Bridging Traditional Knowledge & Natural Products Innovations Towards Wellness and Shared Prosperity



Editors • Mary Khoo Gaik Hong • Chee Beng Jin • Getha Krishnasamy • Mazura Md. Pisar • Firdaus Kamarulzaman





Bridging Traditional Knowledge & Natural Products Innovations Towards Wellness and Shared Prosperity

Bridging Traditional Knowledge & Natural Products Innovations Towards Wellness and Shared Prosperity

Editors

- Mary Khoo Gaik Hong Chee Beng Jin Getha Krishnasamy
 - Mazura Md. Pisar Firdaus Kamarulzaman





Ministry of Energy and Natural Resources

https://www.frim.gov.my/publication/maps_tk 2021

©Forest Research Institute Malaysia 2021

All enquiries should be forwarded to: Director-General Forest Research Institute Malaysia 52109 Kepong Selangor Darul Ehsan Malaysia

Tel: +603-62797000 Fax: +603-62731314 http://www.frim.gov.my

Perpustakaan Negara Malaysia

Cataloguing-in-Publication Data

Bridging Traditional Knowledge & Natural Products Innovations Towards Wellness and Shared Prosperity / Editors: Mary Khoo Gaik Hong, Chee Beng Jin, Getha Krishnasamy, Mazura Md. Pisar & Firdaus Kamarulzaman Mode of access: Internet

eISBN 978-967-2810-05-6

- 1. Ethnoscience.
- 2. Traditional medicine.
- 3. Alternative medicine.
- 4. Traditional ecological knowledge.
- 5. Government publication—Malaysia.
- 6. Electronic books.
- I. Khoo, Mary Gaik Hong. II. Chee, Beng Jin.
- III. Getha Krishnasamy. IV. Mazura Md. Pisar.
- V. Firdaus Kamarulzaman.

306.42

MS ISO 9001:2015

Content

Foreword	viii
Preface	ix
TRADITIONAL KNOWLEDGE, AGRONOMY AND CONSERVATION	
Apparatus used in midwifery practice of traditional Malay medicine in Peninsular Malaysia Ida Farah A <i>et al.</i>	2
An overview of herbal medicines as adjunct treatment for cancer in selected hospitals within Ministry of Health Malaysia Teo CS <i>et al.</i>	7
Evaluation of planting media for mass production and yield of tuba roots Angela THG <i>et al.</i>	12
Effects of good agriculture practice on the growth, total phenolic contents and antioxidant status of Labisia pumila var. alata Syafiqah Nabilah SB et al.	16
Observation on affected areas of inoculated Aquilaria malaccensis Abd Majid J et al.	21
Anatomical characterisation of <i>Phyllanthus emblica</i> L.: A plant with therapeutic potential Ummu Hani B <i>et al.</i>	26
Conservation of medicinal and aromatic plants in the Ethnobotanical Garden of FRIM: An effort in tackling biodiversity loss crisis Tan AL <i>et al.</i>	31
Perubatan traditional: Bukan sekadar Jampi Nik Musa'adah M <i>et al.</i>	35
Kajian awal: Kesan penggunaan baja terhadap kadar pertumbuhan dan hasil tongkat ali di Stesen Penyelidikan FRIM, Maran Pahang Syazwan A <i>et al.</i>	40
Kajian perbandingan penanaman ABP016 secara berkelompok di dua lokasi berbeza oleh suku kaum Temiar dan Semelai Siti Salwana H <i>et al.</i>	45
Komposisi dan analisis vegetasi spesies tumbuhan ubatan berpotensi berasaskan pengetahuan tradisi orang asli dalam Hutan Simpan Bera, Tapak Ramsar, Pahang Madihah MN <i>et al.</i>	51
Status kemandirian koleksi tumbuhan ubatan berasaskan pengetahuan tradisi Melayu di Laman Pengetahuan Tradisi, Taman Ethnobotani, FRIM Norbaiah MY <i>et al</i> .	56
NATURAL PRODUCTS DISCOVERY	
<i>In vitro</i> antiproliferative activity of dichloromethane (DCM) fraction of <i>Brucea javanica</i> (L.) Merr. (melada pahit) root on selected prostate cancer cell line Murizal Z <i>et al.</i>	62

