. mangga* and examine the cytotoxic effects against human cancerous cell lines.

Materials and Methods: The plant material was collected from Johor, Malaysia and extracted with methanol. The crude extract was further partitioned to obtain hexane, chloroform and ethyl acetate-soluble fractions. Chromatographic work on the chloroform fraction resulted in the isolation of chemical constituents which were characterised spectroscopically. Cytotoxic testing was performed using MTT [3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay with HL-60, MCF-7 and HeLa cell lines.

Results: New labdane diterpenoid, 12,17-epoxy-3β,17-dihydroxylabda-13-en-16,15-olide has been isolated along with curcumin, demethoxycurcumin and β-sitosterol. The new compound exists as a pair of epimers. The chloroform extract, labdane diterpenoid and curcuminoids exhibited cytotoxic effect against all selected human cancer cell lines, with IC_{50} values < 21 µg/mL.

Conclusion: The present study showed that the cytotoxic properties demonstrated by the chloroform extract might be due to antagonist effects of the pure compounds since isolated constituents were more active in the assay.

ANTICHOLINESTERASE PROPERTIES OF *DYSOXYLUM ACUTANGULUM*

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Introduction: Dysoxylum is a genus comprising of about a dozen species, distributed from Southeast Asia to Australia, which is rich in compounds like alkaloids and terpenoids. We previously have reported the isolation of six new compounds with mild cytotoxicity. In our continuing studies on the plant, we recently have identified fractions and compounds of active antiacetylcholinesterase based on thin layer chromatography (TLC) bioautographic test. Acetylcholine (ACh) is one of the most important neurotransmitters in animal systems. Increasing the levels of ACh by inhibition of acetylcholinesterase (AChE), the enzyme responsible for its hydrolysis at the cholinergic synapses, serves as a strategy for the treatment of Alzheimer's disease (AD), senile dementia and Parkinson's disease.

Objectives: To identify the anticholinesterase inhibitor(s) from *Dysoxylum acutangulum*.

Materials and Methods: A simple and rapid bioautographic enzyme assay on TLC plate was used to screen for AChE inhibitors from *D. acutangulum* leaves extract. Ellman's method was adopted in determination of inhibition concentration at 50% (IC_{50}).

Results: TLC method gives quick access to information concerning both the activity and the localisation of the activity in complex plant matrices. Based on a bioautographic TLC profile, white colouration showed the presence of anticholinesterase activity on the major solvent fractions of *D. acutangulum*; leaves hexane fraction has shown a promising activity. Further fractionation on the hexane fraction resulted in the isolation of three active compounds.

Conclusion: The present study showed that *D. acutangulum* leaves hexane fraction contains AChE inhibitor(s).

ANGIOTENSIN-CONVERTING ENZYME (ACE) INHIBITOR FROM *FICUS DELTOIDEA* JACK FOR HYPERTENSION

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Introduction: Hypertension is one of the most important health problems in developed countries due to numerous deaths, secondary to cardiopathy, stroke and renal failure, produced by vascular complications. In the treatment of hypertension, inhibition of the angiotensin-converting enzyme (ACE) has been established. Inhibition of ACE is one of the effective screening methods in the search for new antihypertensive agents. Leaf of *Ficus deltoidea* (FD) Jack (Moraceae) have been reportedly used traditionally for treating diabetes, high blood pressure, heart problems, gout, diarrhoea, pneumonia and skin diseases. The decoction of leaves is also used by women after giving birth to improve blood circulation and regain body strength.

Objectives: To evaluate the antihypertensive potential of the leaf extracts of FD.

Materials and Methods: Methanol and water extracts of three types of leaf of FD labelled as FDLNA, FDLSPLS (FD var. *terengganuensis*), FDLTKS (FD var. *angustifolia*) and FDLNHT (FD var. *deltoidea*) were prepared. The extracts were then evaluated for their antihypertensive activity using the ACE inhibition assay. Captopril, a widely used synthetic ACE inhibitor was used as a standard.

Results: All extracts showed potential to inhibit the ACE activity. The water extracts exhibited the percentage inhibition ranging from 11.29 ± 1.17% to 45.37 ± 9.41%, whereas percentage inhibition of methanol extracts ranged from 51.60 ± 9.22% to 73.27 ± 1.10%. The percentage inhibition of methanol extracts was higher when compared to those of the water extracts. Among all extracts, the methanol extracts of FDLNHT showed the highest percentage inhibition of 73.27 ± 1.10%. Captopril showed percentage inhibition of 93.51 ± 6.75%.

Conclusion: The present study showed that FD leaf extracts affected ACE activity with high inhibition and were comparable to captopril, an ACE inhibitor used for the treatment of hypertension and some types of congestive heart failure.

ANTIOXIDANT ACTIVITY OF DICHLOROMETHANE EXTRACT OF *DRACAENA UMBRATICA* LEAVES

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Introduction: Dracaena umbratica is a type of shrub from the Dracaenaceae family which is known as *senjuang* in Malaysia. A decoction of the roots of this plant is used traditionally to treat rheumatism.

Materials and Methods: This plant sample was collected from the northern part of peninsular Malaysia. The crude extract of the leaves of this plant showed high activity in FTC assay but low activity in DPPH assay. The dichloromethane leaves extract of this plant was fractionated using vacuum liquid and column chromatography eluted with hexane with increasing ethyl acetate. The fractions eluted were pooled based on the similarities of their TLC profiles to afford six combined fractions. All fractions were then analysed for their antioxidant activity using FTC and DPPH assay.

