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Antimicrobial effect of medicinal plants used in traditional Indonesian medicine

DOCTORAL THESIS

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CERTIFICATION

I, Andreas Romulo, submitted this dissertation for Ph.D. degree at the Czech University of Life Sciences Prague, Faculty of Tropical AgriSciences, declare that this dissertation is my own work unless otherwise referenced or acknowledged.

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Andreas Romulo, M.Sc.

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LIST OF ABBREVIATION

ATCC	American type culture collection
CA-MRSA	Community-associated methicillin-resistant *Staphylococcus aureus*
CLSI	Clinical and Laboratory Standard Institute
DMSO	Dimethylsulfoxide
EO	Essential oil
FID	Flame ionisation detector
GC	Gas chromatography
EBP	Endocarditis- and biofilm-associated pili
HA-MRSA	Hospital-acquired methicillin-resistant *Staphylococcus aureus*
HPLC	High performance liquid chromatography
KHM	Konsentrasi hambat minimal
LA-MRSA	Livestock-associated methicillin-resistant *Staphylococcus aureus*
MHA	Mueller-Hinton agar
MHB	Mueller-Hinton broth
MIC	Minimum inhibitory concentration
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSCRAMM	Microbial surface component recognizing adhesive matrix molecule
MSD	Mass selective detector
NMR	Nuclear magnetic resonance
MS	Mass spectrometry
QTOF	Quadrupole time-of-flight
RI	Retention indices
TIC	Total ion current
TRSA	Tetracycline-resistant *Staphylococcus aureus*
UTI	Urinary tract infection
WHO	World Health Organisation

ABSTRACT

In many regions of Indonesia, there are numerous traditional herbal preparations for the treatment of infectious diseases. Despite the well-documented traditional use of the plants, their antimicrobial potential has been poorly studied by modern laboratory methods. Therefore, this study investigated: 1) antimicrobial effect of ethanol plant extracts using broth microdilution method, 2) antimicrobial and antistaphylococcal effects of essential oil (EO) of *Curcuma mangga* by broth microdilution volatilisation and modified broth microdilution methods, and 3) chemical composition of antimicrobially active essential oil of *C. mangga* using gas chromatography-mass spectrometry (GC-MS) and gas chromatography with quadrupole and time of flight mass spectrometry (GC-QTOF-MS). The results showed that the extract of *Orthosiphon aristatus* leaf produced the strongest antimicrobial effect, inhibiting the growth of *Candida albicans* at minimum inhibitory concentration (MIC) of 128 µg/mL, *Staphylococcus aureus* at MIC of 256 µg/mL, *Enterococcus faecalis* at MIC of 256 µg/mL and *Pseudomonas aeruginosa* at MIC of 256 µg/mL. The leaf extract of *Woodfordia floribunda* also exhibited significant effect against *C. albicans* (MIC 128 µg/mL), *S. aureus* (MIC 256 µg/mL) and *E. faecalis* (MIC 256 µg/mL). The analysis of rhizome of *C. mangga* EO using broth micro dilution volatilisation method and modified standard broth micro dilution method showed that the EO exhibited antistaphylococcal activity with the MIC ranging from 128 to 1024 µg/mL, whereas tetracycline-resistant *S. aureus* was the most sensitive strain (MIC 128 µg/mL). The identification of *C. mangga* EO by GC-MS and GC-QTOF-MS revealed the major constituents of *C. mangga* were 15,16-dinorlabda-8(17),11-dien-13-one (22.76/13.70%), followed by ambrial (14.90/9.52%), 13-nor-eremophil-1(10)-en-11-one (6.64/5.39%), 15,16-dinorlabda-8(17),12-dien-14-al (5.71/6.68%), and aromadendrene oxide (5.53/3.27%). The findings suggest that *O. aristatus* and *W. floribunda* leaves extract and the EO of *C. mangga* rhizome as promising agents for the development of traditional medicine based anti-infective preparation. However, further researches focused on the isolation and characterisation of antimicrobially effective constituents of *O. aristatus* and *W. floribunda* and the safety and *in vivo* efficacy of *C. mangga* EO, will be needed before its possible use.

Keywords: Antibacterial, anti-candidal, antistaphylococcal, *Curcuma mangga*, essential oil, GC-MS, GC-QTOF-MS, *Orthosiphon aristatus*, plant extract, *Woodfordia floribunda*.