Cytotoxicity and glutathione level of methanolic leaves extract from <i>Canarium odonthophyllum</i> Miq. (dabai) against UVB induced B164A5 melanoma cell lines Ahmad Rohi G <i>et al.</i>	66
Antioxidant activities of ethanolic extract of <i>Vitex negundo</i> leaves dried at different temperature Ihsan Safwan K <i>et al.</i>	71
TPA-induced mouse ear oedema inhibitory activity of <i>Piper betle</i> leaves extract Ong BK <i>et al.</i>	77
<i>In vitro</i> evaluation of antiinflammatory activity of <i>Melaleuca cajuputi</i> Powell Mazura MP <i>et al.</i>	81
Biological profiling of Swietenia macrophylla supercritical carbon dioxide extract Saidatul Husni S <i>et al.</i>	87
Study of different extracts of kadok (<i>Piper sarmentosum</i>) leaves on the flavonoid profiles and selected bioactivities Zunoliza A <i>et al.</i>	92
Evaluation of a topical formulation containing novel active ingredient from basidiomycetes to treat MRSA skin infections Getha Krishnasamy <i>et al.</i>	99
Comparative study on production kinetics of antibacterial metabolites from batch fermentation of basidiomycete strain FRIM550 in stirred-tank bioreactor Shariffah Nurhidayah SAR <i>et al.</i>	105
Cell based assay for toxicity assessment: A case study of <i>Baeckea frutescens</i> Khoo MGH <i>et al.</i>	111
A preliminary toxicity evaluation of <i>Neo</i> Trai in Sprague Dawley rats Chee BJ <i>et al.</i>	118
Single oral dose 14-day toxicity study of <i>Brucea javanica</i> (melada pahit) fruit on Sprague Dawley rats Teh BP <i>et al.</i>	124
Acute toxicity evaluation of <i>Phaleria macrocarpa</i> (mahkota dewa) leaves and fruits crude extracts in Sprague Dawley rats Norzahirah A <i>et al.</i>	129
Acute oral toxicity study on <i>Lignosus rhinoceros</i> and <i>Curcuma zedoaria</i> Lalitha Suganthi S <i>et al.</i>	134
Preliminary <i>in vivo</i> toxicity assessment of <i>Parkia speciosa</i> (petai) seed, <i>Averrhoa bilimbi</i> (belimbing buluh) fruit and <i>Garcinia mangostana</i> (manggis) rind Nor Azlina Z <i>et al.</i>	139
Acute oral toxicity study of Carica papaya, Syzygium polyanthum, Chromolaena odorata and Annona muricata on Sprague Dawley rats Elda Nurafnie IR et al.	145
Flavonoid-saponin extract from leaves of <i>Mitragyna speciosa</i> as hepatoprotective agent against paracetamol-induced toxicity in rats Hidayatul Khamariah ZA <i>et al.</i>	150
GC-MS analysis of terpenoids from leaves of <i>Canarium odontophyllum</i> Miq. (dabai) Muhammad Wahizul Haswan AA <i>et al.</i>	154

Volatile constituents, antiinflammatory and anticollagenase effect of essential oils from four Cymbopogon species Mailina J et al.	159
Analysis of Cymbopogon winterianus essential oil compounds by means of GC-MS and Z-score technique Khairul Anis Athirah K <i>et al.</i>	165
Chemical composition of <i>Melaleuca cajuputi</i> essential oils from three different locations Mohd Shafik Yuzman T <i>et al.</i>	170
Chemical composition of <i>Meistera ochrea</i> essential oils Nor Azah MA <i>et al.</i>	174
Chemical composition of <i>Hevea brasiliensis</i> seeds (RRIM 2001 clone) Nor Hassifi S	178
Penilaian aktiviti antikanser secara <i>in vitro</i> terhadap sampel tumbuhan UGG004 Nurhanan MY <i>et al.</i>	184
Penilaian aktiviti antiinflamasi bagi spesies ABP 016 dari tiga lokasi terpilih Nurul Haslinda M <i>et al.</i>	188
Pencirian nano perak daun semambu (<i>Azadirachta indica</i> A. Juss) melalui sintesis hijau Noor Rasyila MN <i>et al.</i>	192
Penilaian potensi bioaktiviti SBJ 015 berdasarkan pengetahuan tradisi Orang Asli Bateq di Pahang Shalini M <i>et al.</i>	198
STANDARDISATION, PRODUCT DEVELOPMENT & QUALITY CONTROL	
The establishment of three scales of agarwood oils quality index (AOQI) using electronic nose data with Z-score scaling technique Sahrim L et al.	205
Chemical standardisation and quantification of piperine from black peppercorns extract for product development Wong CM & Ling JJ	212
Development of DEET-free mosquito repellent from pepper waste Ling JJ.	217
Real-time stability studies of <i>Baeckea frutescens</i> dried raw materials on quality control element Nurhazwani MH et al.	222
Environment control in natural product quality control laboratory: Air monitoring Amira Rina Nurdiana MS <i>et al.</i>	227
Phenotypic identification of <i>Pseudomonas aeruginosa</i> in herbal based products using biology semi-automated identification system Norulaiman Y <i>et al.</i>	231
Pengoptimuman dan validasi ke atas kaedah ujian bahan campur palsu steroids menggunakan kromatografi cecair bertekanan tinggi Nor Hayati A <i>et al.</i>	235



Foreword

Assalamualaikum WBT and greetings!

The effort to combine traditional knowledge with modern scientific studies is a good endeavour that will empower and promote the importance of traditional knowledge. This effort will also ensure a fair and equitable sharing of resources with resource owners under the Access to Biological Resources and Benefit Sharing (ABS) Act 2017.

As a country with rich biological diversity, efforts to strengthen natural resource management are critical as a form of mitigation to climate change and natural disasters. Various measures have been taken by the Ministry of Energy and Natural Resources (KeTSA) including working towards realising the establishment of the Malaysian Biodiversity Centre, which serves as a biodiversity research and conservation centre as well as the National Competent Authority to enforce the ABS Act. Through the enactment and enforcement of this act, Malaysia can perform its obligations under the Convention on Biological Diversity (CBD).

The richness in natural resources including medicinal plants is a blessing to Malaysia. Meanwhile, our local wisdom of previous generations using plants in traditional treatment is a treasure warrants to be preserved. In recent times, health and cosmetic products of natural origin are increasingly in demand due to the high awareness of environmental protection as well as the risk of harmful chemicals to consumers. This is a good prospect for researchers to produce high value and safe research output to meet the present trends.

In addition, the recent world situation in facing the unexpected COVID-19 pandemic has also opened the eyes of many that research on infectious diseases and public health should take precedence. In this aspect, the Natural Products Division at FRIM can be a key pillar that plays an imperative role in upholding the knowledge of local traditions through its strengths in scientific research, development and commercialisation.

