BIOACTIVE PHENOLIC COMPOUNDS FROM PLANTS TRADITIONALLY USED TO TREAT MICROBIAL SKIN INFECTIONS

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by

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LIST OF SYMBOLS AND ABBREVIATIONS

ABTS	2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)
AChE	Acetylcholinesterase
ANOVA	analysis of variance
ATCC	American Type Culture Collection
CFU	colony-forming unit
COX	Cyclooxygenase
DMSO	dimethyl sulphoxide
DPPH	2,2-diphenyl picryl-hydrazyl
EDTA	ethylene diamine tetracetic acid
EC_{50}	effective concentration at 50% of activity
FRAP	ferric reducing antioxidant power
GAE	gallic acid equivalent
HCl	hydrochloride acid
HPLC	high performance liquid chromatography
LC-MS	liquid chromatography – mass spectrometry
LOX	Lipoxygenase
MIC	minimum inhibition concentration
MS	mass spectrometer
NA	nutrient agar
NB	nutrient broth
nm	Nanometer
NSAIDs	nonsteroidal anti-inflammatory drugs
OH	Hydroxyl
PDA	photo diode array
PDA	potato dextrose agar
PDB	potato dextrose broth
PG	Postraglandin
R^2	linear regression coefficient
ROS	reactive oxygen species
Rt	retention time
SD	standard deviation
SPSS	Statistical Package for the Social Sciences

TPC	total phenolic content
TPTZ	2,4,6-tri (2-pyridyl)-s-triozine
UPLC	ultra performance liquid chromatography
UV	Ultraviolet
v/v	volume to volume
w/v	weight to volume
λmax	lambda maximum

SEBATIAN FENOL BIOAKTIF DARIPADA TUMBUHAN YANG DIGUNAKAN SECARA TRADISI UNTUK MERAWAT JANGKITAN MIKROB PADA KULIT

ABSTRAK

Kajian ini pada mulanya bertujuan untuk mengesahkan nilai terapeutik tumbuhan yang digunakan secara tradisional untuk merawat jangkitan mikrob pada kulit. Berdasarkan hipotesis bahawa ekstrak harus mempunyai aktiviti antimikrob dan antioksida untuk mempercepatkan penyembuhan kulit, sebanyak 120 ekstrak fenolik telah disaring untuk kedua-dua aktiviti. Aktiviti antimikrob telah ditentukan terhadap sembilan mikroorganisma patogenik terhadap kulit. Ekstrak daun *Anacardium occidentale, Cassia alata* dan *Terminalia catappa* telah didapati mempunyai spektrum aktiviti antimikrob yang luas. Potensi antioksidan primer telah ditentukan dengan menggunakan 2,2-difenil-1-pikrilhidrazil (DPPH) dan 2,2'-azino-bis (asid 3-ethylbenzthiazoline-6-sulfonik) (ABTS) yang menyingkirkan radikal bebas, dan potensi antioksidan penurunan ferik (FRAP) asai, dan asai pengkelatan digunakan untuk mengukur aktiviti antioksidan sekunder. Ekstrak daun *T. catappa* menunjukkan aktiviti antioksidan yang lebih tinggi dalam semua asai. Enam ekstrak daun yang mempunyai kedudukan tertinggi bagi kedua-dua aktiviti tertinggi (daripada *Macaranga triloba, Punica granatum, Rosa chinensis, Rhodomyrtus tomentosa, Syzygium jambos* dan *T. catappa*) menunjukkan aktiviti anti-radang yang sederhana apabila diuji menggunakan asai perencatan siklooksigenase dan 5-lipoksigenase. Pelbagai kajian sebelum ini melaporkan aktiviti yang lebih tinggi daripada ekstrak terhidrolisis daripada ekstrak mentah, oleh itu enam ekstrak ini juga telah dihidrolisiskan untuk penilaian lanjut. Ekstrak terhidrolisis *R. tomentosa*

didapati mempunyai aktiviti antimikrob yang paling tinggi terhadap *Corynebacterium minutissimum* ATCC 23348, *Micrococcus luteus* ATCC 4698, *Staphylococcus aureus* ATCC 12600 and *Candida albicans* ATCC 10231, dengan kepekatan perencatan minimum (MIC) 15.63 μg/mL. Aktiviti antioksidan primer tertinggi telah ditunjukkan oleh ekstrak terhidrolisis *P. granatum*, manakala ekstrak mentah *T. catappa* memberikan aktiviti antioksidan sekunder tertinggi. Ekstrak mentah dan terhidrolisis *A. occidentale* dan *C. longa* didapati memberi aktiviti yang lebih tinggi berbanding dengan ekstrak lain dengan nilai MIC antara 125 μg/mL dan 1000 μg/mL terhadap *Pseudomonas aeruginosa* (ATCC 27853, ATCC 15442 dan ATCC 9027). Sebatian fenolik dalam 12 ekstrak terhidrolisis telah dikesan dengan menggunakan Kromatografi Cecair Berprestasi Ultra (UPLC) dengan pengesan diod pelbagai dan telah disahkan dengan menggunakan Kromatografi Cecair – Spektrometri Jisim (LC-MS). Antara empat sebatian fenolik (mirisetin, kuersetin, kaempferol dan asid galik) yang sering dikenal pasti daripada ekstrak terhidrolisis dan glikosidanya (mirisetin-3-*O*-rhamnosida, kuersetin-3-*O*-rhamnosida, kuersetin-3-*O*-glukosida dan kaempferol-3-*O*-glukosida), mirisetin menunjukkan aktiviti yang paling tinggi terhadap semua bakteria ujian dan *C. albicans* dengan nilai MIC adalah antara 7.81 μg/mL dan 62.5 μg/mL. *P. aeruginosa* juga mudah terdedah kepada mirisetin dengan nilai MIC 31.25 μg/mL. Oleh itu, mirisetin telah dikenal pasti sebagai sebatian fenolik bioaktif dalam ekstrak.