ABSTRAKT

V mnoha oblastech Indonésie existuje řada tradičních rostlinných přípravků pro léčbu infekčních onemocnění. Přes dobře zdokumentované tradiční použití rostlin, jejich antimikrobiální potenciál byl doposud nedostatečně studován moderními laboratorními metodami. Tato studie se proto zaměřila na: 1) vyhodnocení antimikrobiálního účinku rostlinných etanolových extraktů pomocí mikrodiluční bujonové metody, 2) hodnocení antimikrobiálních a antistafylokokových účinků silice *Curcuma mangga* pomocí mikrodiluční bujonové volatilizační a modifikované bujonové mikrodiluční metody a 3) charakterizací chemického složení antimikrobiálně aktivní silice *C. mangga* za použití plynové chromatografie a hmotnostní detekce s jednoduchým kvadrupólem (GC-MS) a plynovým chromatografem ve spojení s kvadrupólem a analyzátorem doby letu (GC-QTOF-MS). Výsledky ukázaly, že extrakt z listu *Orthosiphon aristatus* prokázal nejsilnější antimikrobiální účinek proti *Candida albicans* (MIC 128 µg/mL), *Staphylococcus aureus* (MIC 256 µg/mL), *Enterococcus faecalis* (MIC 256 µg/mL) a *Pseudomonas aeruginosa* (MIC 256 µg/mL). Extrakt z listů *Woodfordia floribunda* také vykazoval významný účinek proti *C. albicans* (MIC 128 µg / ml), *S. aureus* (MIC 256 µg/mL) a *E. faecalis* (MIC 256 µg/mL). Analýza silice oddenku *C. mangga* s využitím mikrodiluční bujonové volatilizační a modifikované bujonové mikrodiluční metody ukázala, že silice vykazuje antistafylokokovou aktivitu s MIC v rozmezí od 128 do 1024 µg/mL, zatímco *S. aureus* rezistentní na tetracyklin byl nejcitlivějším kmenem (MIC 128 µg/mL). Analýza *C. mangga* EO pomocí GC-MS a GC-QTOF-MS ukázala, že hlavními složkami *C. mangga* byl 15,16-dinorlabda-8(17),11-dien-13-on (22,76/13,70%), následovaný ambrial (14,90/9,52%), 13-nor-eremophil-1(10)-en-11-on (6,64/5,39%), 15,16-dinorlabda-8(17),12-dien-14-al (5,71/6,68%) a aromadendrene oxid (5,53/3,27%). Tato zjištění naznačují, že extrakty z *O. aristatus* a *W. floribunda* a silice z rizomu *C. mangga* jsou slibným materiálem pro vývoj antiinfekčních přípravků založených na tradiční medicíně. Nicméně další výzkum zaměřený na izolaci a charakterizaci antimikrobiálně účinných složek extraktů z *O. aristatus* a *W. floribunda* a bezpečnost a *in vivo* účinnost silice z *C. mangga* bude zapotřebí před jejich možným použitím.

Klíčová slova: Antibakteriální, anti-kandidózní, antistafylokokový, *Curcuma mangga*, GC-MS, GC-QTOF-MS, *Orthosiphon aristatus*, rostlinný extrakt, silice, *Woodfordia floribunda*.

ABSTRAK

Di berbagai daerah di Indonesia, terdapat beraneka macam ramuan herbal tradisional yang digunakan untuk pengobatan penyakit menular yang disebabkan oleh mikroba patogen. Meskipun banyak literatur yang membahas tentang manfaat tanaman herbal berkhasiat secara tradisional, penelitian ilmiah tentang potensi kandungan antimikroba dalam riset modern masih terbatas. Oleh karena itu, penelitian ini bertujuan untuk: 1) menyelidiki kandungan antimikroba pada ekstrak etanol tanaman dengan menggunakan metode mikrodilusi cair, 2) menyelidiki kandungan antimikroba dan antistafilokokal pada minyak atsiri *Curcuma mangga*, dengan metode volatilisasi mikrodilusi cair dan mikrodilusi cair yang telah dimodifikasi, dan 3) karakterisasi kandungan kimia utama pada minyak atsiri yang memiliki keaktifan sebagai antimikroba dengan menggunakan GC-MS dan GC-QTOF-MS. Hasil penelitian menunjukkan bahwa ekstrak daun *Orthosiphon aristatus* memiliki efek antimikroba yang paling kuat dengan menghambat pertumbuhan *Candida albicans* pada konsentrasi hambat minimal (KHM) 128 µg/mL), *Staphylococcus aureus* (KHM 256 µg/mL), *Enterococcus faecalis* (KHM 256 µg/mL) dan *Pseudomonas aeruginosa* (KHM 256 µg/mL). Ekstrak daun *Woodfordia floribunda* juga menunjukkan efek penghambatan yang signifikan terhadap *C. albicans* (KHM 128 µg/mL), *S. aureus* (KHM 256 µg/mL) dan *E. faecalis* (KHM 256 µg/mL). Analisis miyak atsiri dari rimpang *C. mangga* menggunakan metode volatilisasi mikrodilusi cair dan mikrodilusi cair yang telah dimodifikasi menunjukkan bahwa minyak atsiri memiliki aktivitas antistafilokokal dengan KHM mulai dari 128 hingga 1024 µg/mL, dimana *S. aureus* resisten tetrasiklin merupakan strain yang paling sensitif (KHM 128 µg/mL). Identifikasi minyak atsiri rimpang *C. mangga* dengan menggunakan GC-MS dan GC-QTOF-MS mengungkapkan konstituen utama dari *C. mangga* adalah 15,16-dinorlabda-8 (17),11-dien-13-one (22.76/13.70%) , diikuti oleh ambrial (14.90/9.52%), 13-atau-eremophil-1(10)-en-11-one (6.64/5.39%), 15,16-dinorlabda-8 (17),12-dien-14-al (5.71/6.68%), dan aromadendrene oksida (5.53/3.27%). Hasil ini menunjukkan bahwa ekstrak daun *O. aristatus* dan *W. floribunda* dan minyak atsiri dari rimpang *C. mangga* memiliki prospek untuk dikembangkan sebagai obat berbasis herbal. Namun, penelitian fitokimia lebih lanjut pada *O. aristatus* dan *W. floribunda* dengan focus pada isolasi dan karakterisasi konstituen antimikroba dan keamanan dan keefektifan minyak atsiri *C. mangga* secara *in vivo* diperlukan sebelum dapat digunakan secara luas.

Kata kunci: antibaketeri; anti-kandida, antistafilokokal, *Curcuma mangga*, ekstrak tanaman, GC-MS, GC-QTOF-MS, minyak atsiri, *Orthosiphon aristatus*, *Woodfordia floribunda*.