It is the aspirations and policies of the Malaysian government to increase efforts as well as the use of local natural resources to produce high value products and innovations for the prosperity of the country. I hope that researchers and the policy makers will always work together to shoulder the responsibility of preserving and conserving the country's natural resources as well as biodiversity to ensure the sustainability of our present-day society and the future generations.

This book is a collection of short scientific papers on traditional knowledge and natural product research as well as innovations from researchers in Malaysia. The basis of this publication stemmed from the passion for knowledge-sharing and as a better preservation method of research findings. I hope that this book is able to reach its goal in sharing the work conducted by these scientists to be reviewed as reference by others.

Dr. Khali Aziz Hamzah Director General of FRIM

Preface

Malaysian culture demonstrates a rich and unique potpourri of knowledge and practices originated from its multiracial and diverse cultural society. The traditional knowledge practiced by the Malay, Chinese, Indian and the orang asli continues to sustain and maintain their community livelihood from the olden days to the present age. Their dependence on the forests is vital which serves as their sustainable green pharmacies. Apparently, the maintenance of good wellbeing and prevention of illness are much accentuated on food and usage of natural remedies from the surroundings, especially from plants and other natural resources. In the current fast-paced modern society, traditional knowledge which encompasses traditional remedies and folk cures gradually faced extinction and very soon to be long forgotten.

The Natural Products Division at the Forest Research Institute Malaysia (FRIM) had come a long way in pioneering research and discoveries of medicinal and aromatic plants along with local traditional knowledge after receiving its mandate from the Government of Malaysia in 1995. The institute had forged collaborations with renowned local and international academics, research institutions and herbal industries in technology transfer, training, sharing of expertise and product development.

This book celebrates Natural Products Division's involvement for a period of more than 25 years of research in the importance of natural resources related to medicinal plants and microbes in Malaysia. The content of the book acknowledged the tireless labour, years of hard work and patience of scientists and researchers in the field of traditional knowledge, conservation, agronomy, natural products discovery, standardisation and processing technology, product development and commercialisation, quality control as well as issues on regulatory and standards.

The title "Bridging Traditional Knowledge & Natural Product Innovations Towards Wellness and Shared Prosperity" signifies the effort and aspiration to combine traditional knowledge with new scientific studies to promote measures tending to the betterment of the society in Malaysia. We sincerely hope that the scientific findings contributed by our fellow colleagues and contributors will complement and strengthen each other's discoveries to produce common solutions to a pressing issue for the benefit of this nation.

The Editorial Team Natural Products Division @ FRIM TRADITIONAL KNOWLEDGE, AGRONOMY AND CONSERVATION

GC-MS ANALYSIS OF TERPENOIDS FROM LEAVES OF *Canarium odontophyllum* Miq. (DABAI)

Muhammad Wahizul Haswan AA^{1,2}, Siti Fathiah M¹, Dayang Fredalina B³ & Ahmad Rohi G¹ ¹Centre for Toxicology and Health Risk Studies (CORE), Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia. ²Department of Para-clinical Sciences, Faculty of Medicine and Health Sciences, Universiti Malaysia Sarawak, Jalan Datuk Mohammad Musa, 94300 Kota Samarahan, Sarawak, Malaysia. ³Centre for Diagnostic, Therapeutic & Investigative Studies (CODTIS), Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia.

Tel: 019-2268976 Fax: 03-26914304 Email: rohi@ukm.edu.my

ABSTRACT

Terpenoids are defined as secondary metabolites with carbon backbone molecular structures consisting of isoprene (2-methylbuta-1, 3-diene) units. They demonstrate important biological activities, such as antibacterial, antiviral, antimalarial, antiinflammatory, anticancer and cholesterol synthesis inhibition activities. *Canarium odontophyllum* Miq. or locally known as "dabai" is an endemic plant in Sarawak, Malaysia. Its leaf compositions were examined by using the GC-MS analysis in order to compare and contrast their volatile terpenoids constituents. The terpenoids content were 36.67% and 14% for hexane and ethanol extracts, respectively. n-Hexadecanoic acid, phytol and octadecanoic acid were the major terpenoids constituents from the leaves of *C. odontophyllum* Miq. n-Hexadecanoic acid (20.22%), phytol (8.74%) and octadecanoic acid (7.54%) were found to be predominant in the hexane extract, while phytol (21.02%) and n-hexadecanoic acid (14.52%) were major constituents in the ethanol extract. The *C. odontophyllum* Miq. leaf constituents are also related to their biological activities and would offer promising therapeutic effects. Further investigation should be conducted to develop it as a potential therapeutic drug.

Keywords: Canarium odontophyllum, dabai, GC-MS, biological activities, terpenoids

INTRODUCTION

Terpenoids are classified as secondary metabolites with carbon backbone-containing molecular structures made up of isoprene (2-methylbuta-1,3-diene) units. In growth and development, thousands of terpenoids produced by plants have no discernible role and thus are classified as "secondary" metabolites. Important medicinal activities are shown by the terpenoids group such as antiviral, antibacterial, antimalarial, antiinflammatory, cholesterol synthesis inhibition and anticancer (Mahato & Sen 1997).