BIOACTIVE PHENOLIC COMPOUNDS FROM PLANTS TRADITIONALLY USED TO TREAT MICROBIAL SKIN INFECTIONS

ABSTRACT

This study was initially aimed to verify the therapeutic values of plants that are traditionally used to treat microbial skin infections. Based on hypothesis that the extracts should have antimicrobial and antioxidant activities in order to accelerate the healing of skin, a total of 120 phenolic extracts were screened for both activities. The antimicrobial activities were determined against nine skin pathogenic microorganisms. The leaf extracts of *Anacardium occidentale*, *Cassia alata* and *Terminalia catappa* were found to possess a broad spectrum of antimicrobial activities. The primary antioxidant potentials were determined using 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) free radical scavenging, and ferric reducing antioxidant potential (FRAP) assays, and metal chelating assay was used to measure the secondary antioxidant activity. The extract of *T. catappa* showed higher activities in all assays. Six leaf extracts that have higher rank for both activities (from *Macaranga triloba*, *Punica granatum*, *Rosa chinensis*, *Rhodomyrtus tomentosa*, *Syzygium jambos* and *T. catappa*) showed moderate anti-inflammatory activities when determined using cyclooxygenase and 5-lipoxygenase inhibitory assays. Many earlier studies reported higher activities of hydrolyzed extracts than crude extracts, hence the six extracts were hydrolyzed for further assessments. The hydrolyzed extract of *R. tomentosa* was found to have the highest antimicrobial activity against *Corynebacterium minutissimum* ATCC 23348, *Micrococcus luteus* ATCC 4698, *Staphylococcus aureus* ATCC 12600 and *Candida albicans* ATCC 10231 with minimum inhibition

concentration (MIC) of 15.63 µg/mL. The highest primary antioxidant activity was shown by the hydrolyzed extract of *P. granatum*, while the crude extract of *T. catappa* gave the highest secondary antioxidant activity. The crude and hydrolyzed extracts of *A. occidentale* and *C. longa* were found to give higher activities compared to other extracts with MIC values ranging between 125 µg/mL and 1000 µg/mL against *Pseudomonas aeruginosa* (ATCC 27853, ATCC 15442 and ATCC 9027). Phenolic compounds in 12 hydrolyzed extracts were detected using Ultra Performance Liquid Chromatography (UPLC) with diode array detector and were confirmed using Liquid Chromatography-Mass Spectrometry (LC-MS). Among four phenolic compounds (myricetin, quercetin, kaempferol and gallic acid) that were commonly identified from the hydrolyzed extracts and their glycosides (myricetin-3-*O*-rhamnoside, quercetin-3-*O*-rhamnoside, quercetin-3-*O*-glucoside and kaempferol-3-*O*-glucoside), myricetin showed the highest activity against all the tested bacteria and *C. albicans* with MIC values ranging between 7.81 µg/mL and 62.5 µg/mL. *P. aeruginosa* was also susceptible to myricetin with MIC value of 31.25 µg/mL. Thus, myricetin was identified to be the bioactive phenolic compound in the extracts.

CHAPTER 1:

INTRODUCTION

1.1 Problem statements

Plants are still used traditionally to treat the microbial skin infections. To date, there is still lack of scientific investigation to verify the therapeutic values of the plants. Moreover, there is still lack of comparative study to determine which plant extracts have higher bioactivities. Thus, this comparative study is needed to verify and rank the antioxidant and antimicrobial activities of the plants. Even though many phenolic compounds have been identified from the bioactive extracts of the plants, many of their bioactivities are not yet determined. Therefore, the antioxidant and antimicrobial activities of common phenolic compounds in the bioactive extracts were determined.

1.2 Treatment for microbial skin infections

Skin serves primarily as a protective barrier of human body (Douglas, 2001). Skin infections can be caused by various types of microorganisms. Bacteria, fungi and viruses are reported to be the main causes of skin infections. Bacteria can cause skin infections such as impetigo, abscess and acne. Candidiasis and ringworm are among the common skin infections caused by fungi, and viral infections will generate skin infections such as chickenpox (measles) and shingles (Goodyear, 2014).

Microbial skin infections can be treated using modern or traditional approaches. Modern treatment for mild infections relies on medications such as benzoyl peroxide, salicylic acid, retinoids, antibacterials (bactroban) and antifungal agents (lamisil and lotrimin). The medications are often applied directly to the skin as medicated ointment, lotion and cream to heal the infections as well as to reduce soreness and itchiness. However, when the infection is severe, oral or intravenous prescribed drugs such as antibiotics (erythromycin and tetracycline) and antifungal agents (ketoconazole) may be needed (Tabassum and Hamdani, 2014).

Plants are still used traditionally to treat various microbial skin infections. Some of the plant extracts have been commercialized. For examples, the essential oil from the leaf of tea tree (*Melaleuca alternifolia*) has been commercialized to treat acne (Carson *et al*., 2006). Most of the plants that are used in treating microbial skin infections are finely pounded and applied as a poultice to the infected skin. The remedial values of the medicinal plants and the traditional recipes on how to formulate the plant ingredients into medicinal poultices have been well compiled and published. Table 1.1 shows a list of 120 plants that are used in this study and the types of skin microbial infections that can be treated by each plant. The selection was made based on their traditional uses to treat various types of microbial skin infections as documented by Ong (2003, 2004a, 2004b, 2008a, 2008b, 2011, 2013); Ong *et al*. (2011) and Sabda (2011).

Treatment using antioxidant agents from plants may involve in healing of the skin infections by assisting in the stimulation of collagen deposition, activation of immune cells, increasing macrophage migration to the injured areas and enhancing tensile strength (Cerci *et al*., 2008). Antimicrobial agents are needed to treat microbial skin infections by inhibiting the skin pathogenic microorganisms.

Table 1.1 List of selected plants and their uses in treating microbial skin infections.

Scientific name	Family	Local name	Part used	Types of microbial skin infection	Reference
Acalypha indica	Euphorbiaceae	Cika mas	Leaves	Abscess, sore	Ong (2013)
Ageratum conyzoides	Asteraceae	Rumput tahi ayam	Leaves	Abscess, itch, sore	Ong (2013)
Allamanda cathartica	Apocynaceae	Bunga loceng	Leaves	Sore, itch	Sabda (2011)
Allium sativum	Amaryllidaceae	Bawang putih	Bulbs	Abscess, eczema, acne	Ong (2008a)
Aloe barbadensis	Xanthorrhoeaceae	Lidah buaya	Leaves	Sore	Sabda (2011)
Alpinia galanga	Zingiberaceae	Lengkuas	Rhizome	Acne	Ong (2008a)
Alstonia scholaris	Apocynaceae	Pulai	Leaves	Acne, ringworm, eczema	Ong (2004b)
Alternanthera sessilis	Amaranthaceae	Keremak	Whole plants	Abscess, eczema	Ong (2003)
Amaranthus spinosus	Amaranthaceae	Bayam duri	Leaves	Abscess, eczema, itch	Ong (2003)
Anacardium occidentale	Anacardiaceae	Gajus	Leaves	Eczema	Ong (2008a)
Antigonon leptopus	Polygonaceae	Bunga berteh	Leaves	Rashes	Ong (2008b)
Archidendron jiringa	Fabaceae	Jering	Leaves	Itch	Ong (2003)
Ardisia crispa	Primulaceae	Mata pelandok	Leaves	Itch	Ong (2004b)
Asystasia gangetica	Acanthaceae	Rumpai jejentik	Leaves	Abscess	Ong (2013)
Averrhoa bilimbi	Oxalidaceae	Belimbing buluh	Leaves	Abscess, acne, itch	Ong (2003)
Averrhoa carambola	Oxalidaceae	Belimbing	Leaves	Abscess, itch	Ong (2004a)
Azadirachta indica	Meliaceae	Mambu	Leaves	Abscess, sore, ringworm	Ong (2008b)
Barringtonia racemosa	Lecythidaceae	Putat	Leaves	Itch	Ong (2004b)
Bridelia insulana	Phyllanthaceae	Kenidai	Leaves	Itch	Ong (2004b)
Caesalpinia pulcherrima	Fabaceae	Bunga merak	Leaves	Acne, rashes	Ong (2008b)
Calophyllum inophyllum	Calophyllaceae	Bintangor laut	Leaves	Abscess, itch	Ong (2004b)
Cananga odorata	Annonaceae	Kenanga	Barks	Abscess, rashes, eczema	Ong (2004b)