Results: Fractions 2–6 showed high antioxidant activity when assayed by FTC while in DPPH assay, all the fractions exhibited low radical scavenging activity. Under long UV wavelength (366 nm), almost all the components appeared as red spot on TLC plate which indicated they are chlorophyll except for those in fraction 1. This result indicates that the antioxidant activity of the dichloromethane fractions in the FTC assay was primarily due to chlorophyll.

IDENTIFICATION OF SOME LOCAL HERBS IN INHIBITING *AEDES AEGYPTI* LARVAE GROWTH

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Introduction: Herbs/botanicals are simply any plant, tree or shrubs that are consumable by other living beings. Due to the toxicity and environmental impact a synthetic insecticide causes, insecticides of botanical origin may serve as suitable alternative for vector control in the future.

Objectives: The study was done to investigate the effectiveness of some local herbs in inhibiting the growth of *Aedes aegypti* larvae.

Materials and Methods: Aqueous solvent of fresh *Allium sativum, Curcuma domestica, Cinnamomum verum, Murraya koenigii* and *Ocimum basilicum* Linn were tested against *A. aegypti* larvae.

Results: The fastest 100% mortality of *A. aegypti* larvae was recorded by the aqueous solvent of *A. sativum* at the mean of 6 minutes followed by *O. basilicum* at 8 minutes, *C. verum* at 11 minutes, *M. koenigii* at 43 minutes and *C. domestica* at 65 minutes after exposure, while the control larvae were most likely to live until adulthood.

Conclusion: The study showed that *A. sativum* has significant larvacidal property that offer promises as a potential growth inhibiting agent of *A. aegypti* larvae, whereas, the remaining four botanicals have slower efficiency in getting the job done.

THE EFFECT(S) OF *FICUS DELTOIDEA* AQUEOUS EXTRACT ON SPONTANEOUSLY HYPERTENSIVE RATS DETERMINED BY SELDI-TOF SERUM PROTEIN PROFILING

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Introduction: *Ficus deltoidea* also known as *mas cotek* is a traditional medicine widely used in Malaysia to treat many diseases. Consumption of this plant is popular among the locals due to its reported benefits which have been scientifically researched. Epidemiologically, consumption of *F. deltoidea* has been suggested to control high blood pressure or hypertension. Our preliminary study showed that *F. deltoidea* extract promotes the in vitro inhibition of angiotensin converting enzyme (ACE) activity.

Objectives: Thus, the aim of this current study was to monitor the effect of *F. deltoidea* leaf aqueous extract on spontaneously hypertensive rats' (SHRs) serum protein profile.

Materials and Methods: SHRs were treated with the aqueous extract for two weeks and later sacrificed. Sera obtained were subjected to surface enhanced laser desorption/ionisation–time of flight (SELDI-TOF) to monitor changes in the low molecular weight serum protein profile.

Results: SELDI spectra obtained revealed that peak intensities of 16 protein mass/charge ratios (m/z) were significantly decreased in SHRs compared to normotensive rats. Administration of the leaf aqueous extract to SHRs affected 4 proteins in which m/z 8303.3, 4180.6 and 37294.0 showed a reversed expression pattern compared to SHRs while m/z 5059.7 demonstrated a continuous increase.

Conclusion: Identification of these proteins might lead to better understanding of the mechanism connecting *F. deltoidea* and hypertension.

EVALUATION OF CYTOTOXIC, APOPTOSIS AND ANTIOXIDANT PROPERTIES OF *PHYSALIS MINIMA* L. EXTRACTS

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Introduction: A combination of antioxidant- and apoptosis-based anticancer effects of natural compounds have become a promising approach in enhancing the efficacy of cancer remedy by neutralising chemotherapy/carcinogenesis-generated radicals as well as triggering a selective cell death mode.

Objectives: The purpose of this study was to systematically evaluate anticancer-related bioactivities elicited by *Physalis minima*.

Materials and Methods: Cytotoxic (methylene blue assay), apoptosis (TEM and DeadEnd™ assay) and antioxidant (FRAP and DPPH assays) analyses were performed using the extracts derived from different polarity of the solvent system.

Results: Cytotoxicity screening revealed that the chloroform extract has the strongest dose-dependent inhibitory effect on human breast carcinoma T-47D cells with the lowest EC_{50} at 3.80 µg/mL, followed by hexane (EC_{50} at 10.42 µg/mL) and ethyl acetate (EC_{50} at 25.76 µg/mL) extracts, however, a non-cytotoxic activity was shown in the methanol extract. With the remarkable cytotoxicity, the effect of the chloroform extract on T-47D cells was elucidated to be apoptotic in nature based on a clear indication of DNA fragmentation, which is a biochemical hallmark of apoptosis. Morphological observation has further supported this notion by displaying apoptotic characteristics in the treated cells, including clumping and margination of chromatins, followed by convolution of the nuclear and budding of the cells to produce membrane-bound apoptotic bodies. Meanwhile, only ethyl acetate and methanol extracts exerted the pronounced DPPH radical scavenging capacity (EC_{50} at 749.00 µg/mL and 258.40 µg/mL, respectively) and ferric reducing activities (46.10 mgTE/g and 42.54 mgTE/g extract, respectively), indicating that the synergistic effects of the polar constituents of these extracts may contribute to the antioxidant properties of this plant.