1. INTRODUCTION

Despite the advance progress of human medicine combating the infectious diseases in 20th century, reflected by the development of antimicrobial drugs (Weatherall et al. 2006), they are still the major threat of morbidity and mortality, especially in developing countries. For example in Indonesia, the prevalence of lower respiratory infections and tuberculosis is high and become the leading causes of death (World Health Organisation 2015a). Improper prescription, irrational used and uncontrolled access to antibiotics (Abdulah 2012) have driven the rapid emergence of multidrug-resistant pathogens (Golkar et al. 2014; Wright 2014). Consequently, infected patients are likely to have higher health expenditure, longer hospital stays and require a second- or third-line drugs treatment that may be less effective, more toxic and more expensive.

Among the rapid emergence of antimicrobial-resistance *Staphylococcus aureus* is the major concern. It is the common pathogenic bacteria that responsible for community and hospital-acquired infections such as lower respiratory tract infections, pneumonia and nosocomial bacteraemia (Richards et al. 1999; Wisplinghoff et al. 2004). Historically, β-lactam antibiotics have been used for the treatment of staphylococcal infections (Bæk et al. 2014). However, the emergence of resistant microorganisms such as methicillin-resistant *S. aureus* (MRSA), poses a great challenge to the prevention and treatment of staphylococcal infections (Arias & Murray, 2009). Following this reason, there is an urgent need for discovering new therapeutic antimicrobial agents.

During the last few years, medicinal plants have attracted the attention of pharmaceutical and scientific communities as sources of antimicrobial substances (Ginsburg & Deharo 2011). A phytochemical screening based on ethnomedicinal data is considered as an effective approach for the search of new therapeutic agents (Savithramma et al. 2012). For instance, the discovery of artemisinin from *Artemisia annua* in 1971 by Chinese scientists using data from ancient texts in Traditional Chinese Medicine (Tu 2011) has already saved millions of people from malarial infection (Bhatt et al. 2015). Currently, WHO recommend artemisinin-based combination therapy for treatment of this life-threatening disease and being used worldwide (World Health Organisation 2015b). Since medicinal plants have demonstrated great efficacy as anti-infective remedies in the past (Rahmatullah et al. 2012), they may also indicate to be a valuable reservoir for novel solutions against other microbial-related diseases.

Blessed with the richness of local phytocoenoses, there are numerous traditional

herbal preparations in Indonesia, known as "jamu", used for treating diseases, maintaining health and wellness since centuries ago (Stevensen 1999). "Jamu" medicines are usually prepared in form of infusion or decoction by mixing of different plant parts such as leaves, barks, roots and flowers. Species belonging to the family Zingiberaceae are the most frequently used "jamu" ingredients. For example "jamu kunir asam", which mainly consists of turmeric (*Curcuma longa*) and tamarind (*Tamarindus indica*), has been used to cure several diseases associated with pathogenic microorganism such as diarrhoea and dysentery (Beers 2001).

Despite the number of recent ethnobotanical inventories reporting the folk use of Indonesian medicinal plants for the treatment of infectious diseases (Grosvenor et al. 1995; Himmi et al. 2014; Roosita et al. 2008; Silalahi et al. 2015; Sujarwo et al. 2015; Zumsteg & Weckerle 2007), only limited number of studies have assessed their anti-infective potential. Up to now, the specific studies targeting anti-acne (Batubara et al. 2009), anti-candidal (Kusuma et al. 2014), anti-biofilm (Pratiwi et al. 2015), and resistant isolates inhibition (Wikaningtyas & Sukandar 2016) were performed by several laboratories. However, no systematic screening covering standard representatives of the most common bacterial and yeast pathogens have previously been done.

This dissertation reports *in vitro* antimicrobial activity of ethanol extracts of 37 plant species used in traditional Indonesian medicine and chemical composition and antistaphylococcal activity of essential oil from *Curcuma mangga* rhizome.

2. REVIEW OF LITERATURE

2.1. Microbial infections

Microbial infection is defined as the ability of microorganism to invade organism's body tissue and cause cellular damage, overcoming the host immune system, survive inside a host, multiplicate, production of extracellular substances that promote the immediate invasions (e.g. toxins), which results in clinical signs with an outcome of either morbidity or mortality (Petterson 1996). Organisms such as bacteria, viruses, fungi or parasites mainly cause it. In fact, numerous microorganisms already live in the skin and mucosal surfaces, to form the normal flora of the human body. They cause no harm, but often provide benefit to the host, by competing with potential pathogens for attachment sites and nutrients, and by producing antimicrobial substances toxic to pathogens. The infectious diseases could spread to others (communicable), transmitted by many routes: direct person-to-person transfer; respiratory transmission; sexual or mucosal contact; parenteral inoculation; by insect vectors; or by means of fomites (inanimate objects) (Bannister et al. 2006).

Regardless of previous successful prevention and control efforts, communicable diseases are still the leading causes of morbidity and mortality worldwide. It also seriously threatens the public health, not only in high-income countries but also in low and middle-income countries such as Indonesia. According to World Health Organization (WHO) (MacPherson & Ghusulak 2010), lower respiratory infections and tuberculosis account for 9.5 % of annual mortality in adult. Moreover, the prevalence of other infectious diseases such as acute respiratory infections, diarrhoea, measles, malaria and HIV/AIDS are higher in children under 5 years of age, accounting for 30% of annual death. Even though the intensive effort to eradicate the spread of infections such as development of antimicrobial drugs, the overuse and misuse of these medications has attributed to the rapid emergence of multidrug-resistant microorganisms (Ventola 2015; Hemaiswarya *et al.* 2008). Beside inappropriate use of antibiotics, several concurrent factors that affect the rapid emergence of microbial infections are as follow:

1. Demographic changes have resulted in a growing proportion of vulnerable populations, especially elderly people, needing hospital-based interventions who are thus at risk of exposure to highly resistant pathogens found in these environments.