Table 1.1 Continued

Scientific name	Family	Local name	Part used	Types of microbial skin infection	Reference
Cardiospermum halicacabum	Sapindaceae	Peria bulan	Leaves	Abscess, itch	Ong (2013)
Cassia alata	Fabaceae	Gelenggang	Leaves	Ringworm	Ong (2013)
Cassia fistula	Fabaceae	Berkesa	Barks	Sore	Ong (2008b)
Casuarina equisetifolia	Casuarinaceae	Ru laut	Barks	Acne	Ong (2004b)
Centella asiatica	Apiaceae	Pegaga	Leaves	Acne	Ong *et al*. (2011)
Chromolaena odorata	Asteraceae	Payung terjun	Leaves	Itch, acne	Ong (2013)
Cleome gynandra	Cleomaceae	Maman	Leaves	Itch	Ong (2003)
Clitoria ternatea	Fabaceae	Bunga telang	Leaves	Abscess, acne	Ong (2008b)
Cordyline terminalis	Asparagaceae	Jenjuang	Leaves	Abscess, sore	Ong (2008b)
Crinum asiaticum	Amaryllidaceae	Bakong	Bulbs	Abscess	Ong (2004b)
Curcuma longa	Zingiberaceae	Kunyit	Rhizome	Abscess, itch, acne	Ong (2008a)
Curcuma zedoaria	Zingiberaceae	Temu lawak	Rhizome	Acne	Ong (2008a)
Dendrobium crumenatum	Orchidaceae	Anggerik merpati	Leaves	Abscess, acne	Ong (2013)
Dicranopteris linearis	Gleicheniaceae	Resam	Leaves	Itch, rashes	Ong (2013)
Diplazium esculentum	Athyriaceae	Paku benar	Leaves	Abscess, acne	Ong (2011)
Donax canniformis	Marantaceae	Bemban	Twigs	Abscess	Ong (2004b)
Drymoglossum piloselloides	Polypodiaceae	Duit-duit	Leaves	Itch	Ong *et al*. (2011)
Duranta repens	Verbenaceae	Duranta	Leaves	Abscess, sore	Ong (2008b)
Eleusine indica	Poaceae	Rumput sambau	Whole plants	Abscess	Sabda (2011)
Emilia sonchifolia	Asteraceae	Tetambak merah	Leaves	Abscess, sore	Ong (2013)
Erythrina variegata	Fabaceae	Dedap	Leaves	Abscess, sore	Ong (2004b)

Table 1.1 Continued

Scientific name	Family	Local name	Part used	Types of microbial skin infection	Reference
Etlingera elatior	Zingiberaceae	Bunga kantan	Leaves	Abscess	Ong (2008a)
Euphorbia hirta	Euphorbiaceae	Ara tanah	Whole plants	Abscess	Ong (2013)
Ficus pumila	Moraceae	Ara jalar	Leaves	Abscess, acne	Ong (2008b)
Gliricidia sepium	Fabaceae	Bunga jepun	Leaves	Itch, sore	Ong (2008b)
Gomphrena globosa	Amaranthaceae	Bunga butang	Whole plants	Sore, ringworm	Ong (2008b)
Gynura procumbens	Asteraceae	Akar sebiak	Leaves	Rashes	Ong (2008b)
Hedyotis corymbosa	Rubiaceae	Siku dengan	Leaves	Abscess, sore	Ong (2013)
Hibiscus rosa-sinensis	Malvaceae	Bunga raya	Leaves	Abscess, itch	Ong (2008b)
Hibiscus tiliaceus	Malvaceae	Bebaru bulu	Leaves	Abscess, rashes	Ong (2004b)
Homalomena sagittifolia	Araceae	Kemoyang	Leaves	Itch	Sabda (2011)
Hyptis suaveolens	Lamiaceae	Kemangi	Leaves	Abscess, acne, rashes	Ong (2008a)
Ipomoea cairica	Convolvulaceae	Seri pagi jalar	Leaves	Abscess	Ong (2008b)
Ixora chinensis	Rubiaceae	Jarum-jarum	Leaves	Abscess	Ong (2008b)
Justicia gendarussa	Acanthaceae	Gandarusa	Leaves	Abscess, itch	Ong (2008b)
Kaempferia galanga	Zingiberaceae	Cekur	Rhizome	Sore, acne	Ong (2008a)
Lawsonia inermis	Lythraceae	Inai	Leaves	Abscess, ringworm	Ong (2008b)
Leucaena leucocephala	Fabaceae	Petai belalang	Peels	Rashes	Ong (2003)
Lygodium flexuosum	Lygodiaceae	Ribu-ribu besar	Leaves	Abscess, ringworm	Ong (2013)
Macaranga triloba	Euphorbiaceae	Mahang	Leaves	Abscess	Sabda (2011)
Mallotus macrostachyus	Euphorbiaceae	Balik angin	Leaves	Abscess	Ong (2004b)
Melaleuca cajuputi	Myrtaceae	Gelam	Leaves	Abscess, itch	Ong (2004b)
Melastoma malabathricum	Melastomataceae	Senduduk	Leaves	Abscess, sore	Ong (2013)