Conclusion: These results strongly proposed that the anticancer effect of this plant was mainly ascribed to the interrelation between cytotoxic, apoptotic and antioxidant activities of different polar phytochemicals in the plant extracts. This finding could provide a valuable insight for its application in cancer therapy and prevention.

IN VITRO CYTOTOXICITY ACTIVITY OF THE ESSENTIAL OILS OF *MYRISTICA FRAGRANS, MORINDA CITRIFOLIA* AND *CYMBOPOGAN CITRATUS* IN HUMAN COLON AND BREAST CARCINOMA CELL LINES

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Introduction: *Myristica fragrans* (nutmeg), *Morinda citrifolia* (noni) and *Cymbopogan citratus* (lemongrass) are widely distributed throughout tropical and subtropical regions. Their essential oils and volatile constituents are widely used as antioxidants, antidiabetic agents and for the prevention and treatment of different human diseases such as cancer, cardiovascular diseases (including atherosclerosis, thrombosis), bacterial and viral infections.

Objectives: The objective of the present study was to screen and compare the cytotoxic activity of the essential oils of these plants using two different cell lines, human colon carcinoma (HCT-116) and breast carcinoma cell lines (MCF-7).

Materials and Methods: The study was determined using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2,4-tetrazolium bromide] assay.

Results: The results showed that essential oil of lemongrass possessed most potent cytotoxic effect on HCT-116 and MCF-7 with the IC_{50} value of 27.41 µg/mL and 41.90 µg/mL respectively and followed by the nutmeg essential oil sample with IC_{50} values of 78.12 µg/mL and 69.45 µg/mL. The noni essential oil showed the least cytotoxic activity against both cell lines compared to two other essential oil samples. The IC_{50} values determined were 92.34 µg/mL for HCT-116 and 76.68 µg/mL for MCF-7.

Conclusion: Following the finding on promising cytotoxic activity of lemongrass essential oil against the HCT-116 and MCF-7 cell lines, further studies on apoptosis and bioactivity-guided fractionation are on-going to isolate and identify the most potent anticancer compound.

EVALUATION OF ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *DERRIS ELLIPTICA*

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Introduction: Fabaceae is the second largest family of dicotyledons and is rich in flavonoids. Flavonoids exhibit wide range of therapeutic applications. *Derris* is one of the largest genus of *Fabaceae. Derris elliptica* a woody climber with large red flowers belongs to this family. In Malaysia, it is found growing in the Rimba Ilmu Botanic garden up to the altitude of 3500 ft.

Objectives: To determine the antimicrobial activities of ellipticachalcone and lupinifolin, the active constituents isolated from *D. elliptica* and to determine the antioxidant activity of the whole extract.

Materials and Methods: The antimicrobial activity of the active constituents was determined by two fold serial dilution method. Furthermore the antioxidant activity for the leaf extract was performed by Fe/Ascorbate system. Inhibition of lipid peroxidation was determined by the thiobarbituric acid method at 1000 µg, 3000 µg, 4000 µg and 6500 µg concentrations of the extract and compared with ascorbic acid at the same concentrations.

Results: The minimum inhibitory concentration of the two isolates by 2 fold serial dilution method against *B. cereus, S. aureus, E. coli, P. vulgaris* was found to be 20.0 µg/mL, 8.2 µg/mL, 12.5 µg/mL, 25.0 µg/mL for ellipticachalcone and 20 µg/mL, 25 µg/mL, 35 µg/mL, 50 µg/mL for lupinifolin, respectively. The percentage of in vitro inhibition of lipid peroxide for the extract was found to be 7.39 ± 0.39, 22.35 ± 1.04, 31.52 ± 1.25 and 59.35 ± 1.48 and for ascorbic acid 8.64 ± 0.29, 13.41 ± 0.58 and 47.82 ± 0.91 at 1000, 3000, 4000, 6500 µg/mL concentrations respectively.

Conclusion: The extract showed significant antioxidant activity and the isolated active constituents showed significant antimicrobial activity.

CYTOTOXIC ACTIVITY OF *VINCA ROSEA* (APOCYNACEAE) STANDARDISED CRUDE EXTRACTS AND SELECTED ISOLATED COMPOUNDS AGAINST HUMAN COLON CARCINOMA CELL LINE

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Introduction: The search for better cytotoxic agents continues to be important in the discovery of modern anticancer drug. *Vinca rosea* (Apocynaceae), in Malaysia known as *kemuning cina* is well known for being rich in such agents.

Objectives: To investigate and evaluate *V. rosea* standardised crude extracts and selected isolated compounds for cytotoxic activity using MTT assay against human colon carcinoma cell line.

Materials and Methods: *V. rosea* was cultivated under controlled conditions at the Herbal Farm joint venture between USM-UNIMAP at Perlis, Malaysia. Fresh leaves were ground into fine powder and subjected to sequential extraction. Quantitative HPLC profiling was done using Agilent Technologies Series 1100 system using validated method. Four anticancer markers namely vindoline, vincristine, catharanthine and vinblastine were used to evaluate the standardised crude extracts. Cytotoxicity activity of crude extracts and isolated compounds were investigated using MTT assay against human colon carcinoma cell line (HCT 116).