2. The urbanization of human populations with its associated overcrowding and poor sanitation greatly facilitates the spread of such diseases as typhoid, tuberculosis, respiratory infections, and pneumonia.
3. Pollution, environmental degradation, and changing weather patterns can affect the incidence and distribution of infectious diseases, especially those such as malaria, which are spread by insects and other vectors.
4. The AIDS epidemic has greatly enlarged the population of immune-compromised patients at risk of numerous infections, many of which were previously rare.
5. The resurgence of specific drug-resistant infectious diseases, such as malaria and tuberculosis, is now in total responsible for many millions of infections each year. Much of this burden is carried by low-income countries that lack social investments in infrastructure, training and education, and other resources to adequately contain and control emerging drug resistance (MacPherson & Ghusulak 2010).

The development of resistance to antimicrobial drugs commonly used to treat infectious diseases has caused severe consequences. Failure to respond to the prescribed antibiotics increase the emergence of the resistant microorganism, prolonged illness, and greater risk of death. It also prolongs the periods of infectivity, which increase the opportunities of resistant strains exposure to the community. When the resistant to first-line antimicrobials becomes prevalent, the second- or third-line drugs are administered, which are usually more expensive and possibly more toxic as well (Farrell et al. 2005; Levy 2005). In many countries, the high cost of such replacement drugs is prohibitive. Along with poor healthcare services and unaffordable hospital care, some diseases can no longer be treated in areas where resistance to first-line drugs is widespread (Institute of Medicine Forum on Emerging Infections 2003).

2.2. Microorganisms

The incidence of microbial infections resulting in diarrhoea, dysentery, urinary tract infections, respiratory tract infections, and hospital-acquired infections, remains high in Indonesia. Even some commensal microorganism become pathogens in case of patients with immunodeficiency problem. The most common bacterial and yeast found causing

these infection are *Candida albicans*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Not only causing the infections, these microorganism are also become the burden in Indonesia as they can develop the resistance to antibiotics such as *E. coli* and *S. aureus* (Parathon et al. 2017).

2.2.1. *Candida albicans*

This member of the genus *Candida* is a commensal yeast frequently occurring as a benign member of the skin, mouth, gastrointestinal tract, and women genital tract (Gow & Yadav 2017). *C. albicans* typically grows as spherical to oval budding yeast cells 3-5 × 5-10 µm in size (Samaranayake 2012). A unique feature of *C. albicans* is its ability to form unicellular budding yeast or in filamentous pseudohyphal and hyphal forms. Morphologically, pseudohyphae are identifiable because pseudohyphae have impediment at the sites of septation and are wider than hyphae. By contrast, hyphae form long tube-like filaments with parallel sides and no constrictions at the site of septation (Sudbery et al. 2004). Typically, *C. albicans* live as harmless commensal; however, the overgrowth of this fungus could lead to a serious situation as it has the ability to colonize nearly every human tissue and up to the systemic organ invasion organ, causing life-threatening systemic infections, particularly in immune compromised patients (Tsui et al. 2016).

Several diseases related to *Candida* infections are as follows: oral candidiasis, vulvovaginal candidiasis, and invasive candidiasis. Oral candidiasis is an opportunistic infection of the oral cavity (Singh et al. 2014). It occurs frequently among the elderly, particularly in those who wear dentures. Among the immunocompromised patients, this disease is quite common to spread and could be a sign of systemic disease such as diabetes mellitus (Akpan & Morgan 2002). *C. albicans* also responsible for the high prevalence of vulvovaginal candidiasis in women of childbearing ages, especially HIV-infected patients (Fidel 1998). Invasive candidiasis (candidemia) corresponds to the most important opportunistic mycosis in the world and one of the major causes of nosocomial infections (Pfaller & Diekema 2007). The manifestations of nosocomial invasive infections may present in various forms: bloodstream infections (fungaemia), urinary tract infection (UTI), surgical wound infections, skin abscesses related to insertion of the catheter, and infection of the heart muscle (Mavor et al. 2005).

C. albicans containing various known virulence factors which responsible for the pathogenesis. The virulence factors of the *C. albicans* have the great role in the

pseudohyphae formation by attached with epithelial cells (in the respiratory tract), endothelial cells (in blood vessels), hyphal switching, surface recognition molecules, and phenotypic switching. Extracellular hydrolytic enzymes (phospholipase, protease) play an important role in candidal profusion, as these enzymes facilitate adherence, tissue penetration, invasion and hence destruction of the host tissue. The haemolysin is another most common virulence factor, which contributes to the pathogenesis. The lysis of red blood cells caused by the action of a haemolysin promotes the acquisition of iron, which is essential for the development of yeast and infectious process (Souza et al. 2014; Lu et al. 2014; Tsang et al. 2007; Sundstrom 2006; Schaller et al. 2005). Candidalysin, a newly discovered peptide toxin secreted by *C. albicans* hyphae during the invasion, contribute to tissue penetration, epithelial damage, immune activation and phagocyte attraction (Li et al. 2017; Richardson et al. 2017; Moyes et al. 2016).