Table 1.1 Continued

Scientific name	Family	Local name	Part used	Types of microbial skin infection	Reference
Michelia alba	Magnoliaceae	Cempaka putih	Leaves	Abscess	Ong (2008b)
Microcos tomentosa	Malvaceae	Cenderai	Twigs	Itch	Sabda (2011)
Mikania cordata	Asteraceae	Selaput tunggul	Leaves	Abscess, itch, rashes, sore	Ong (2013)
Mimusops elengi	Sapotaceae	Tanjung	Barks	Acne, skin disorders	Ong (2004b)
Mirabilis jalapa	Nyctaginaceae	Bunga pukul empat	Leaves	Abscess, acne, sore	Ong (2008b)
Momordica charantia	Cucurbitaceae	Peria katak	Fruits	Abscess, sore	Ong (2011)
Morinda citrifolia	Rubiaceae	Mengkudu	Leaves	Abscess, itch	Ong (2003)
Murdannia nudiflora	Commelinaceae	Rumput aur	Leaves	Abscess, sore	Ong (2013)
Murraya paniculata	Rutaceae	Kemuning	Leaves	Abscess, itch	Ong (2008b)
Nigella sativa	Ranunculaceae	Jintan hitam	Seeds	Abscess, eczema	Ong (2008a)
Oxalis corniculata	Oxalidaceae	Sikap dada	Whole plants	Abscess, itch, sore	Ong (2013)
Paederia foetida	Rubiaceae	Akar sekentut	Leaves	Abscess	Ong (2013)
Passiflora foetida	Passifloraceae	Leletup	Leaves	Eczema, itch, rashes	Ong (2013)
Peltophorum pterocarpum	Fabaceae	Batai laut	Barks	Abscess	Ong (2004b)
Peperomia pellucida	Piperaceae	Ketumpangan air	Whole plants	Abscess, sore	Ong (2013)
Phyllanthus niruri	Phyllanthaceae	Dukung anak	Leaves	Abscess, itch, sore	Ong (2013)
Phyllanthus pulcher	Phyllanthaceae	Naga buana	Leaves	Itch	Sabda (2011)
Pilea microphylla	Urticaceae	Ketumpangan	Whole plants	Abscess	Ong (2013)
Piper nigrum	Piperaceae	Lada	Seeds	Abscess	Ong (2008a)
Piper sarmentosum	Piperaceae	Kaduk	Leaves	Itch, eczema	Ong (2011)
Pisonia alba	Nyctaginaceae	Putih pulu	Leaves	Abscess	Ong (2008b)
Plantago major	Plantaginaceae	Ekor anjing	Leaves	Abscess, sore	Ong (2013)

Table 1.1 Continued

Scientific name	Family	Local name	Part used	Types of microbial skin infection	Reference
Pluchea indica	Asteraceae	Beluntas	Leaves	Abscess, itch, sore	Ong (2004b)
Plumeria acuminata	Apocynaceae	Kemboja	Leaves	Abscess	Sabda (2011)
Pongamia pinnata	Fabaceae	Mempari	Barks	Itch	Ong (2004b)
Portulaca grandiflora	Portulacaceae	Ros jepun	Leaves	Abscess, rashes	Ong (2008b)
Portulaca oleracea	Portulacaceae	Gelang pasir	Whole plants	Abscess, eczema	Ong (2003)
Psidium guajava	Myrtaceae	Jambu batu	Leaves	Abscess, acne	Ong (2004a)
Psophocarpus tetragonolobus	Fabaceae	Kacang botol	Leaves	Abscess, sore	Ong (2003)
Pterocarpus indicus	Fabaceae	Angsana	Barks	Abscess, eczema, thrush	Ong (2004b)
Punica granatum	Lythraceae	Delima	Leaves	Itch	Ong (2004a)
Rhodomyrtus tomentosa	Myrtaceae	Kemunting	Leaves	Sore	Sabda (2011)
Rosa chinensis	Rosaceae	Mawar	Leaves	Abscess	Ong (2008b)
Salix babylonica	Salicaceae	Dalu-dalu	Leaves	Abscess, sore	Ong (2008b)
Sandoricum koetjape	Meliaceae	Sentul	Leaves	Itch	Ong (2004a)
Scoparia dulcis	Plantaginaceae	Teh makau	Leaves	Abscess, rashes, sore	Ong (2013)
Sesuvium portulacastrum	Aizoaceae	Tapak hantu	Leaves	Abcsess, ulcers	Sabda (2011)
Sida rhombifolia	Malvaceae	Sedeguri	Whole plants	Abscess, itch	Ong (2013)
Solanum torvum	Solanaceae	Terung pipit	Roots	Acne	Ong *et al*. (2011)
Spondias cytherea	Anacardiaceae	Kedondong	Leaves	Sore, abscess	Ong (2004a)
Stachytarpheta jamaicensis	Verbenaceae	Selasih dandi besar	Leaves	Abscess, eczema, rashes, sore	Ong (2013)
Syzygium polyanthum	Myrtaceae	Samak	Leaves	Itch	Ong (2008a)
Syzygium jambos	Myrtaceae	Jambu mawar	Leaves	Itch	Ong (2004a)

Table 1.1 Continued

Scientific name	Family	Local name	Part used	Types of microbial skin infection	Reference
Tamarindus indica	Fabaceae	Asam jawa	Barks	Abscess, itch, rashes	Ong (2008a)
Terminalia catappa	Combretaceae	Ketapang	Leaves	Abscess, sore, thrush	Ong (2004a)
Tetracera indica	Dilleniaceae	Mempelas	Leaves	Abscess, itch, sore, thrush	Ong (2004b)
Thespesia populnea	Malvaceae	Bebaru	Leaves	Itch, sore	Ong (2004b)
Thunbergia laurifolia	Acanthaceae	Akar tuau	Leaves	Abscess	Ong (2008b)
Trachyspermum ammi	Apiaceae	Jemuju	Fruits	Abscess, sore	Ong (2008a)
Tridax procumbens	Asteraceae	Kancing baju	Leaves	Abscess	Ong (2013)
Turnera ulmifolia	Passifloraceae	Lidah kucing	Leaves	Abscess, sore	Ong (2008b)
Urena lobata	Malvaceae	Pulut-pulut	Flowers	Abscess, ringworm, itch	Ong (2013)
Vitex trifolia	Lamiaceae	Lenggundi	Leaves	Abscess	Ong (2004b)
Wedelia biflora	Asteraceae	Serunai	Leaves	Abscess, itch	Ong (2004b)
Zingiber officinale	Zingiberaceae	Halia	Rhizome	Abscess, itch, ringworm	Ong (2008a)

Therefore, the antioxidant and antimicrobial activities of some plants that are used to treat microbial skin infections had been reported previously. For examples, the effectiveness of plants that are used traditionally to treat ringworm such as *Azadirachta indica* (Pandey *et al*., 2014; Tiwari *et al*., 2014; Viji Chandran *et al*., 2015), *Cassia alata* (Benjamin and Lamikanra, 1981; Pesewu *et al*., 2008; Nayak *et al*., 2015), *Lawsonia inermis* (Berenji *et al*., 2010; Liou *et al*., 2013; Shivakumar Singh and Vidyasagar, 2015; Tekin *et al*., 2015) and *Zingiber officinale* (Ahmad *et al*., 2008; Sharma and Sharma, 2011; Shivakumar Singh and Vidyasagar, 2015), have been verified by *in vivo* and *in vitro* studies. The bioactive compounds from these plants also had been identified.

1.3 Objectives of research

The specific objectives of this study are:

1. to collect and extract 120 plants that are traditionally used to treat microbial skin infections.
2. to evaluate the *in vitro* biological activities associated with microbial skin infections, that are antimicrobial (against skin pathogenic microorganisms) and antioxidant activities of these extracts.
3. to determine the anti-inflammatory activities of the selected antimicrobial and antioxidant extracts.
4. to determine the effects of hydrolysis towards the antimicrobial and antioxidant activities of the selected active extracts.