Results: Extractive value of sequential extracts of n-hexane, chloroform, methanol and water were found to be 4%, 8%, 9% and 10%, respectively. n-hexane extract contains vindoline (1.38%), vincristine (0.003%) and catharanthine (0.46%). Chloroform extract contains vindoline (0.16%), vincristine (0.09%), catharanthine (0.22%) and vinblastine (0.06%). Methanol extract contains vindoline (0.004%), vincristine (0.003%), catharanthine (0.007%) and vinblastine (0.01%) while the water extract showed no significant markers. Chloroform, n-hexane and methanol fractions showed independent cytotoxic activity with chloroform fraction showing the highest activity whilst the water fraction showed minor cytotoxic activity. Catharanthine showed the most promising activity and a dose dependent cytotoxic activity of its IC_{50} value was found to be 60 µg/mL.

Conclusion: The preliminary study demonstrated that the chloroform fraction of *V. rosea* extract has the highest cytotoxic activity when screened against colon carcinoma cell line. Although this plant has been exhaustively studied, there are potential for new cytotoxic agents using standardised extracts.

CYTOTOXIC AND ANTIOXIDANT ACTIVITIES OF METHANOLIC EXTRACT OF *PITHECELLOBIUM JIRINGA* (JACK) PRAIN

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Introduction: Pithecellobium jiringa (Jack) Prain is a traditional medicinal plant belonging to the family Legumnasea originating from Southeast Asia. *P. jiringa* is locally known as *jering* in Malaysia, as well as *djengkol* in Indonesia. This plant is a kind of raw vegetable which is normally consumed with rice. *P. jiringa* is traditionally used to induce urination in which the seeds are crushed and mixed with water before drinking. Old folks have also been using its crushed leaves and bark for chest pain, skin ailments, gum pains and toothache. In treating wounds and cuts, the young leaves are burned and its ashes are applied onto the injured area. Breast cancer make up around 10% of all cancer occurrences among women worldwide while in Malaysia, breast cancer formed 31% of newly diagnosed cancer cases in Malaysian women. In protecting health as well as preventing cancer, the exploration of new antioxidants especially from plants has been on a rise.

Objectives: The present work is aimed to evaluate the cytotoxic property of the methanolic extract of *P. jiringa's* skin against an isogeneic human tumour cell line namely, MCF 7 derived from estrogen dependent human breast cancer cells and antioxidant property of the methanolic extract against DPPH free radical.

Materials and Methods: The cell proliferation assay was performed on MCF 7 human breast cancer cell line using tetrazolium (MTT) method. The antioxidant property was assessed using DPPH free radical assay.

Results: The methanolic extract exhibited significant cytotoxicity against MCF 7 cells with an IC_{50} of 26.87 µg/mL. The methanolic extract displayed potent DPPH free radical scavenging activity with an IC_{50} of 9.411 µg/mL.

Conclusion: The findings in the present study thus highlights the potent cytotoxic nature of *P. jiringa* against breast cancer as well as a new source of natural antioxidant.

MIXING HERBS IN A NEWLY FORMULATED POLYHERBAL REMEDY MAY INCREASE THE RISK OF ITS TOXICITY

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Introduction: Toxicity of newly formulated polyherbal products cannot be deduced from the information of the toxicity of each individual component of the polyherbal products. *Eurycoma longifolia* Jack is an example of a popular herb that is commonly mixed with other reputedly nontoxic herbs in a non-traditional (new) formulation.

Objectives: In this study, the effect of mixing herbs in a polyherbal product on the overall toxicity status of the polyherbal products was studied using *E. longifolia*-based products.

Materials and Methods: Three commercial polyherbal products containing mixture of *E. longifolia* with other herbs and a product which contains only *E. longifolia* were purchased from a retail shop while powder of an authenticated *E. longifolia* was obtained from a local research institute. *E. longifolia* or the herbal products were extracted with methanol-chloroform and the extracts were challenged with human cells, Hep2. Effects of the extracts on Hep2 cells, viability was analysed using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2,4-tetrazolium bromide (MTT) assay. The extracts were also tested for mutagenicity using Ames test employing *Salmonella* TA98 and *Salmonella* TA100. Cd, As, Pb, Mn and Cu content of the products were analysed by flame atomic absorption spectrometry.

Results: IC_{50} of crude extract of pure *E. longifolia* and a product containing only *E. longifolia* was 28.23 μg mL^{-1} and 50.00 μg mL^{-1}, respectively. Two of the polyherbal products had IC_{50} between 15.20 μg mL^{-1} and 18.89 μg mL^{-1}. All extracts, except a product containing mixture of *E. longifolia* and *Cistanche deserticola,* was not mutagenic. Heavy metals were detected in very low concentrations in all products.

Conclusion: Under the condition of this study, it can be concluded that there is a risk of increased cytotoxicity and mutagenicity of extract of polyherbal remedies compared to the toxicity of a single herbal remedy.

TOXICOLOGY STUDY OF METHANOL EXTRACT OF *CINNAMON BURMANNII*

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Introduction: Cinnamon burmannii is native to Southeast Asia, widely used as a spice and employed in cookery as a condiment and flavouring material.

Objectives: The present study was conducted to evaluate the safety and toxicity of the methanol extract of *C. burmannii* by acute and sub-chronic toxicology study using Sprague–Dawley rats (SD) rats in vivo method.

Materials and Methods: For acute toxicity studies, a methanol extract of *C. burmannii* was orally administered to SD rats (female and male) at a dose range of 500, 1000 and 2000 mg/kg. For sub-chronic toxicity studies, the rats were orally administered the methanol extract of *C. burmannii* at the doses of 500, 1000, and 2000 mg/kg (per day) for a period of 28 days. The animals were sacrificed, followed by examination of their organs and blood serum.