It has been shown that plants of the genus Canarium contain different biological activities, such as antioxidant, antibacterial, antifungal, antitumour, antiinflammatory, hepatoprotective, analgesic and antidiabetic (Mogana & Wiart 2011; Basri & Nor 2014). To date, only several biological studies had been conducted to investigate the properties of *C. odontophyllum* Miq. *Canarium odontophyllum* Miq. or locally known as "dabai" is an indigenous fruit to Sarawak, Malaysia and devoured as snack food by the natives (Latiff *et al.* 2000). Dabai fruit comprises of edible skin (5–6%) and flesh (54–60%), and kernel (35–40%). However, *C. odontophyllum* Miq. is classified as an underutilised fruit and has not been fully explored due to lack of promotion. Our study investigated and determined the terpenoids from *C. odontophyllum* leaf hexane and ethanol crude extracts.

MATERIALS AND METHODS

Plant Sample



Figure 1: Leaves of C. odontophyllum Miq. (dabai).

Fresh leaves of *C. odontophyllum* Miq. (Figure 1) were collected from Kuching, Sarawak, Malaysia in December 2019. The permit for export, and the permit for research and development were obtained from Sarawak Biodiversity Centre with permit number SBC-2020-EP-58-MWH and SBC-2019-RDP-20-MWH, respectively. The leaf was deposited in Universiti Kebangsaan Malaysia (UKM) Herbarium with voucher number ID028/2020.

Preparation of C. odontophyllum Miq. Leaf Extracts

The plant leaves were air dried for about 3 days at room temperature. A commercial grinder was used to grind the dried leaves. In order to obtain different extracts, extraction was done using solvents of different polarities, specifically n-hexane and ethanol. Then, the extracts were evaporated using Rotavapor (Buchi) to dryness (Basri *et al.* 2014).

GC-MS Determination of Terpenoids

The Marina *et al.* (2013) method was used. Analyses were performed on an Agilent 7890 series Gas Chromatograph coupled to an Agilent 5975 N MSD quadrupole mass spectrometer (Agilent Technologies). Compounds extracted from various extracts were identified based on the GC retention time on the HP-5MS column and the matching of the spectra with standard computer software data (Replib and Mainlab GC-MS systems data) and cross-matched with the massfinder terpenoids library (Dr Hochmuch scientific consulting).

RESULTS AND DISCUSSION

Various crude extracts from *C. odontophyllum* Miq. leaves have unique physical features. The ethanol extract was green to dark green with a sweetened, strong scent. Extraction using n-hexane yielded 0.624% while ethanol yielded 9.9%.

Figure 2 shows the chromatograms for both *C. odontophyllum* Miq. leaf hexane and ethanol extracts after GC-MS analysis.



Figure 2: GC-MS chromatogram of (a) hexane extract and (b) ethanol extract of C. odontophyllum Miq. leaf.

Based on the GS-MS analysis, the terpenoids content were 36.67% and 14% for hexane and ethanol extracts, respectively. The terpenoids detected are listed in Table 1. It was shown that hexane extract contains richer amount and number of terpenoids compared to the ethanol extract. Hence, for any study regarding plant terpenoids, hexane extract will be the best choice to study properties of terpenoids as major constituent.

· · · · ·	C. odontophyllum Hexane Extract	C. odontophyllum
		Ethanol Extract
Terpenoids	alpha-cadinol, alpha-pinene, beta-bisabolene, beta- humulene, gamma-himachalene, gamme-muurolene, 1-nonadecene, 2-methyltetracosane, 6,10,14- trimethyl 2-pentadecanone, alloaromadendrene, dehydro-aromadendrene, caryophyllene, copaene, D- limonene, decane, dodecane, humulene, methyl stearate, n-hexadecanoic acid, nonadecane, nonanal, nonanoic acid, 3-ethyl-5-(2-ethylbutyl)-octadecane & octadecanoic acid.	beta-bisabolene, aromandendrene, camphene, caryophyllene, n- hexadecanoic acid, octadecanoic acid, pentadecanoic acid, phytol & tetradecanoic acid.

hullum Mig loof (a) h al (la) at la

n-Hexadecanoic acid, phytol and octadecanoic acid were the major terpenoids constituents of C. odontophyllum Miq. leaf (Table 2). n-Hexadecanoic acid (20.22%), phytol (8.74%) and octadecanoic acid (7.54%) were predominant in hexane extract, while phytol (21.02%) and n-hexadecanoic acid (14.52%) were major in ethanol extract.

Table 2: Major terpenoids in hexane and ethanol extracts of Canarium odontophyllum Miq. leaf with claimed biological activities

Retention Time	Library/ID	Qual	Biological Activities Claimed
(RT)	Terpenoids		
30.527 (Hex)	n-Hexadecanoic	99	Antioxidant, antitumor (nasopharynx),
26.344 (EtOH)	acid ($C_{16}H_{32}O_2$)	99	tumor necrosis production inhibitor factor.
			(U.S. Department of Agriculture, Agricultural
			Research Service 2019)
26.792 (Hex)	Phytol (C ₂₀ H ₄₀ O)	91	Antimicrobial, antiinflammatory, diuretic,
28.066 (EtOH)		96	anticancer. (U.S. Department of Agriculture,
			Agricultural Research Service 2019)
29.132 (Hex)	Octadecanoic	91	Antifungal, antitumor activity, antibacterial.
	acid ($C_{18}H_{36}O_2$)		(Hsouna <i>et al.</i> 2011; Geha <i>et al.</i> 2009)

The C. odontophyllum Miq. leaf would offer promising therapeutic effects based on the content of terpenoids found in it. Our study could also help to predict the terpenoids structure and formula of biomolecules. In addition, further research may lead to the isolation and purification of terpenoids from bioactive compounds and their structural elucidation by screening for their biological activities will be beneficial for further drug development.