5. to evaluate the antibacterial activity of selected extracts that are active against *Pseudomonas aeruginosa* ATCC 27853 and their hydrolyzed extracts against three different strains of *Pseudomonas aeruginosa*.

6. to identify the phenolic compounds present in the active hydrolyzed extracts using Ultra Performance Liquid Chromatography (UPLC) with photo diode array detector and Liquid Chromatography-Mass Spectrometry (LC-MS).

7. to compare the antimicrobial and antioxidant activities of commonly identified phenolic compounds of the active hydrolyzed extracts and their glycosides that were identified in the crude extracts.

1.4 Scopes of study

Figure 1.1 shows the flow chart of this study. In Chapter 3 of this thesis, the antimicrobial (against skin pathogenic microorganisms) and antioxidant activities of 80% methanol extracts of 120 plants that are traditionally used in treating microbial skin infections were determined. Then, the potent extracts that gave better results for both activities were selected for further analysis. In some circumstances, microbial skin infections that cause the alterations in the skin immunological balance may induce inappropriate defensive responses, which lead to an inflammatory process (Lee and Hwang, 2012). Hence, in Chapter 4, the potent extracts were further analyzed for their anti-inflammatory activities using enzyme inhibition assays

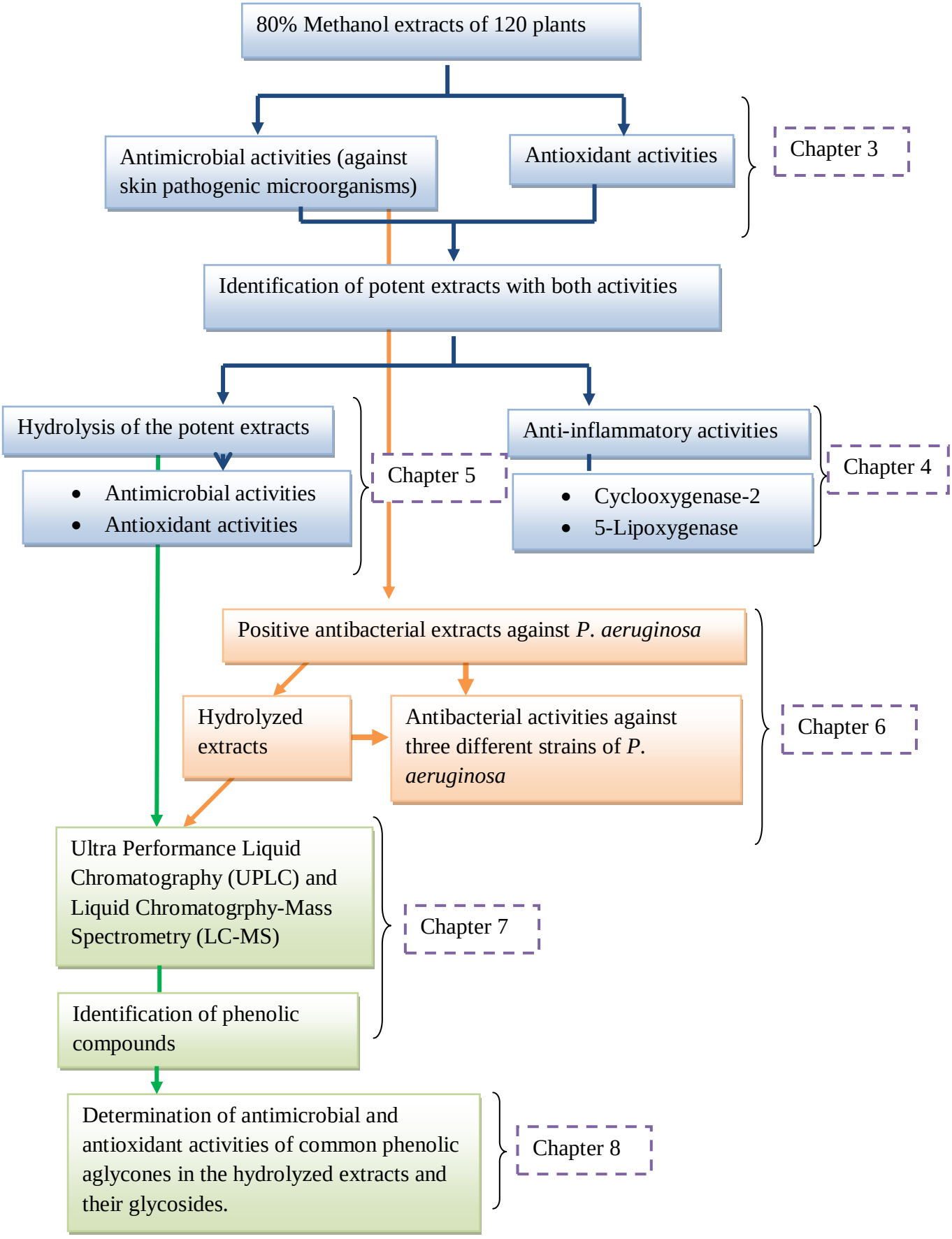

Figure 1.1 Overall flow chart on how this study has been carried out.

(cyclooxygenase-2 and 5-lipooxygenase). The findings were correlated with their antioxidant and antimicrobial activities.

Many studies had reported higher antioxidant (Bahorun *et al*., 2004; Sroka and Belz, 2009; Slavin *et al*., 2009; González-Peña *et al*., 2013) and antimicrobial activities of hydrolyzed extracts than that of the crude extracts (Branen and Davidson, 2000; Sihombing *et al*., 2014; Min *et al*., 2014b). Thus, in Chapter 5, the potent extracts were hydrolyzed and tested for their antimicrobial and antioxidant activities. The activities were compared with crude extracts. In addition, as reported by many researchers, *Pseudomonas aeruginosa* is a well-known multidrug resistant pathogens and is very difficult to be treated (Denys and Relich, 2014; Martinez and Baquero, 2014). Based on the antibacterial screening results in Chapter 3, this bacterium (*P. aeruginosa* ATCC 27853) was found to be the most resistant microorganism. In Chapter 6 the extracts that have positive results against this strain were selected for further hydrolysis and the antibacterial activities against three different strains of *P. aeruginosa* of the crude and hydrolyzed extracts were determined.

In Chapter 7, the potent hydrolyzed extracts were analyzed using UPLC and LC-MS to identify the phenolic compounds. The findings of this chapter were related to the bioactivities of the extracts and were used for future analysis. In Chapter 8, the antimicrobial and antioxidant activities of the commonly identified phenolic compounds in the active hydrolyzed extracts and their glycosides were determined.