Results: The results showed that no toxicity was found in both acute and sub-chronic toxicity studies. The methanol extract of *C. burmannii* given orally at a dose of 500, 1000 and 2000 mg/kg caused neither visible signs of toxicity nor mortality. All the rats survived until the end of the experiment period and there were no significant differences in the general condition, growth, organ weights, hematological parameters, clinical chemistry values, or gross and microscopic appearance of the organs from the treatment groups as compared to the control group.

Conclusion: The methanol extract of *C. burmannii* did not cause any death nor did it cause abnormalities; in necropsy and histopathology findings. There were no acute or sub-chronic toxicity observed.

ANTIACETYLCHOLINESTERASE ACTIVITY OF *ACMELLA ULIGINOSA (POKOK GETANG)*

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Introduction: *Acmella uliginosa* (*pokok getang*) is an annual herb belonging to the Compositae family, traditionally used for relieving toothache.

Objectives: The study was carried out to investigate the antiacetylcholinesterase (anti-AChE) activity of hexane, ethyl acetate (EA) and water fractions from methanolic extract of *A. uliginosa* (flowers).

Materials and Methods: Hexane, EA and water fractions from methanolic extract of flowers were tested for anti-AChE activity by using Ellman's colorimetric method in 96-welled microplates and fast blue TLC bioautography methods.

Results: The results showed that all fractions exhibited dose-dependent AChE inhibitory activity. Whereas at the concentration of 0.05 mg/mL, AChE activity was respectively inhibited at 66.16% (hexane fraction), 50.39% (EA fraction) and 21.0% (water fraction).

Conclusion: This study suggests that the flowers from *A. uliginosa* may provide a potential source of natural anti-AChE inhibitors.

THE EFFECT OF *MANGIFERA INDICA* FRUIT EXTRACT AS A NEURAMINIDASE INHIBITOR IN AVIAN INFLUENZA A THERAPEUTICS (H5N1)

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Introduction: Avian influenza has been a disease that affects man for centuries and up till now a standard drug for its cure or a vaccine for immunisation from the disease has not yet been discovered. The currently available drugs are not up to expectation especially in a pandemic situation where the disease is not easy to control. The only hope is drug discovery from natural products, and if a potent drug is found and safety level for the use is established, then the drug can undergo clinical trial before it can be accepted to be used worldwide. *Mangifera indica* L. (Anacardiaceae) is a plant with numerous chemical constituents used as antioxidants, antidiarrhoeal and antiinflammatory. The fruit extract

was able to inhibit the activity of key enzyme neuraminidase and as such have the potential to cure the disease. It is an overwhelming approach knowing the fact that, the active site of the enzyme is antigenically conserved in all clinically relevant strains and also being critical to viral replication.

Objectives: The study was carried out to check if *M. indica* (mango) constituents have bioactivity against the neuraminidase enzyme.

Materials and Methods: Soxhlet extraction apparatus and rotary evaporator were used to extract and evaporate the methanol extract of matured mango and the fractions of the extracts. A microplate reader was used to read the inhibition of neuraminidase by the extracts.

Results: The ethyl acetate and chloroform fractions of the matured mango showed inhibition of the neuraminidase enzyme at 1 mg/mL. The pure compound, 3-hydroxy-3-(methoxycarbonyl) pentanedioic acid however did not show any inhibition of the enzyme activity.

Conclusion: The study confirmed that *M. indica* has chemical constituents that can be used to treat Avian influenza, but further studies are required to find the pure compounds with such activity.

ANTIOXIDANT ACTIVITY AND ANTIPROLIFERATIVE EFFECT OF DIFFERENT PARTS OF *CARICA PAPAYA*

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Introduction: Antioxidant is a chemical agent that prevents the oxidation of other chemicals. A free radical can badly injure or kill cells of various parts of the body.

Objectives: This study was carried out to determine the antioxidant activity, total phenolic and total flavonoid content and antiproliferative activity of different parts of *Carica papaya* including the ripe and unripe fruit, young leaves and seeds.

Materials and Methods: The antioxidant capacities were measured by two different assays. The total phenolic content was determined spectrometrically according to the Folin-Ciocalteu's method and expressed as gallic acid equivalents (GAE). The growth of viable cells was evaluated by using microculture-tetrazolium (MTT) assay.

Results: The radical scavenging activity was in the order of leaves > unripe > ripe > seeds. The unripe pulp had the highest antioxidant activity that inhibited the oxidation of β-carotene efficiently followed by ripe pulp, seeds, and young leaves. The phenolic content was significantly different ($p < 0.05$) among the samples. The highest phenolic content was found in young leaves followed by unripe, ripe pulp and seeds. The different pattern of antioxidant activities can be observed in total flavonoids where leaves still

contribute to the highest flavonoids content followed by ripe fruit, seed and the least was unripe fruit. A linear positive relationship existed between antioxidant activity and total phenolic content (r = 0.78). MCF-7 (hormone-dependent breast cancer) and MDA-MB-231 (non-hormone-dependent breast cancer) cell cultures were used to determine the antiproliferative activities. The ripe fruit was found to inhibit 50% of MCF-7 cancer cell's proliferation at 50 µg/mL. The ripe and unripe fruit were found to cause 50% cell death of MDA-MB-231 at 25 and 50 µg/mL respectively.

Conclusion: The findings of this study showed that the methanol extract especially of the ripe fruit have great potentials in antioxidant and antiproliferative activities which could be attributed to their phytochemicals contents.