CONCLUSION

Canarium odontophyllum Miq. leaf hexane extract contains higher amount of terpenoids than the ethanol extract and will be beneficial for further research and drug development. Nevertheless, the isolation and biological activity of individual terpenoids will certainly yield rewarding findings, thus will open up new areas of research into particular compounds and their pharmacological potential.

ACKNOWLEDGEMENTS

We would like to acknowledge the financial support from Universiti Kebangsaan Malaysia via Dana Impak Perdana (DIP-2018-034).

REFERENCES

- Basri DF & Nor NHM. (2014). Phytoconstituent screening and antibacterial activity of the leaf extracts from *Canarium odontophyllum* Miq. *American Journal of Plant Sciences* 2014(5): 2878–2888.
- Basri DF, Heng KY, Meng CK & Ghazali AR. (2014). Screening of antioxidant phytoextracts of *Canarium odontophyllum* Miq. leaves *in vitro*. *IOSR Journal of Pharmacy* 4(12): 1–6.
- Gehan MA, Hanan AE, Hassan AHI, & Okbah MA. (2009). Marine natural products and their potential applications as antiinfective agents. *World Sciences Journal* 7(7): 872–880.
- Hsouna AB, Trigie M, Mansour RB, Jarraya RM, Damak M & Jaoua S. (2011). Chemical composition, cytotoxicity effect and antimicrobial activity of *Ceratonia silisqua* essential oil with preservative effects against listeria inoculated in minced beef meat. *International Journal of Food Microbiology* 148(1): 66–72.
- Latiff A, Faridah HI & Zakri AH. (2000). The importance of biodiversity conservation research and collaboration in the ASEAN: A view from ASEAN scientists. Pp. 24 in *Proceedings of the First Scientific Planning Conference & Report of the Second Scientific Experts Meeting (SEC) Meeting of ARCBC*, Kuala Lumpur.
- Mahato SB & Sen S. (1997). Advances in triterpenoid research, 1990–1994. *Phytochemistry* 44: 1185–236.
- Marina DC, Carmela I & Emmanuel M. (2013). Determination of terpenoids in plant leaves by GC-MS: Development of the method and application to *Ocimum basilicum* and *Nicotiana langsdorffii*. *Analytical Letters* 46(4): 630–639.
- Mogana R. & Wiart C. (2011). *Canarium* L.: A phytochemical and pharmacological review. *Journal* of Pharmaceutical Research 4(8): 2482–2489.
- U.S. Department of Agriculture, Agricultural Research Service. (2019). Dr. Duke's Phytochemical and Ethnobotanical Databases. Retrieved on 12 September 2018 from https://phytochem.nal.usda.gov/phytochem/search/list

VOLATILE CONSTITUENTS, ANTIINFLAMMATORY AND ANTICOLLAGENASE EFFECT OF ESSENTIAL OILS FROM FOUR CYMBOPOGON SPECIES

Mailina J¹, Mazura MP¹, Shalini M¹, Mohd Shafik Yuzman T¹, Abd Majid J¹, Saidatul Husni S¹, Mohd Faridz ZP¹, Khairul Anis Athirah K², Husna Sabrina M³ & Nor Azah MA¹

¹Natural Products Division, Forest Research Institute Malaysia (FRIM), 52109 Kepong, Selangor.
²Faculty of Electrical Engineering, Universiti Teknologi MARA (UiTM), 40450 Shah Alam, Selangor.
³Department of Plant Science, Kulliyyah of Science, International Islamic University Malaysia (IIUM), Jalan Sultan Ahmad Shah, Bandar Indera Mahkota, 25200 Kuantan, Pahang.

Tel: 03-62797349 Fax: 03-62729805 Email: mailina@frim.gov.my

ABSTRACT

The genus of Cymbopogon is a member of the Poaceae (Gaminae) family, popularly known for their high content of essential oil. In this study, leaves of C. winterianus, C. martini, C. nardus and C. citratus were extracted to produce the essential oil through lab-scale hydrodistillation technique. GC and GC/MS analyses revealed the presence of monoterpene and sesquiterpene compounds in each of the essential oils. Geranyl acetate (57.6%), linalool (3.6%), citronellal (3.2%) and citronellol (0.3%) were identified as major compounds in C. winterianus oil. The main compounds for C. martini oil were geraniol (15.6%), methyl eugenol (10.2%), (E)-methyl isoeugenol (10.1%), borneol (4.4%), citronellal (4.2%) and citronellol (3.4%). Cymbopogon nardus oil contained geraniol (40.8%), citronellal (15.7%), (E)-citral (9.4%), citronellol (8.8%) and (Z)-citral (5.7%) as major compounds. Meanwhile, C. citratus oil was rich in (Z)-citral (30.7%) and (E)-citral (37.9%), followed by geranial (5.1%) and myrcene (3.0%). These oils were screened for antiinflammatory activity by in vitro assays, namely antihyaluronidase, antixanthine oxidase, antilipoxygenase and antiprotein denaturation assays, as well as anticollagenase assay for antiaging effect. Cymbopogon citratus oil showed the highest percentage of inhibition against lipoxygenase activity (84.45 ± 2.85%), followed by C. martini which exhibited moderate inhibition effect (41.08 \pm 2.70%). All essential oils showed low activity for inhibition of xanthine oxidase and hyaluronidase. No inhibition of protein denaturation was observed for C. winterianus and C. citratus, while C. martini and C. nardus demonstrated negligible activity. All essential oils exhibited profound anticollagenase activity (57–74%) except C. nardus oil (21%). These findings provided evidence that the Cymbopogon oils could be used as an active ingredient in product formulation for antiinflammatory and antiaging effects.