The azole antifungals are the most frequent class used to treat *Candida* infections. They are preferred for treatment of many *Candida* infections as they are inexpensive, less toxic, and readily available for oral administration. Based on their mechanism of action, there are four different classes of antifungal drugs currently available for the treatment of invasive mycoses: (1) alteration of membrane function (amphotericin B); (2) inhibition of DNA or RNA synthesis (flucytosine); (3) inhibition of ergosterol biosynthesis [azoles (fluconazole, itraconazole, and the newer agents voriconazole, posaconazole, and ravuconazole)]; and (4) inhibition of glucan synthesis [echinocandins (caspofungin, micafungin, and anidulafungin)] (Whaley et al. 2017; Morio et al. 2010; Pfaller et al. 2010; Eliopoulos et al. 2002). In the past decade, there has been a significant increase in the prevalence of emerging drug-resistant fungal infections in hospitalized patients, especially with the first and second-line antifungal drugs such as fluconazole, miconazole, clotrimazole, and tioconazole, amphothericin B, and echinocandins (Perlin 2015; Lortholary et al. 2011; Oxman et al. 2010; Sterling & Merz 1998). In principle, the mechanisms of antifungal resistance fall into distinct categories, including (1) decrease of effective drug concentration, (2) drug target alterations, and (3) metabolic bypasses (Sanglard 2016).

2.2.2. *Enterococcus faecalis*

E. faecalis is a Gram-positive, anaerobe facultative, oval (0.5-1 μm in diameter), non-motile and non-sporulating bacterium (Gilmore et al. 2013) commonly found in the

gastrointestinal tract of humans, which is densely colonized with up to 10^{11} bacterial cell/ gram feces (Van Tyne et al. 2013). The genus *Enterococcus* consists of over 40 ecologically diverse species, yet more than 90 percent of Enterococci infections are caused by two species: *E. faecalis* and *E. faecium* (Hidron et al. 2008). In the normal healthy host, the Enterococci are seldom causing infections. However, surveillance data indicate that *E. faecalis* is becoming one of the major cause of nosocomial infections, including endocarditis, sepsis, surgical wound infections, urinary tract infections, secondary bacteraemia, and inflammatory bowel diseases (Richards et al. 1999; Jett et al. 1994).

Many factors determine the virulence of Enterococci species, for example (1) ability to colonize the gastrointestinal tract, which is the normal habitat; (2) the presence of aggregation substance; and 3) ability to adhere to a range of extracellular matrix proteins (Fisher & Phillips 2009). The formation of biofilm by Enterococci is fundamental in causing endodontic and urinary tract infections, as well as endocarditis. The gene cluster associated with the biofilm formations is EBP (endocarditis- and biofilm-associated pili), consists of EBPa, EBPb, EBPc, and an associated encoding sortase C gene (Singh et al. 2007). The secreted *E. faecalis* virulence factors play active roles in weakening host immunity such as toxin production (e.g. cytolysin), and degrading host tissue by extracellular enzymes (e.g. hyaluronidases, gelatinase and serine protease) (Van Tyne et al. 2013; Mohamed & Huang 2007).

The clinical importance of the Enterococci is directly related to its antibiotic resistance, which contributes to the risk of colonization and infection. All Enterococci exhibit decreased susceptibility to penicillin, ampicillin, cephalosporins, and all semi-synthetic penicillins, as the result of the expression of low-affinity penicillin-binding proteins (Hollenbeck & Rice 2012). Among the most distinct examples of this adaptability is the acquisition of the genes encoding glycopeptide-vancomycin resistance. Vancomycin use was associated with the emergence and spread of methicillin-resistant *Staphylococcus aureus* (MRSA) in the 1960s. However, unlike MRSA, Enterococci have been able to recruit and maintain a variety of gene clusters encoding the biochemical machinery for vancomycin resistance. Antibiotic resistance in Enterococci can be transferred by pheromone-mediated conjugative plasmids or transposons, not only to antibiotic-susceptible Enterococci, but also to other pathogens such as *S. aureus*, an event

considered a serious public health threat, causing vancomycin-resistant *S. aureus* (Périchon & Courvalin 2009). One requirement for the conjugative transfer of mobile genetic elements is cell-to-cell contact between donor and recipient, which occurred, in the microbial biofilm mediated by the secretion of bacterial sex pheromones, small peptides that induce a mating response resulting in the aggregation or clumping of the cells (Weigel et al. 2007).

2.2.3. *Escherichia coli*

This bacterium is a Gram-negative, rod-shaped, facultative anaerobic, coliform bacterium of the genus *Escherichia*, and commonly found in the lower intestine of warm-blooded organisms (Tenaillon et al. 2010). Theodor Escherich first described this microorganism in 1885. Most *E. coli* strains harmlessly colonize the gastrointestinal tract of humans and animals as a normal flora. However, some serotypes have evolved into pathogenic *E. coli* by acquiring virulence factors through plasmids, transposons, bacteriophages, and/or pathogenicity islands (Lim et al. 2010). The pathogenic *E. coli* could induce broad spectrum of human diseases such as watery diarrhoea, haemorrhagic colitis, haemolytic-uremic syndrome, urinary tract infection (UTI), neonatal meningitis, and sepsis, as well as infections in other bodily sites (Smith & Fratamico 2017).

The *E. coli* genome is composed of a conserved core of genes that provides the backbone of genetic information required for essential cellular processes and a flexible gene pool that retains genetic information and enables the bacterium to adapt to special environmental conditions (Dobrindt 2005). To date, eight pathovars and their mechanisms of disease have been extensively studied. These pathovars can be broadly classified as either diarrhoeagenic *E. coli* or extraintestinal *E. coli*. Six pathovars are diarrhoeagenic (enteropathogenic *E. coli*, enterohaemorrhagic *E. coli*, enterotoxigenic *E. coli*, enteroinvasive *E. coli*, enteroaggregative *E. coli*, and diffusely adherent *E. coli*). Two pathovars are extraintestinal *E. coli* (uropathogenic *E. coli* and neonatal meningitis *E. coli*) (Croxen & Finlay 2010).