NATURAL STATINS PRODUCTION BY FILAMENTOUS FUNGI OF THE GENUS *PENICILLIUM* ISOLATED FROM LOCAL SOIL SAMPLES

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Introduction: Statins are a group of drugs that lower the cholesterol level in blood due to the inhibition of 3-hydroxy-3-methylglutaryl-CoA reductase, the essential enzyme in cholesterol biosynthesis. Such anticholesterolemic activity of statins decreases the risk of heart attack. Natural statins are mainly produced by filamentous fungi of the genera *Aspergillus* and *Penicillium*. At present, development of novel biotechnological production processes for natural statins and their derivatives is essential.

Objectives: The purpose of this study was to screen fungal cultures of the *Penicillium* genus isolated from soils for natural statins production.

Materials and Methods: Statins-producing ability of examined fungi was estimated by the spectrophotometric method using a spectrophotometer U-1800 (Hitachi, Japan). Absorbance was monitored at 237 nm.

Results: Screening work was carried out to identify the fungi of the *Penicillium* genus, which produces natural statins. Statins yields in tested fermentation samples were evaluated based on the absorbance levels. Of 20 local penicillia screened in this study, 2 cultures, *Penicillium* sp. FI2 and *Penicillium* sp. FI19, were found to be the best natural statins producers. These two fungal isolates were recommended for further studies.

Conclusion: The present investigation showed that some local fungal cultures of the *Penicillium* genus tested can produce natural statins. Fungal isolates with high statins-producing ability can be considered as potential producers of hypocholesterolemic agents.

CYTOTOXICITY STUDIES OF *BOESENBERGIA ROTUNDA* ON SELECTED CANCER CELL LINES

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Introduction: *Boesenbergia rotunda*, locally known as *temu kunci* in Indonesia or *kra-chai* in Thailand, is a rhizomatous herb belonging to the Zingiberaceae family. The rhizomes are traditionally used as a food ingredient and treatment for aphtous ulcers, dry mouth, stomach discomfort, leucorrhoea and dysentery.

Objectives: The interest of this study is to conduct further cytotoxicity studies in order to validate the use of the rhizomes in the treatment of cancer.

Materials and Methods: An investigation on this effect of the rhizomes of *B. rotunda* on five human carcinoma cell lines, namely hormone-dependent breast carcinoma (MCF-7), human cervical carcinoma cell line (Ca Ski), human colon adenocarcinoma (HT-29), human nasopharyngeal epidermoid carcinoma cell (KB), human ovarian carcinoma cell line (SKOV-3) and one non-cancer human lung fibroblast cell lines (MRC-5) was conducted using the neutral red cytotoxicity assay.

Results: The chloroform fraction showed remarkable inhibition on the growth of MCF-7, Ca Ski, HT-29, KB and SKOV-3 with IC_{50} values of 7.25, 11.35, 9.5, 6.0 and 10.0 µg/mL respectively. Pinostrobin, a flavone isolated from the chloroform fraction was found partly responsible for the ability to inhibit the growth of Ca Ski (IC_{50} value of 4.00 µg/mL, 14.8 µM), KB (IC_{50} value of 2.00 µg/mL, 7.40 µM) and MRC-5 (IC_{50} value of 25.0 µg/mL, 92.6 µM).

Conclusion: These results show that pinostrobin has the potential in inhibiting the growth of both cervical and nasopharyngeal cancer cells without much damage to normal cells. The present findings thus provide important preliminary data for future work.

MITRAGYNA SPECIOSA (KRATOM) CAN INHIBIT NEOVASCULARISATION VIA WNT, TGF-β AND MAPK/ERK PATHWAYS

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Introduction: Mitragyna speciosa Korth (Rubiaeceae) is commonly referred to as *kratom*, can be found mainly in Southeast Asian countries such as Malaysia, Thailand and Myanmar. It is traditionally used as a pain killer, immune system booster, treatment for diarrhoea, as a sexual stimulant as well as to help reduce addiction to opiates. Angiogenesis plays an important role in the formation and growth of new blood vessels, forms a necessary component of normal tissue repair, tumor growth and a wide variety of other inflammatory and pathological process including Alzheimer disease. Inhibition of angiogenesis is vital in tumor growth suppression as it can halt the disease is progression. Previous studies revealed that leaf extracts of *M. speciosa* are good source of natural antioxidants. Antioxidants are well known for their antitumor properties with inhibition of angiogenesis being one of the primary routes.

Objectives: The study was undertaken to investigate the antiangiogenic activity of different extracts of *M. speciosa* and the mechanisms underlying this activity.

Materials and Methods: Using ex vivo techniques employing tissue explants of rat aorta, antiangiogenic activity of aqueous, chloroform, methanolic and total alkaloid extracts of *M. speciosa* were screened. In order to explore the mechanism of anticancer activities of *M. speciosa*, the effects of the most potent extract were studied on the transcriptional activity of 10 carcinogenesis pathways in a signal reporter assay system.

Results: The results obtained revealed significant suppression of microvessel sprouting from the rat aorta by these extracts. The total alkaloid extract displayed strongest antiangiogenic property (100%) followed by the methanol (60%), chloroform (42%) and water (40%) extracts in descending order of reactivity. The total alkaloid extract caused significant down-regulation of Wnt pathway and up-regulation of tgf-β and MAPK/ERK pathways.