Keywords: Cymbopogon spp., essential oil, volatile compounds, antiinflammatory, antiaging

INTRODUCTION

Cymbopogon spp. belong to the family Poaceae (Graminae). They comprised of nearly 140 species reported to be found in Africa, India, Australia, America, Europe and South Asia (*Nakahara et al. 2013; Wany et al. 2013). They are known worldwide for their high content of essential oil.* In different countries, traditional use of *Cymbopogon* spp. showed a range of applications including as insect repellent, insecticide, common tea, flu control and medicinal supplement (Shah *et al.* 2011; Wany *et al.* 2013; Avoseh *et al.* 2015). Pharmacological activities such as antiamoebic, antibacterial, antidiarrhoeal, antiinflammatory, antiobesity, antinociceptive, antimalarial, antifungal, antianxiety and antioxidant have been reported in *Cymbopogon* spp. (Wannissorn *et al.* 2005; Shah *et al.* 2012; Nishijima *et al.* 2014; Kusmardiyani *et al.* 2016; Miral *et al.* 2016). Essential oils from *Cymbopogon* spp. are commonly used in the formulation of skincare products. The discovery of new natural inhibitors of pro-inflammatory and pro-aging enzymes could be interesting for the formulation of active and safe cosmetic ingredients for skin protection. In this

study, the essential oils from selected *Cymbopogon* species, namely *C. winterianus*, *C. nardus*, *C. martinii* and *C. citratus* were screened for volatile compounds and *in vitro* antiinflammatory as well as antiaging activities. The results obtained will be useful to justify the use of these plants as antiinflammatory and antiaging agents for further studies in product development.

MATERIALS AND METHODS

Collection and Preparation of Sample

The fresh leaf samples of *C. winterianus, C. nardus* and *C. martinii* were collected from MARDI Linggi, Melaka. While *C. citratus* leaf samples were collected from Branang, Negeri Sembilan. The samples were air-dried for 2 days until the moisture content of samples reached 30–40%. Then, they were cut into small pieces before being weighed and subjected to water distillation technique for 6 hours using *Clavenger*-type apparatus. The oils were collected and isolated from their hydrosol using anhydrous sodium sulphate. The pure oils were kept in a fridge prior to further analysis.

GC and GC/MS Analysis

Analysis of the oils was conducted by Gas Chromatography (GC) using Shimadzu GC-2010 Plus capillary chromatograph which was equipped with a flame ionisation detector (FID) and the split/splitless mode injection technique was used under the following conditions: carrier gas helium; similar temperature for injector and detector at 250° C. A non-polar capillary column BP-5 (30 m by 0.25 mm, film thickness 0.25 µm) was used and the operating conditions were as follows: initial oven temperature, 60° C for 10 min, up to 230° C at 3° C/min and then 230° C for 10 min. Gas Chromatography/Mass Spectrometry (GC/MS) analysis was conducted on Agilent Technologies GCMS 7890A/5975C Series MSD under similar conditions as described in GC programs using HP-5MS column (30 m by 0.25 mm, film thickness 0.25 µm). The chemical constituents were identified by comparison of retention times and calculated Kovats indices with reference and matching their mass spectra with database library (HPCH2205.L; Wiley7Nist05.L; NIST05a.L).

Bioactivity Tests

In vitro Antiinflammatory Activity

The antiinflammatory activity of essential oils of *Cymbopogon* spp. were evaluated using 4 *in vitro* assays, namely lipoxygenase inhibition, xanthine oxidase inhibition, hyaluronidase inhibition and protein denaturation inhibition assays according to methods of Azhar *et al.* (2004), Noro *et al.* (1983), Ling *et al.* (2003) and Williams *et al.* (2008), respectively, with minor modifications. The results were expressed as mean of the percentage inhibition ± standard error of mean (SEM) of at least 3 separate independent experiments measured in triplicate.

Collagenase Inhibitory Assay (Antiaging Activity)

The anticollagenase assay was adopted from Thring *et al.* (2009) and was slightly modified. This assay was performed in tricine buffer pH 7.5 (50 mM tricine with 10 mM CaCl₂ and 400 mM sodium chloride, pH 7.5 at 25°C). The reaction mixture (150 μ L total volume) contained 37 μ L tricine buffer and 20 μ L of collagenase enzyme (0.1 U/MI) in a 96 well plate, in triplicates. A 50 μ L of essential oil (10 mg/mL) was added into 96 well microtiter plate and pre-incubated for 10 min at 25°C. Then, 60 uL of FALGPA (1 mM) substrate was added into all the wells except blank and the absorbance was measured at 340 nm using a spectrophotometer.

RESULTS AND DISCUSSION

The essential oil from the leaves of *C. winterianus, C. martini, C. nardus* and C. *citratus* were extracted by hydrodistillation and yielded 3.36, 1.31, 3.27 and 0.87% v/w, respectively (on dry weight basis). The essential oils were subjected to GC and GC/MS analysis. Table 1 shows the major compounds of each essential oil. Geranyl acetate (57.6%), linalool (3.6%), citronellal (3.2%) and citronellol (0.3%) were identified as major compounds in *C. winterianus* oil. The main compounds for *C. martini* oil were geraniol (15.6%), methyl eugenol (10.2%), (*E*)-methyl isoeugenol (10.1%), borneol (4.4%), citronellal (4.2%) and citronellol (3.4%). *Cymbopogon nardus* oil contained geraniol (40.8%), citronellal (15.7%), (*E*)-citral (9.4%), citronellol (8.8%) and (*Z*)-citral (5.7%) as major compounds. Citronellal is responsible for their distinctive lemony scent (Wany *et al.* 2013). Meanwhile, *C. citratus* oil was rich in (*Z*)-citral (30.7%) and (*E*)-citral (37.9%). Citral is one of the widely used raw material in perfumery, confectionery and vitamin A production industries (Khanuja *et al.* 2005).