CHAPTER 2:

LITERATURE REVIEW

2.1 Introduction

Traditional medicine has made great contributions to human healthcare and in the development of modern medicine. Currently, traditional medicines are still playing an important role in the primary healthcare sector of many countries. Traditional herbal formulations to treat various diseases are practiced worldwide especially in China, India and Europe. In Malaysia, medicinal plants are added into herbal formulations or can be prepared individually. Each plant has its own efficacy. The incorporation of medicinal plants into herbal formulations is mostly guided by experience and expertise inherited from the ancestors. Different parts of plants are collected to prepare medicinal herbal formulations. The aerial parts, leaves, fruits and seeds are those most frequently used. Roots and bulbs are also used in many remedies. Sometimes, these are mixed with other ingredients, such as sugar, honey, alcohol or flour to enhance their activity (Carretero Accame *et al*., 2011).

Diseases that are still treated using plant extracts are diarrhea, headache, fever, wounds, skin disorders, asthma, diabetes, gout, cough and toothaches (Nordin, 1993). Different parts of a plant can be used to cure different diseases. For instance, the fruits of *Rhodomyrtus tomentasa* (kemunting) can be consumed to treat diarrhea, while the roots and leaves are added in the bath water for women during confinement and their decoction is drank to reduce blood pressure. The leaf poultice is applied to treat skin disorders (Sabda, 2011). Another example is *Nephelium*

lappaceum (rambutan), whereby its roots, is used in treating high fever while its leaves are used in treating headache. Moreover, the peels of the fruits are used in treating dysentery and high fever (Ong, 2004a).

Scientific verifications of the therapeutic properties of medicinal plant extracts are still being thoroughly carried out. Plant-based drug can be discovered from medicinal plants through bioactivity guided isolation procedure. The drugs are used worldwide for the treatment of various diseases (Nordin, 1993; Thippeswamy *et al*., 2015).

2.2 Microbial skin infections

Microorganisms have two fundamentally different types of cells, which are eukaryotic and prokaryotic. Eukaryotic cell has a membrane-enclosed nucleus and membrane-enclosed organelles. Microorganisms with eukaryotic cells are protists, algae and fungi. Prokaryotic type of cell is lacking a membrane-enclosed nucleus and membrane-enclosed organelles. Microorganisms with prokaryotic cells are bacteria and archaea (Prescott *et al*., 2005).

Human skin serves as a shield against microbial infection (McAdam and Sharpe, 2005). The skin may get infected by the pathogenic microorganisms when the surface of the skin is broken due to injuries, wounds, insect bites, scratches and lacerations. Antiseptics are applied to the skin to prevent microorganisms from getting into the broken skin (Drosou *et al.*, 2003). Most microbial skin infections can cause symptoms such as itching, soreness, erythema (redness), edema (swelling) and inflammation (Marks, 2003).

Microbial skin infections can be classified according to the causing (aetiological) agents and severity. The invasion of microbial into the skin can be ranged from mild or uncomplicated or superficial or acute infections to deep or complicated or chronic or life-threatening infections (Esposito *et al.*, 2016). Several types of microbial skin infections caused by bacteria, fungi and viruses are indicated in Table 2.1.

2.3 Bacteria and fungi that cause skin infections

The skin is colonized by various microorganisms as the resident biota and the species of which vary depending on the site of the body, the degree of moisture and other factors such as the pH and temperature. Among the resident biota are the species of bacteria (from genus *Staphylococcus, Streptococcus, Corynebacterium, Propionibacterium* and *Pseudomonas*), fungi (from genus *Microsporum, Trichophyton, Alternaria* and *Trichospron*) and yeasts (from genus *Candida, Rodotorula* and *Torulopsis*). However, some opportunistic resident biota may also act as pathogenic microbes to the host by taking advantage of the broken skin or have evolved mechanisms to breach in the host barriers (McAdam and Sharpe, 2005; Dryden, 2009).

All the skin bacteria that were selected for this study are aerobic bacteria. Among them are *Staphylococcus aureus* and *Staphylococcus epidermidis*. Even though these species were found as resident biota, they are considered as the primary causatives of skin infections, especially in people who have suppressed in immunity. Examples of skin infections that are caused by *S. aureus* are folliculitis at the hair follicles, acne, carbuncles, impetigo, ‘athlete’s foot’ (tinea pedis), abscess

Table 2.1. Types of microbial skin infections.

Skin infection	Aetiological agent	References
Bacteria infections (Uncomplicated)		
Impetigo	*Streptococcus pyogenes*, *Staphylococcus aureus*	Esposito *et al.* (2016)
Ecthyma	*Streptococcus pyogenes*	Esposito *et al.* (2016)
Folliculitis	*Staphylococcus aureus*	Esposito *et al.* (2016)
Carbuncles	*Staphylococcus aureus*	Esposito *et al.* (2016)
Abscess	*Staphylococcus aureus*	Esposito *et al.* (2016)
Erythrasma	*Corynebacterium minutissimum*	Blaise *et al*. (2008)
Bacteria infections(Complicated)		
Erysipelas	*Streptococcus pyogenes*	Esposito *et al.* (2016)
Lymphadenitis	*Streptococcus pyogenes*, *Staphylococcus aureus*	Esposito *et al.* (2016)
Diabetic foot infection	Gram-positive and Gram-negative, including *Pseudomonas aeruginosa*	Esposito *et al.* (2016)
Cellulitis	*Staphylococcus aureus*	Esposito *et al.* (2016)
Myositis	*Staphylococcus aureus*	Esposito *et al.* (2016)
Necrotising fasciitis	*Streptococcus pyogenes*, *mixed anaerobic flora*	Esposito *et al.* (2016)
Gangrene	*Clostridium perfringens*	Esposito *et al.* (2016)
Wound infection	*Staphylococcus aureus*	Esposito *et al.* (2016)
Leprosy	*Mycobacterium leprae*	Goodyear (2014)
Fungi and Yeast Infections		
Tinea capitis	*Microsporum audouinii* *Microsporum canis*, *Trichopython tonsurans*	O'Dell (1998); Goodyear (2014)
Tinea corporis (ringworm)	*Microsporum canis*, *Trichophyton tonsurans*, *Trichophyton rubrum*	O'Dell (1998); Goodyear (2014)
Tinea barbae	*Trichophyton rubrum* *Trichophyton verrucosum*	Havlickova *et al.* (2008)
Tinea versicolor (pityriasis)	*Malassezia furfur*	O'Dell (1998); Goodyear (2014)
Candidiasis	*Candida albicans* or other *Candida* species	O'Dell (1998); Goodyear (2014)
Virus Infections		
Warts	Human papillomavirus (HPV)	O'Dell (1998); Goodyear (2014)
Molluscum contagiosum	Poxvirus	Goodyear (2014)
Chickenpox and shingles	Varicella zoster virus (VZV)	Goodyear (2014)

and ‘jock itch’ (tinea cruris) (Rhody, 2000; Skov and Baadsgaard, 2000; Esposito *et al*., 2016). It can also cause complicated infections such as cellulitis, myositis and wound infection (Esposito *et al.*, 2016). *S. epidermidis* and *Propionibacterium acnes* were often isolated from acne lesions and are considered as bacteria causing acne vulgaris (pimples) (Nishioka *et al*., 1977). In addition, infection by *S. epidermidis* is regarded as the most important skin nosocomial infection (Otto, 2009).