Conclusion: Hence our findings suggest a promising outlook on the use of total alkaloid extract of *M. speciosa* as potential antitumor agent and given the important role of the Wnt, tgf-β and MAPK/ERK pathways in neuronal protection, the total alkaloids of *M. speciosa* may also be useful for treatment of neurodegenerative diseases such as Alzheimer disease.

MORINDA CITRIFOLIA LEAVE EXTRACT REDUCED PLATELET AGGREGATION OF HUMAN BLOOD IN VITRO

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Introduction: Morinda citrifolia (Mc) known as *noni* is traditionally used to treat many ailments including infections, asthma, menstrual cramps, arthritis, gastric and indigestion, inflammation, cancer and to reduce pain. The leaf extract has been shown by us to increase clotting time, prothombin, thrombin and activated thomboplastin time.

Objectives: The aim of the study is to determine the effect of Mc hot water leaf extract on human platelet aggregation in vitro.

Materials and Methods: Ten healthy volunteers participated in the study. Citrated blood was added to the Mc dose and incubated at 37°C. The blood was analysed using the multiplate analyzer (Dynabyte Medical, Munich) for platelet aggregation activity, initiated by different agonists (ADP, ristocetin, collagen and arachidonic acid). The results, presented in units of area under the curve/min (AUC/min) and subsequently transformed into ratio (platelet aggregation of the treated sample divided by the platelet aggregation of its own control, PBS or phosphate buffer).

Results: Platelet aggregation studies were conducted with different doses of Mc and control. Platelet aggregation for Mc 100 and 125 mg/mL using 4 different agonists compared to their controls was inhibited more than 98%. Mc (1 mg/mL) increased platelet aggregation with arachidonic acid significantly.

Discussion: Effect of Mc 10 to 62.5 mg/mL could not be carried out because the plasma clotted before coagulation assays could be conducted, showing Mc had a procoagulant effect at these doses, reversing the anticoagulant effect of citrate. Mc showed a procoagulant effect with agonist arachidonic acid at 1 mg/mL. However, antiplatelet effect was seen with all the higher doses (100 mg/mL and 125 mg/mL) using all agonists (ADP, ristocetin, collagen and arachidonic acid) in the platelet aggregation studies. This suggests possible interference with many factors contributing to platelet aggregation. These results show that the leaf extract has antiplatelet activity.

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BIOASSAYS OF LOCAL PLANTS FOR ANTIFUNGAL ACTIVITY USING COLORIMETRIC BROTH MICRODILUTION METHOD

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Introduction: The prevalence of immune-suppressing disease and the emergence of widespread drug resistance have resulted in an increases in incidences of opportunistic fungal infections. Consequently, the hunt for new antifungal agents from natural resources such as plants has intensified in response to the limitations of the therapies currently available.

Objectives: Five local plants including the leaves extracts of *Cocos nucifera*, *Clerodendrum calamitosum*, *Citrus microcarpa,* flowers extracts of *Bougainvillea spectabillis* and fruits extracts of *Passiflora edulis* were evaluated for antifungal properties against 5 medically important fungi, namely *Candida albicans* ATCC 90028*, Issatchenkia orientalis* ATCC 6258, *Cryptococcus neoformans* ATCC 90112*, Aspergillus brasiliensis* ATCC 16404 and *Trichophyton mentagrophytes* ATCC 9533.

Materials and Methods: Sequential maceration method with hexane, chloroform, ethyl acetate, ethanol, and methanol was performed for plant extraction. Colorimetric broth microdilution method, using p-iodonitrotetrazolium as a growth indicator was applied for the antifungal activity assay.

Results: Out of 125 bioassays of the plant extracts, 90.4% of them demonstrated fungistatic activity; however, only 52.8% of them possessed fungicidal activity. *B. spectabilis* was found to be the most active species (92% of bioassays) against the fungal strains tested. Ethanol and methanol extracts of *C. nucifera* showed the greatest antifungal potency against *I. orientalis* with the lowest minimum inhibitory concentration (MIC) of 0.02 mg/mL. The lowest minimum inhibitory concentration (MBC) at 0.08 mg/mL was showed by the hexane extract of *C. microcarpa* and the hexane and ethyl acetate extracts of *B. spectabilis* against *C. neoformans.* All the extracts from *C. nucifera* and *P. edulis* were inactive against *A. brasiliensis.* The fungal susceptibility indexes indicated that the most susceptible fungal species was *C. neoformans* with 100% towards the tested plant extracts while *A. brasiliensis* possessed the lowest susceptibility index of 32%.

Conclusion: *B. spectabilis* warrants further phytochemical investigation to ascertain fully its medicinal properties and potential toxicity.

MECHANISMS OF ANTIINFLAMMATORY EFFECTS OF SYNTHETIC CURCUMINOID AND CHALCONE DERIVATIVES

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Introduction: Overproduction of NO can provoke serious effects leading to cellular accumulation of reactive nitrogen species causing cell death of surrounding tissues and destruction of tissue homeostasis. Various chronic inflammatory diseases related to the conditions above can be commonly found in those suffering from rheumatoid arthritis and asthma. Therefore several pathways leading to the conditions above were identified as a potential therapeutic target including the nuclear factor-kappa B (nF-κβ) and mitogen activated protein (MAP) kinases pathways.

Objectives: This study was undertaken to investigate the mechanisms of synthetic curcuminoids and chalcone derivatives on the nF-κβ and MAP kinases pathways.