Virulence factors involve mechanisms that enable pathogenic bacteria to cause infections. They have been positively linked with the pathogenicity of *E. coli* such as the ability to colonize, attach and form distinctive lesions on the surfaces of intestinal epithelial cells, secretion of adhesin protein, secretion of enterotoxin (e.g. shiga toxin, heat-stable enterotoxins, heat-labile enterotoxin, cytotoxin), invasion of the intestinal

epithelium cell wall, suppress the host immune response, and spread directly from cell to cell (Farfan & Torres 2012; Isidean et al. 2011; Croxen & Finlay 2010; Peng et al. 2009).

The prevalence of emerging resistance in *E. coli* is consistently high against antimicrobial agents that have been in use the longest time in human and veterinary medicine. *E. coli* has been reported as a major cause of nosocomial UTI and bacteraemia (Tuon et al. 2014). The conditions are worse than expected as many studies reported the resistance of nosocomial *E. coli* to the extended spectrum β-lactamase, fluoroquinolones, and third- and fourth-generation cephalosporins (Karagöz et al. 2016; Lautenbach et al. 2002). Similar occurrence pattern can also be detected in community-acquired *E. coli* where the prevalence of resistance to antibiotic are gradually increasing (Nicolle 2013; Swami et al. 2012). The resistance to antibiotics in *E. coli* are primarily mediated by the production of β-lactamase enzymes, alterations of the molecular target, expression of modifying enzymes, and overexpression of efflux pumps (Bajaj et al. 2016; Anes et al. 2015; Hardy & Cozzarelli 2003).

2.2.4. *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is a Gram-negative, rod-shaped, asporogenous, and monoflagellated bacterium. It has a pearlescent appearance and grape-like or tortilla-like odour. It grows well at 25°C to 37°C, and its ability to grow at 42°C helps distinguish it from many other *Pseudomonas* species. *P. aeruginosa* is a ubiquitous microorganism, which has the ability to survive under a variety of environmental conditions (Wu et al. 2015). Luke, who observed rod-shaped particles in the blue-green pus of some infections, likely first, reported it in human infections in 1862. Similar coloration had been previously observed by Sedillot on surgical dressings, by the pigment pyocyanin produced by *P. aeruginosa*. The microorganism was first isolated from infections in 1882 by Gessard, who called it *Bacillus pyocyaneus.* Given the widespread occurrence of *P. aeruginosa* in the environment, it is noteworthy that human disease attributable to it is quite rare in otherwise healthy individuals (Lyczak et al. 2000). *P. aeruginosa* continues to persist in the community and hospital settings, as it is able to survive on minimal nutritional requirements and tolerate a variety of physical conditions (Lister et al. 2009).

P. aeruginosa is one of the most important nosocomial pathogens, especially in intensive care units. The pathogen is also the most frequently associated with morbidity and mortality causing cystic fibrosis, pneumonia, urinary tract infections, surgical site

infections, and bloodstream infections (Bhagirath et al. 2016; Nathwani et al. 2014; Vincent 2003; Lyczak et al. 2002; Stover et al. 2000; Vincent et al. 1995). The high rates of *P. aeruginosa* during hospitalization, especially among patients who have experienced trauma to or a breach in cutaneous or mucosal barriers by mechanical ventilation, tracheostomy, catheters, surgery, or severe burns, also occurred on patients with weak immune system (Erol et al. 2004; Thuong et al. 2003; Blanc et al. 1998). There were also report on the high prevalence of *P. aeruginosa* in-patient under antimicrobial therapy (Takesue et al. 2002; Bonten et al. 1999; Blanc et al. 1998).

The prevalence of this microorganism are mainly caused by its virulence factors, which manipulate host physiology and overcome host defences such as adhesins, pyocyanin, elastase, proteases, haemolysins, exotoxin and exoenzyme (Matroș et al. 2016; Ballok & O'Toole 2013; Döring et al. 2011; Livermore 2002; Van Delden & Iglewski 1998). The virulence factors, especially the exotoxins, proteases and exoenzymes, they cause extensive host tissue damage by disrupting normal cytoskeletal structure, depolymerisation of actin filaments and cleavage of the immunoglobulin G and A, thus facilitates invasion, dissemination, and development of chronic infections (Chatterjee et al. 2016; Sadikot et al. 2005).

P. aeruginosa presents a serious therapeutic challenge for treatment of both community-acquired and nosocomial infections. This bacterium has the ability to develop resistance to multiple classes of antibacterial agents by acquisition of resistance genes encoding extended spectrum β-lactamases and aminoglycoside-modifying enzymes via horizontal gene transfer, mutations of chromosomal genes in efflux site, and target site (Poole 2011; Ramirez & Tolmasky 2010; Lister et al. 2009; Strateva & Yordanov 2009; Gupta 2008).

2.2.5. *Staphylococcus aureus*

It is a Gram-positive bacteria, with diameters of 0.5 - 1.5 µm, characterised by individual cocci, form grape-like clusters, non-motile, non-spore forming, facultative anaerobes that grow by aerobic respiration or by fermentation (Götz et al. 2006; Harris and Richards 2006). The cell wall of *S. aureus* is a tough protective coat, which is relatively amorphous in appearance, about 20- 40 nm thick (Shockman and Barrett 1983). Underneath the cell wall is the cytoplasm that is enclosed by the cytoplasmic membrane. Peptidoglycan is the basic component of the cell wall, makes up 50% of the cell wall

mass, located in the formation of the tight multi-layered cell wall network, and bear up the high internal osmotic pressure of staphylococci. Phosphate-containing polymers called teichoic acids, contributes about 40% of cell wall mass (Harris et al. 2002). The pathogenicity of bacteria is influenced by the complexity of cell surface (Atilano et al. 2011). These include acquisition of specific adhesion factors, formation of biofilms, adaptation to an intracellular environment, production of a protective capsular polysaccharide or evasion of innate immune defences (Foster 2005).

