THE CHEMICAL CHARACTERISATION AND BIOACTIVE POTENTIAL OF SECONDARY METABOLITES IN CURRY LEAF (*Murraya koenigii* L. Spreng)

THILAHGAVANI NAGAPPAN

INSTITUTE FOR TROPICAL BIOLOGY AND CONSERVATION
UNIVERSITI MALAYSIA SABAH

2014

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AUTHENTICATION

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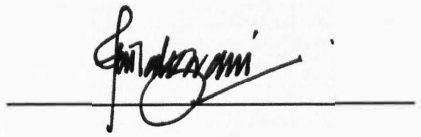
30th January 2014



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DECLARATION

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1. SUPERVISOR

Prof. Dr. Charles S. Vairappan Signature

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ABSTRACT

Murraya koenigii L. Spreng (curry leaf) is a small perennial shrub in the family Rutaceae. Widely distributed throughout tropical Asia, its aromatic leaves are used in culinary and folk medicine. Extraction efficiency using three different solvent systems was investigated using soxhlet system where ethanol produced the best quality and quantity. Hence, specimens from seven locations in Malaysia were investigated for their chemical fingerprint and bioactive potentials. Essential oil was extracted using hydro-distillation technique and Gas Chromatography Mass Spectroscopy (GCMS) analysis revealed the presence of 61 types of volatile metabolites. Major chemotaxonomical markers were β-caryophyllene and α-humulene and the minor chemotaxonomical markers were β-elemene, aromadendrene, β-selinene, spathulenol, caryophyllene oxide, viridiflorol, 2-naphthalenemethanol, trivertal and juniper camphor. High Performance Liquid Chromatography (HPLC) technique yielded three major metabolites and their structures elucidated as mahanine (**I**), mahanimbicine (**II**) and mahanimbine (**III**). Preparative Thick Layer Chromatography (PTLC) technique isolation gave koenimbine (**IV**), koenidine (**V**) and neo-mahanine (**VI**) were isolated and elucidated. Antibacterial activity of extract revealed potent inhibition against four environmental strains and pathogenic strains with the MIC between 0.25 to 0.063 mg/ml while mahanine (**I**), mahanimbicine (**II**), mahanimbine (**III**) and essential oil activity against antibiotic resistant strains showed promising MIC and MBC values. Essential oil exhibits bacteriostatic potential against *Escherchia coli* and *Salmonella thypimurium* by suppressing the growth to 10^5 and 10^4 CFU/ml respectively within 12 hours at 25 °C and to 10^4 CFU/ml ranged from Day 1 to Day 3 at 4 °C in food model. Cytotoxicity against mahanine (**I**), mahanimbicine (**II**), mahanimbine (**III**) and essential oil were evaluated against MCF-7, HeLa and P388. The IC_{50} values of mahanimbine (**III**) against MCF-7 cell line, P388 cell line and HeLa cell line were 2.12 µg/ml, 5.0 µg/ml and 1.98 µg/ml respectively while the IC_{50} values of essential oil against MCF-7 cell line, P388 cell line and HeLa were 6.00 µg/ml, 7.01 µg/ml and 2.83 µg/ml. Mahanimbine (**III**) was found to cause cell arrest in sub G1 phase of MCF-7 cells and induce apoptosis *via* up-regulation of pro-apoptotic proteins, down-regulation of anti-apoptotic protein, activation caspase-7 and cleavage of PARP. Wound treated with mahanimbicine (**II**) and extract showed the highest rate of collagen deposition with well-organized collagen bands, formation of fibroblasts, hair follicle buds and reduced number of inflammatory cells as extract promotes significant wounds contraction by Day 4. This plant is an ideal candidate for further pharmacological investigation.

ABSTRAK

PENENTUAN PROFIL KIMIA DAN POTENSI BIOAKTIF SEBATIAN METABOLIT SEKUNDAR DAUN KARI (*Murraya koenigii* L. Spreng)

*Murraya koenigii L.Spreng (daun kari) ialah sejenis pokok saka kecil tergolong dalam famili Rutaceae. Tumbuhan ini tersebar secara meluas di kawasan Asia beriklim tropika dimana daun beraromanya digunakan dalam masakan dan perubatan tradisional. Efisiensi pengekstrakan menggunakan tiga sistem pelarut berlainan telah dikaji menggunakan sistem soxhlet dimana etanol menghasilkan kualiti dan kuantiti yang terbaik. Oleh itu, spesimen dari tujuh lokasi dari Malaysia telah dikaji untuk mendapatkan cap jari kimia dan potensi bioaktifnya. Minyak pati telah diekstrak menggunakan teknik pemeringkatan hidro dan kandungannya dianalisa menggunakan Kromatografi Gas Jisim Spektroskopi dimana 61 jenis sebatian kimia meruap telah dikenalpasti. Penanda kimotaksonomikal utama ialah β-elemene dan α-humulene manakala penanda kimotaksonomikal kecil ialah aromadendrene, β-selinene, spathulenol, caryophyllene oksida, viridiflorol, 2-napthalenemethanol, trivertal dan juniper camphor. Melalui teknik Kromatografi Cair Kinerja Tinggi, tiga metabolit utama dipencil dan difahami sebagai mahanine (**I**), mahanimbicine (**II**) dan mahanimbine (**III**) manakala melalui teknik kromatografi preparative lapisan tebal, koenimbine (**IV**), koenidine (**V**) dan neo-mahanine (**VI**) telah dipencil dan ditentukan strukturnya. Aktiviti antibakteria ekstrak mendedahkan perencatan kuat ke atas empat jenis bakteria persekitaraan dan pathogen dengan nilai perencatan minima diantara 0.25 hingga 0.063 mg/ml manakala aktiviti mahanine (**I**), mahanimbicine (**II**), mahanimbine (**III**) dan minyak pati terhadap bakteria ketahanan antibiotik menjanjikan nilai perencatan kepekatan minimum dan kepekatan bakteriacidal minimum yang positif. Minyak pati menunjukkan potensi bakteriastatik ke atas Esherchia coli dan Salmonella thypimurium dengan merencatkan pertumbuhan kepada 10^5 dan 10^4unit koloni/ml dalam masa 12 jam pada 25 °C dan kepada 10^4 unit koloni/ml dari hari pertama kepada hari ke-3 pada 4 °C dalam model makanan. Sitotoksik mahanine (**I**), mahanimbicine (**II**) dan minyak pati ke atas sel-sel MCF-7, P388 dan HeLa turut dikaji. Nilai IC_{50} minyak pati ke atas sel-sel MCF-7, P388 dan HeLa ialah 6.00 µg/ml, 7.01 µg/ml dan 2.83 µg/ml. Mahanimbine (**III**) dikesan menyebabkan penangkapan sel di fasa sub G1 dalam sel MCF-7 dan mendorong 'apoptosis' melalui peningkatan protin pro-apoptatik, pengurangan protin anti-apoptatik, mengaktifan caspase-7 dan belahan PARP. Luka dirawat menggunakan mahanimbicine (**II**) dan ekstrak menunjukkan deposi kolagen yang tinggi berserta susunan kolagen yang teratur, pembentukkan fibroblast, kudup folikal rambut dan pengurangan sel radang sekaligus ekstrak merangsang pengecutan luka pada hari ke-4. Tumbuhan ini ialah calon ideal untuk investigasi farmakologikal lanjutan.*

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