Materials and Methods: Standard protocols including RT-PCR, western blot analysis, cytokine immunoassay, immunoprecipitation and in vitro kinase activity assays were carried out to investigate the mechanism of action for the active compounds.

Results: BHMC (curcuminoid derivative) and HMP (chalcone derivative) showed potential results to treat inflammation. HMP showed selective potent activity against p38a MAP kinase as compared to SB203580 (positive control) and significant inhibition occurring at doses as low as 6.25 µM on inducible nitric oxide synthase (iNOS) protein level in murine macrophages. Meanwhile BHMC showed potent activities against IL-10 and MCP-1 gene expression compared to dexamethasone. The IC_{50} of BHMC upon TNF-a, IL-6, IL-10 and MCP-1 secretions by LPS induced U937 cells are 5.537 ± 0.477 µM, 6.542 ± 0.613 µM, 2.448 ± 0.177 µM and 0.981 ± 0.241 µM, respectively. BHMC also inhibited the translocation of nf-κβ p65 from cytoplasm into nucleus at the concentration of 12.5 µM and dose-response against p38 and JNK. Finally, treatment of BHMC in murine provides a higher degree of protection from lethal sepsis compared to curcumin at a comparable dose.

Conclusion: The present study showed the selective inhibition of the synthesis of proinflammatory mediators by BHMC and selective inhibition of the expression of the enzyme iNOS and p38-MAP kinase and its activity by HMP, both an active ingredients for the prevention or treatment of inflammatory related diseases.

MONOGRAPH AND DOCUMENTATION

DIVERSITY AND DISTRIBUTION OF MEDICINAL PLANTS IN FARASAN ARCHIPELAGO, RED SEA, SAUDI ARABIA

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Introduction: Farasan Archipelago in the Red Sea of Saudi Arabia is part of a transition zone between Africa and Asia. It is one of the important centers of medicinal plants in the south western region of the country.

Objectives: The current study aims to assess plant diversity and distribution of medicinal plants on 20 islands and 5 main habitats namely mangroves, salt marshes, sand formations, wadi channels and coral rocks in the Farasan Archipelago.

Results: The results identified a total of 191 species belonging to 129 genera and 53 families, with 38 species belonging to the Monocotyledoneae and 153 species to the Dicotyledoneae classes respectively. The study area has one of the highest species-to-area ratios when compared to other regional floras with high taxonomic diversity (species/genera = 1.5) and high percentage of rare and very rare species (72%). Medicinal plants constitute 58% of the Farasan flora. The largest family is Poaceae with 27 species, followed by Convolvulaceae, Fabaceae and Capparaceae with 13, 12 and 11 species, respectively. The large islands such as Farasan Alkabir, Sajid and Zuifaf are more diverse than the small islands such as North Reef, South Reef and Sulyn. The diversity indices indicated that coral rocks are the most diverse habitats followed by sand formation. On the other hand, the mangroves and wadi channels have the lowest diversity indices. The main dominant species include *Arthrocnemum macrostachyum, Cyperus conglomerartus, Zygophyllum coccineum* and *Zygophyllum simplex.* Approximately 57% of medicinal plants are threatened due to intensive collection and other human activities. The threatened medicinal plants are *Amaranthus spinosus, Cadaba farinosa, Caralluma sinaica, Citrullus colocynthis, Commiphora gileadensis* and *Salvadora persica.*

Conclusion: Initial priority for management and conservation plan of medicinal plants in Farasan Archipelago is to protect their habitats. Public and private sectors involvement in management and utilisation of medicinal plants in a sustainable way is recommended in order to combat human pressures on these valuable natural resources.

EDUCATION AND PRACTICE

AN ANALYSIS OF THE EXTENT AND TYPES OF RESEARCH EMPLOYED AND PUBLISHED IN THE SCIENTIFIC LITERATURE ON HERBAL MEDICINE BETWEEN 1986–2009

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Introduction: Medicinal plants are plants that provide people with medicines to prevent disease, maintain health or cure ailments. In one form or another, they benefit virtually everyone on Earth. Plants have been used for medicinal purposes for as long as history has been recorded. Over 80% of the population in developing countries depend directly on plants for their medical requirements. Traditional medicine is a key element among the rural communities in developing countries for the provision of primary health care especially where there are inadequate primary health care systems. Herbal research is needed in order to evaluate and prove scientifically the traditional experience on the safety and efficacy of herbal medicines. It may also be conducted to validate a new-found plant material or a new combination of herbal medicines, or even a new indication, a new dosage form or a new administrative route for an existing herbal medicine.

Objectives: The objective of this study is to evaluate the extent and types of research carried out and published on herbal medicine in Pubmed database between 1986–2009.

Materials and Methods: The study focuses on research publications of herbal medicine used worldwide. Data were collected through the Pubmed database for the period of 1986 to 2009. The literature were searched by keying the search term, herbal medicine and the total number of publication cited was then recorded and analysed.

Results and Discussion: Analysis from the study showed that journal article contributed the highest number of citation (3913 citations), followed by randomised control trial (205 citations), case reports (195 citations) and in vitro (83 citations). The interest and appreciation on herbal medicines increases with time as can be seen in the number of research publication cited. The quality of research also increases with time.

Conclusion: The interest on medicinal plant or herbs as a traditional and alternative medicine and the need to evaluate its safety and efficacy have resulted in an increase in the number of research publications as seen in Pubmed database. Meta analyses and RCT study design were also observed and this is seen as a positive trend towards producing strong evidence as to the safety and efficacy of herbal medicines.