	Chemical Name	RT	Percentage (%)			
No.	No. Chemical Name		C. winterianus	C. martini	C. nardus	C. citratus
1	Camphene	946	-	3.28	-	-
2	6-Methyl-5-hepten-2-one	981	0.06	0.04	0.17	1.72
3	Myrcene	988	-	0.31	-	2.97
4	Limonene	1024	1.74	4.21	-	-
5	Linalool	1095	3.61	0.78	1.09	1.48
6	Citronellal	1148	3.29	4.24	15.73	-
7	Borneol	1165	-	4.36	-	-
8	α -Terpineol	1186	0.16	1.06	-	-
9	Citronellol	1223	0.27	3.43	8.81	0.44
10	Neral	1235	1.87	0.76	5.65	30.73
11	Geraniol	1249	2.79	15.6	40.75	5.04
12	Geranial	1264	3.16	1.18	9.93	38.31
13	Citronellyl acetate	1350	3.66	0.88	0.83	-
14	Eugenol	1356	1.20	0.03	1.94	-
15	Geranyl acetate	1379	57.62	2.98	2.39	2.02
16	β-Elemene	1389	-	1.12	0.04	-
17	Methyl eugenol	1403	-	10.18	-	-
18	β -Caryophyllene	1417	4.45	0.21	1.15	0.11
19	α -cis-Bergamotene	1432	-	6.43	-	0.73
20	(E)-Methyl isoeugenol	1491	-	10.08	0.09	-
21	γ-Cadinene	1513	4.04	-	2.93	-
22	Elemol	1548	7.00	4.19	0.84	-
23	γ-Eudesmol	1630	0.43	1.54	0.09	-
24	α -Eudesmol	1652	0.59	1.41	0.15	-

Table 1: Major chemical constituents of Cymbopogon spp. essential oils

Table 2 shows the findings for antiinflammatory and antiaging effect of all essential oils. They were screened for antiinflammatory activity by means of in vitro assay of antihyaluronidase, antixanthine oxidase, antilipoxygenase and antiprotein denaturation as well as anticollagenase assay for antiaging effect. At the final concentration of 100 µg/mL, C. citratus oil elicited the highest inhibition of the enzyme lipoxygenase (84.45 ± 2.85%) while C. martini demonstrated moderate inhibition effect (41.08 ± 2.70%). The possible association between the observed inhibition of enzyme lipoxygenase and essential oil composition (Table 1) could be due to 2 main components identified as geranial and neral, representing more than 30% of the total content in C. citratus. Liao et al. (2015) showed that both neral and geranial demonstrated better efficacy in inhibiting the expression of the proinflammatory mediators. These compounds also elicited significant in vivo antiallergic and antiinflammatory effects by supressing an immunoglobulin E (IgE)-induced passive cutaneous anaphylactic reaction in mice and a 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced inflammatory mouse ear oedema, respectively (Mitoshi et al. 2014). All essential oils showed low activity for inhibition of xanthine oxidase and hyaluronidase enzymes. There was no inhibition of protein denaturation of C. winterianus and C. citratus. While, C. martini and C. nardus only inhibited at low percentage. For anticollagenase assay, all essential oils at the final concentration of 2.5 mg/mL had high collagenase inhibiting activity (57–74%) except for C. nardus oil (21%). Thus, the essential oils of Cymbopogon spp. have potential capabilities to protect the degradation of collagen from collagenase, and slows down aging symptoms.

		Antiaging			
Species	Lipoxygenase (% ± SEM) ^a	Xanthine Oxidase (% ± SEM) ^a	Hyaluronidase (% ± SEM) ^a	Protein Denaturation (% ± SEM) ^a	Anticollagenase (% ± SEM) ^b
C. winterianus	5.97 ± 3.45	5.04 ± 3.59	2.38 ± 0.23	NA	71.99 ± 6.53
C. nardus	22.51 ± 4.93	8.49 ± 1.76	2.33 ± 0.90	3.64 ± 1.47	21.78 ± 0.20
C. martinii	41.08 ± 2.70	3.33 ± 1.95	1.63 ± 0.33	1.20 ± 0.75	57.14 ± 3.43
C. citratus	84.45 ± 2.85	3.99 ± 2.00	1.80 ± 0.19	NA	74.40 ± 5.23
Positive controls					
NDGA	97.62 ± 1.19	-	-	-	-
Allopurinol	-	99.77 ± 0.22	-	-	-
Apigenin	-	-	82.58 ± 6.04	-	-
Diclofenac sodium	-	-	-	93.18 ± 0.93	-
EGCG	-	-	-	-	> 100.00

Table 2: In vitro antiinflammato	ry and antiaging activities

Notes: Values are expressed as mean inhibition (%) \pm Standard Error Mean (SEM) of triplicate measurements from 3 independent experiments. ^aFinal concentration of samples/positive controls in reaction mixture was fixed at 100 µg/mL. ^bFinal concentration of samples/positive controls in reaction mixture was fixed at 2.5 mg/mL.