S. aureus is a common human opportunistic pathogen, which is capable to colonize the skin and mucosa surfaces, whereas approximately 30 % of the individuals carry *S. aureus* in the skin, skin glands, anterior nares, nasal passage, oropharynx, and axillae of humans (Wertheim et al. 2005). The infections are initiated by the entrance of the microorganism through a breach of the skin or mucosa, involving local structures or spread to distant organs to generate life-threatening invasive infections such as bacteraemia, pneumonia, sepsis, septic arthritis, and osteomyelitis. (Corrado et al. 2016; Hal et al. 2012; Ragle et al. 2010; Kowalik et al. 2001). It also causes toxin-mediated diseases when they are introduced into the bloodstream or ingested through contaminated food. *S. aureus* toxin-mediated diseases include toxic-shock syndrome, scalded-skin syndrome, and food poisoning (Boswihi & Udo 2018; Mishra et al. 2016; Hennekinne et al. 2012). *S. aureus* is easily spread between animals and human. Under certain conditions, the spread is initiated through contact with excretions such as saliva or aerosols released during sneezing and coughing. Moreover, this bacterium could spread by animal products such as non-pasteurized milk (Werckenthin et al. 2001). Livestock such as swine, cattle, and chickens may also contract *S. aureus* infection and develop mastitis, arthritis, septicaemia, and so forth (Lin et al. 2009).

The virulence factor of *S. aureus* are divided according to their mechanisms into: invasion and colonization, synthesis of extracellular molecules, toxins, and ability to form biofilms (Lacey et al. 2016; Kong et al. 2016; Bien et al. 2011). The pathogenesis of infection is usually preceded by cell adhesion, followed by bacterial invasion and colonization of the host tissue which released numerous adhesin proteins such as microbial surface component recognizing adhesive matrix molecules (MSCRAMMs) (George et al. 2006). MSCRAMMs function in helping bacteria adhere to the plasma or different host extracellular matrices such as collagen, fibrinogen, and fibronectin. In

addition to adhesion properties, they also participate in other aspects of pathogenesis such as invasion of host cells and tissues, evasion of immune responses, and biofilm formation. The formation of biofilm is a multistep process, starting with transient adherence to a surface. MSCRAMMS promote the actual attachment causing the bacteria stick to each other, produce extracellular polymeric substances, and/or incorporation of host-derived components, such as platelets, resulting in a mature biofilm (Al-Mebairik et al. 2016). In contrast to the protective and passive role of cell wall associated virulence factors, secreted *S. aureus* virulence factors play active roles in disarming host immunity by disrupting host cells and tissues and interfering with the host immune system to release nutrients and facilitate bacterial dissemination (Olivier et al. 2009). The secreted virulence factors comprise four main categories such as superantigens, various exoenzymes, miscellaneous proteins, and pore-forming toxins (Kong et al. 2016; Bien et al. 2011; Burnside et al. 2010; Fraser & Proft 2008). Penicillin and other β-lactam antibiotics, cephalosporins, and clindamycin remain as effective drugs for treatment of *S. aureus* infection in areas where the prevalence of methicillin resistance is low (Moellering 2012). However, the dependence on non β-lactam antibiotics for treatment of *S. aureus* infections has increased dramatically because of the problem with the emergence of antibiotic-resistant strains, most notably MRSA (Lu & DeLeo 2015).

S. aureus rapidly acquired the status of resistance to methicillin in 1961; only two years after it was first used clinically (Nordmann et al. 2007). Intrinsically, this microorganism is susceptible to β-lactam agents that inhibit cell wall formation due to binding with proteins involved in the formation of peptidoglycan (Bæk et al. 2014). The mechanism of resistance to β-lactam drugs including methicillin, isoxazolylpenicillins and cephalosporins in MRSA is an altered target site due to an acquired penicillin-binding protein 2a encoded by the gene mecA (Catry et al. 2010; Wielders et al. 2002). MRSA has been known as nosocomial pathogen for decades (Graffunder & Venezia 2002). This microorganism can colonize and form biofilms on biomaterials that contribute spread of hospital-acquired (HA-MRSA). HA-MRSA is generally defined as a MRSA infection detected by a positive culture taken more than 48 hours after hospital admission and treated in the inpatient setting (Bhattacharya et al. 2015; Liu et al. 2011). Not only observed in hospital setting, currently the MRSA has spread into the community, known as community-associated MRSA (CA-MRSA). The outbreaks of CA-MRSA have been seen among athletes, prisoners, military recruits, day-care attendees, injection drug users,

and other groups of people who live in crowded settings and/or routinely share contaminated items (Braun et al. 2016; Camargo et al. 2013). Humans who acquired MRSA and have a closed contact with animal can also spread the bacteria, causing livestock-associated MRSA infection (LA-MRSA). LA-MRSA are found in a wide range of other animals, including cattle, horses, chickens, turkeys, rats, dogs, and cats (Sørensen et al. 2017).

2.3. Ethnopharmacological approach to anti-infective drugs discovery

Plants have been known as the largest biochemical and pharmaceutical reserve ever known on our planet. These living stores are able to generate endless biochemical compounds, which its products are not essential for their living. These compounds have been known as secondary metabolite, for which no role has yet been found in growth, photosynthesis, reproduction, or other "primary" functions. Secondary metabolites are produced as a defence mechanism against conditions of biotic and abiotic stress (Rosado-Aguilar et al. 2017), including bacterial and fungal pathogens (Lewis & Ausubel 2006).