Micrococcus luteus that is also the resident biota of human skin and eye, but its overgrowth could lead to liver abscess (Edmond *et al.*, 2005). Meanwhile, erythrasma, a common infection of the skin, is usually caused by *Corynebacterium minutissimum* (Blaise *et al*., 2008). Most of the *Corynebacterium* sp. bacteria do not respond to a wide range of antibiotics as they are less common and not well investigated (Bannister *et al*., 2000). *Pseudomonas aeruginosa*, which is the only Gram-negative bacterium that was used in this study, generally can cause several skin diseases, such as diabetic foot infection, folliculitis, intertrigo and nail infection (Lacour *et al*., 1994; Esposito *et al.*, 2016).

Several dermatophytes and yeasts are typical aetiological agents for fungal infection associated with the skin. Dermatophytosis is an infection of the keratinized tissues such as hair, nails and the skin, and is commonly known as ringworm or tinea. The common dermatophytes that were also used in this study are *Candida albicans*, *Malassezia furfur*, *Microsporum canis* and *Trichophyton rubrum*. As opportunistic pathogens, the overgrowth of *C. albicans* may lead to intertrigo, thrush (oral candidiasis) and candidiasis (Weckesser *et al*., 2006). Eczema, tinea versicolor (pityriasis) and dandruff are usually caused by *M. furfur*, which grows in skin areas rich in sebaceous glands such as neck, face and arms (Faergemann, 2004; Goodyear, 2014). *M. canis* is well known to cause ringworm of the scalp (tinea

capitis) and tinea corporis (ringworm at any part of the skin). These infections involve animals as host and common carriers are known to be cats and dogs (Ilkit *et al*., 2007; Goodyear, 2014). *T. rubrum* is the common dermatophyte that causes 'athlete's foot', tinea corporis and tinea barbae (an infection of the bearded area of the face and neck) (Havlickova *et al*., 2008; Goodyear, 2014).

2.3.1 *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a major nosocomial pathogen, particularly harmful to humans with weak immune system (Rassolini and Mantengoli, 2005). Infections of *P. aeruginosa* on the skins will cause different microbial skin infections such as dermatitis, wound infections; burn wound sepsis, folliculitis and unmanageable forms of acne vulgaris (Pier and Ramphal, 2010). In addition, it also can be associated with infections like pneumonia, bloodstream, urinary tract and surgical site infections (Hidron *et al*., 2008; Zhanel *et al*., 2010).

Moreover, *P. aeruginosa* is well-known to be resistant to many antibacterial drugs and have lost susceptibility toward fluoroquinolones, aminoglycosides, cephalosporins and carbapenems. The mechanisms of resistant either intrinsically (because of constitutive expression of β-lactamases and efflux pumps, combined with low permeability of the outer-membrane) or following acquisition of resistance genes (such as genes for β-lactamases or enzymes inactivating aminoglycosides or modifying their target), over-expression of efflux pumps, decreased expression of porins, or mutations in quinolone targets (Mesaros *et al*., 2007; Riou *et al*., 2010; Poole, 2011; Castanheira *et al*., 2014). Thus, because of multidrug resistance and dual resistance are major problems in developing a definitive therapy for *P.*

aeruginosa infections, nowadays, many researchers have focused on natural products in order to look for new anti-pseudomonal agents (Aburjai *et al*., 2001; Zhou *et al*., 2011; Batta *et al*., 2013; Bouharb *et al*., 2014).

2.4 Natural products

Natural resources have been used as medicines since ancient times. In this modern age, approximately one-third of the top-selling pharmaceuticals are natural products. Natural products can be described as chemical compounds or substances produced by living organisms, which often have biological activities after being confirmed using various scientific validation tests. Some of the bioactive compounds can serve as chemical models for the design, synthesis and semi synthesis of novel substances for treating diseases (Strohl, 2000; Manly *et al*., 2002; Lu *et al*., 2009; Veeresham, 2012).

2.4.1 Natural antimicrobial agents from plants

Antimicrobial agents are substances that can inhibit the growth and/or kill certain strain of microbes (Atlas, 1997). Among the antimicrobial agents are those having antibacterial, antifungal and antiviral activities. There are derived from a variety of sources including plants, animals and microorganisms. Earlier studies revealed that plants used in traditional medicines are proven to be a more reliable source of antimicrobial compounds (Lai *et al*., 2009; Taviano *et al*., 2011; Mousavi and Kazemi, 2015; Thippeswamy *et al*., 2015; Zomorodian *et al*., 2015). Many natural antimicrobial extracts from plants have been commercialized. For examples,

the essential oils of the dried flower buds of *Syzygium aromaticum* (clove) (that contains 75 to 90% of eugenol) and fresh flowers of *Thymus vulgaris* (thyme) (that contains 40% of thymol) are widely used as antiseptics and food flavouring agents (Dewick, 1997). Among the plant extracts, essential oils are the most well-known sources of antimicrobial agents. However, the major limitation to incorporate essential oils in the food preservation system as compare with the synthetic preservatives is that their effective concentrations (dosages) may alter the organoleptic properties of food such as the appearance, taste, scent and texture.

Phenolics, terpenoids and alkaloids are the main classes of antimicrobial compounds from plants. The mechanisms involved in inhibiting the microorganisms are different, depend on the compounds and types of microorganisms involved (Cowan, 1999). Phenolics such as apigenin, luteolin, myricetin, quercetin, caffeic acid and chlorogenic acid from Finnish berries have been reported to have antibacterial properties against Gram-positive and Gram-negative tested bacteria (Puuponen-Pimiä *et al*., 2001). Isoflavonoid pterocarpans such as medicarpin from lucerne (*Medicago sativa*) and pisatin from pea (*Pisum sativum*) are well-known having antifungal activity (Dewick, 1997).

Diterpenoids such as ferruginol, viridone and candidissiol isolated from Syrian sage (*Salvia syriaca*) were found to have pronounced antibacterial activity against *Bacillus subtilis, S. aureus, S. epidermidis* and *Proteus mirabilis* (Ulubelen, 2003). The synergistic effect of terpenoids such as monoterpenoids and diterpenoids in the essential oils from cinnamon, lime, rosemary, orange and clove also were reported to contribute to strong antibacterial activities against *P. aeruginosa, Escherichia coli, Proteus vulgaris, Klebsiella pneumonia, Bacillus subtilis* and *S. aureus* (Prabuseenivasan *et al*., 2006). Among the four tested monoterpenoids (that

are citral, eugenol, nerolidol and α-pinene), citral showed higher antifungal activity against seven opportunistic pathogenic yeasts (*Candida krusei*, *C. albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, *Cryptococcus neoformans*, and *Trichosporon asahii*) and four dermatophyte species (*Trichophyton mentagrophytes*, *T. rubrum*, *Microsporum gypseum* and *M. canis*) (Miron *et al*., 2014).