CONCLUSION

The monoterpene composition of essential oils of *Cymbopogon* species markedly varied among the species. Monoterpenes are responsible for the characteristic odours of essential oils and scents of *Cymbopogon* species. The fact that antiinflammatory and antiaging assays used in this study involved different inflammatory mechanisms, led us to suggest a possible mechanism of action for these essential oils. This finding suggests that the mechanism involved in the antiinflammatory effect of essential oil from *C. citratus* may be related to inhibition of enzymes involved in the production of proinflammatory leukotrienes. Neral and geranial which represented major constituents in the plant, are the bioactive components conferring the biological activity.

ACKNOWLEDGEMENT

We thankfully acknowledge the financial support of FRGS grant (FRGS/1/2018/WAB01/NRE/1). We would like to express heartfelt thanks to all staff from MARDI Linggi, Melaka involved in collecting the plant materials and to all staff from Natural Product Division FRIM for their technical assistance and encouragements during the work.

REFERENCES

- Avoseh O, Oyedeji O, Rungqu P, Nkeh-Chungag B & Oyedeji A. (2015). *Cymbopogon* Species; ethnopharmacology, Phytochemistry and the pharmacological importance. *Molecules* 20: 7438–7453.
- Azhar-Ul-Haq, Malik A, Anis I, Khan SB, Ahmed E, Ahmed Z, Nawaz SA & Choudhary MI. (2004). Enzymes inhibiting lignans from *Vitex negundo*. *Chem. Pharm. Bull*. 52(11): 1269–1272.
- Khanuja SPS, Shasany AK, Pawar A, Darokar RKLMP, Naqvi AA, Rajkumar S, Sundaresan V, Lal N & Kumar S. (2005). Essential oil constituents and RAPD markers to establish species relationship in *Cymbopogon* spreng. (Poaceae). *Biochemical Systematics and Ecology* 33: 171–186.
- Kusmardiyani S, Fitria A & Irda F. (2016). Antioxidant profile and phytochemical content of three kinds of lemon grass grown in West Java-Indonesia. *Asian Journal of Pharmaceutical and Clinical Research* 9(4): 381–385.
- Liao PC, Yang TS, Chou JC, Chen J, Lee SC, Kuo YCH, Ho CL & Chao LKP. (2015). Antiinflammatory activity of neral and geranial isolated from fruits of *Litsea cubeba* Lour. *J. Functional Food* 19: 248–258.
- Ling SK, Takashi T & Isao K. (2003). Effects of iridoids on lipoxygenase and hyaluronidase activities and their activation by β -Glucosidase in the presence of amino acids. *Biol. Pharm. Bull.* 26(3): 352–356.
- Miral RT, Jigisha KP & Meghal AD. (2016). Isolation of essential oil from the leaves of *Cymbopogon martinii* using hydrodistillation: Effect on yield of essential oil, yield of geraniol and antimicrobial activity. *Journal of Essential Oil Bearing Plants* 19(8): 1943–1956.
- Mitoshi M, Kuriyama I, Nakayam H, Miyazato H, Kobayashi Y, Jippo T, Yoshida H & Mizushima Y. (2014). Supression of allergic and inflammatory responses by essential oils derived from herbal plants and citrus fruits. *Int. J. Molec. Med.* 33: 1643–1651.
- Mukherjee PK, Maity N, Nema NK & Sarkarm BK. (2011). Bioactive compounds from natural resources against skin ageing. *Phytomedicine* 19: 64–73.
- Nakahara K., Alzoreky NS, Yoshihashi T, Nguyen HTT & Trakoontivakorn G. (2013). Chemical composition and antifungal aactivity of essential oil from *Cymbopogon nardus* (citronella grass). *Japan Agricultural Research Quarterly* 37 (4): 249–252.
- Nishijima CM, Ganev EG, Mazzardo-Martins L, Martins DF, Rocha LRM, Santos ARS & Hiruma-Lima CA. (2014). Citral: A monoterpene with prophylactic and therapeutic anti-nociceptive effects in experimental models of acute and chronic pain. *European Journal of Pharmacology* 736: 16–25.
- Noro T, Oda Y, Miyase T, Ueno A & Fukushima S. (1983). Inhibitors of xanthine oxidase from flowers and buds of *Daphne genkwa*. *Chem. Pharm. Bull.* 31: 3984–3987.
- Shah G, Kaur M, Dhabiliya F ,& Shri R. (2012). Pharmacognostic Standardisation of *Cymbopogon citratus* (dc.) stapf leaves. *Pharmacognosy Journal* 2 (29): 19–25.
- Shah G, Shri R, Panchal V, Sharma N, Singh B ,& Mann AS. (2011). Scientific basis for the therapeutic use of Cymbopogon citratus, stapf (lemon grass). Journal of Advanced Pharmaceutical Technology & Research 2(1): 3–8.
- Wannissorn B, Jarikasem S & Siriwangchai T. (2005). Antifungal activity of lemon grass and lemon grass oil cream. *Phytotherapy Research* 10: 551–554.

- Wany A, Jha S, Vinod KN & Dev MP. (2013). Chemical analysis and therapeutic uses of citronella oil from *Cymbopogon winterianus*: A short review. *International Journal of Advanced Research* 1(6): 504–521.
- Williams LAD, Connar AO, Latore L, Dennis O, Ringer S, Whittaker JA, Conrad J, Vogler B, Rosner H & Kraus W. (2008). The *in vitro* anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals in the early stages of drug discovery process. *West Indian Medical Journal* 57(4): 327–331.

This book is a collection of short scientific papers on traditional knowledge and natural product research and innovations from researchers in Malaysia. The basis of this publication stemmed from the passion for knowledge-sharing. It is our humble desire to share the work conducted by these scientists to be reviewed as reference by others.