Alkaloids such as berberine, β-hydrastine, canadine and canadaline from goldenseal (*Hydrastic canadensis*), a multipurpose herbal remedy were found having antibacterial properties against *S. aureus*, *P. aeruginosa* and *E. coli* (Scazzocchio *et al*., 2001). Flindersine is the most effective antifungal alkaloid isolated from swallowtail (*Haplophyllum sieversii*) against *Colletotrichum fragariae*, *Colletotrichum gloeosporioides*, *Colletotrichum acutatum*, *Botrytis cinerea*, *Fusarium oxysporum*, and *Phomopsis obscurans*. (Cantrell *et al*., 2005).

2.4.2 Natural antioxidants from plants

Antioxidants can be defined as substances that can prevent or delay oxidative damage of lipids, proteins and nucleic acids by reactive oxygen species (ROS), which include reactive free radicals such as superoxide ($O_2^{\bullet}$), hydroxyl ($OH^{\bullet}$), peroxyl ($RO_2^{\bullet}$), hydroperoxyl ($HO_2^{\bullet}$), alkoxyl ($RO^{\bullet}$), nitric oxide ($NO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$) and lipid peroxyl ($LOO^{\bullet}$) and non-radicals such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and ozone (O_3). These scavenge radicals by inhibiting initiation and breaking chain propagation or suppressing the formation of free radicals by binding to the metal ions, reducing hydrogen peroxide and quenching superoxide and singlet oxygen (Shi *et al*., 2001).

Human body is well equipped with two important ways of defense against oxidative stress, which are antioxidant enzymes (catalase, glutathione peroxide and peroxide dismutase) and non-enzymatic molecules (vitamins, ubiquinone and glutathione) (Bickers and Athar, 2006). However, often the endogenous defense system against ROS is often insufficient. Thus, it is recommended to increase the amount of natural antioxidants through consumption of antioxidants from plant materials (Valko *et al*., 2007).

Fruits and vegetables are well-known sources of antioxidant agents to human. Consumption of fresh fruits and vegetables is a diet rich with vitamins, phytochemicals, enzymes, minerals and fibers. Natural antioxidants from plants is believed to help in delaying the sign of aging, preventing degenerative diseases (such as cancer and diabetes), and improving physical fitness (Mohamed *et al*., 2005). Phenolics and vitamin C play an important role in enhancing the antioxidant activity of fruit juices. Several earlier comparative antioxidant studies of tropical fruits had highlighted higher antioxidant activity of guava fruit (*Psidium guajava*) (Sulaiman and Ooi, 2014). Among the vegetables that are eaten raw in Malaysia (ulam-ulaman), pronounced antioxidant activity was obtained from kantan (*Etlingera elatior*) and mata pelanduk (*Ardisia crenata*) extracts (Sulaiman *et al*., 2011).

Antioxidant activities are contributed the synergistic effects of various phytochemicals in the extracts. The major contents of flavonols such as rutin and flavones such as luteolin in guava fruit were found to be responsible for the antioxidant activities of the juice extract (Sulaiman and Ooi, 2014). Khalaf *et al*. (2007) reported that caffeine, theobromine and theophylline are major contributors to the antioxidant properties of tea (*Camellia sinensis*). Antioxidant activities of fig

(*Ficus microcarpa*) were correlated with the presence of triterpenoids and phenolic compounds in the extract (Ao *et al*., 2008). The presence of gallic acid, tannic acid, catechin, caffeic acid, ferulic acid and benzoic acid in bitter gourd (*Momordica charantia*) was found to contribute to the antioxidant activity of the extract (Kubola and Siriamompun, 2008).

2.4.3 Natural anti-inflammatory agents from plants

Inflammation is a basic pathological mechanism that responded by vascular cells to harmful stimuli such as pathogens, damaged cells or irritants. The inflammatory reaction involves the complex interactions between inflammatory cells (neutrophils, lymphocytes and macrophages) and vascular cells (endothelial cells and smooth muscle cells) (Tedgui and Mallat, 2001). Cyclooxygenase is an enzyme responsible for inflammatory processes expressed as two isomers COX-1 and COX-2, with COX-2 induced in inflamed tissue. During the complex process of inflammation, free radicals are produced. The purpose of the release of large amounts free radicals is to kill or destroy the pathogens (Cerutti *et al*., 1992). However, in some skin disorders, free radicals can act as second messengers that activate NF-κB, a nuclear transcription factor, resulting in secretion of signaling molecules, such as pro-inflammatory and interleukins (IL), may lead to the skin inflammation (Briganti and Picardo, 2003, Wolfle *et al*., 2011; Dzialo *et al*., 2016).

Inflammation-related disorders include muscle swelling, rheumatism, cut wound, accidental bone fracture, insect bites and pains (Namsa *et al*., 2009). In Thailand, the entire plant of pegaga (*Centella asiatica*) is used traditionally to treat inflammatory infections (Punturee *et al*., 2004). The anti-inflammatory activities of

the water extract of this plant and its active triterpenoid, asiaticoside were reported to be due to the inhibition of nitric oxide synthesis (Jin *et al*., 2004). In Malaysia, the leaf of bakong (*Crinum asiaticum*) is used traditionally to treat muscle swelling (Ong, 2004b). The anti-inflammatory effect of this plant extract was scientifically verified using carrageenan-induced paw edema in mice (Samud *et al*., 1999). Consequently, formulation comprising the extract of *C. asiaticum* as an active ingredient to treat and alleviate inflammatory disorders was patented (Jin *et al*., 2007).

The natural anti-inflammatory compounds are mostly derived from phenolic, terpenoid and alkaloid groups. A study done by Hsu *et al*. (2013) found that phenolic compounds namely vanillic acid, quercetin, kaempferol and naringenin from the flowers of water lily (*Nymphaea mexicana*) showed strong inhibitory activity of cyclooxygenase-2 (COX-2). A review publication by Akihisa *et al*. (2003) reported the anti-inflammatory properties of terpenoid compounds such as agnuside, peduncullariside, xanthorrhizol, furanoligularenone, petasin, ergolide and lagascatriol especially in the COX-2 inhibitory assay. Alkaloid compounds such as ailanthamide, berbamine, berberine, caulerpin, indirubin and skimmianine are reported as very potent anti-inflammatory agents based on various *in vivo* tests. Carrageenan-induced pedal edema was the most utilized experimental model for evaluating anti-inflammatory activity of the alkaloids (Souto *et al*., 2011).