Since ages, plants products have always become the backbone of all life on earth and the essential resource for human well-being. Human has utilized plants with healing properties as the basis of sophisticated traditional medicine for treatment of infectious diseases in various part of the world (Karunamoorthi et al. 2013; Graham et al. 2000; Cowan 1999). Earliest evidence record on utilization of medicinal plants have been shown in ancient text from Egypt in 1500 BCE; “Shen Nong Ben Cao”, a Chinese medical text from 200 BCE; and Dioscorides’ *De Materia Medica*, which documents the Mediterranean pharmacopoeia from 50-70 CE (Quave 2016). Even nowadays, World Health Organization (WHO) estimates that 80% of the people living in developing countries rely on traditional herbal preparations as the primary source of medicine (Ekor 2014; Chitmee al. 2004).

Recognizably, the researches focused on searching of new drugs (including antimicrobials) from 1981 to 2007 revealed that almost half of the drugs approved since 1994 are based on natural products (Harvey 2008). Currently, it is estimated that about 80% of molecules used in drugs sold worldwide are derived from natural products and that over hundred new natural product-based leads are in clinical development. In addition, despite the tremendous development of chemical synthesis today, at least 25% of drugs presently used in modern medicine could be traced of plant origins, including

60% of anticancer and 75% of anti-infective drugs approved from 1981-2002 (Patwardhan & Vaidyab, 2010; Bhutani & Gohil 2010; Balunas & Kinghorn 2005; Butler, 2008).

In the process of drug development, the selection of plant species is the key role in the successful of novel anti-infectives discovery. There are three main approaches used to select plants for biological/phytochemical investigation: random selection, chemotaxonomical, and ethnopharmacological (Lahlou 2003). Random approach usually involves collection of plants in a given locality to generate a large number of plant materials, in hope that having a large pool of plant material will provide a great chemical diversity, therefore increases the chance of obtaining a biologically active molecule (Iwu 2012). In this approach, the sample of plant parts are usually selected and evaluated without prerequisite ethnobotanical and chemotaxonomical knowledge (Lahlou 2007). The selection of plants based on chemotaxonomical approach usually considering the presence of certain chemotaxonomic markers in a given family or genus (Iwu 2012). In this method, the phytochemical screening is based on the search for biological active markers present in the plant, which are already isolated previously (e.g. flavonoids, alkaloids, etc.) (Albuquerque & Hanazaki 2006). In the ethnopharmacological approach, either oral or written information on the traditional medicinal use of a plant forms the basis for selection and focused evaluation (Cos et al. 2006).

Ethnopharmacology is a highly diversified approach for novel antimicrobial discovery, which involves the observation, description and experimental investigation of folk medicine and their growth-inhibitory activities that based on botany, chemistry, biochemistry, pharmacology, and other disciplines (anthropology, archaeology, history, and linguistics). Information from organized traditional medical systems (e.g. Ayurveda, Unani, Kampo and traditional Chinese medicine) can be acquired from various sources, such as books, herbals, review articles (usually involving surveys of medicinal plants by geographic region or ethnic culture), notes placed on voucher herbarium specimens, field work, and computer databases (Cos et al. 2006). This approach has been extremely useful in screening and identification of plants with bioactive compounds with potential application in development of anti-infective products. The approach begins by selecting the plant species according to the indication of specific population groups for treatment of infectious diseases (e.g. diarrhoea, malaria). Then, the selected plants will be extracted

(e.g. crude extract or essential oil) and tested on their antimicrobial properties against pathogenic microorganisms. Once the inhibitory activity of the extract is demonstrated, including dose-dependence [minimum inhibitory concentration (MIC)], the searching for active ingredients can be explored using bioguided assay. In this strategy, the most active fraction(s) is chosen, and further refined until there is no further gain in activity. The composition of the final fraction is then determined using modern pharmacognosy and chemical analytical methods The molecules identified can then be tested and validated on disease-specific biological targets (Moore et al. 2017).

In case of plant extract, the bioactive compounds in the extract could be separate, identify, and quantify using high performance liquid chromatography (HPLC). HPLC could be divided into two objectives, analytical HPLC and preparative HPLC. Analytical HPLC is focus on the qualitative and quantitative determination of a compound, while preparative HPLC is intended for the isolation and purification of a valuable product (Huber & Majors 2007). The preparative HPLC is much applied in the process of searching of biologically active substances. The purified compounds usually be identified using mass spectrometry (MS) or nuclear magnetic resonance (NMR). MS is an analytical technique that ionizes chemical compounds and sorts the ions based on their mass-to-charge ratio. A mass spectrum is a plot of the ion signal as a function of the mass-to-charge ratio. The spectra is used to determine the elemental or isotopic signature of a sample, the masses of particles and of molecules, and to elucidate the chemical structures of molecules. NMR spectroscopy is one of the most powerful tools that chemists use to determine the structure of compounds, reveal the number of carbon and proton atoms and their connectivities, the conformations of the molecules, as well as relative and absolute stereochemistries (Cynthia 1999). The analysis of essential oil can be done using gas chromatography coupled with mass spectrometry (GC-MS). In this instrument, the sample is injected into a stream of an inert gas, which is the mobile phase. The vaporized sample is swept through the stationary phase, which is held on an inert support in the capillary column and the separated analytes/compounds flow through a mass spectrometry detector, whose response is displayed on a computer or recorder.

The adoption of ethnophamacological approach for screening bioactive components has several advantages, such as providing a mechanism for pre-screening on the species collected based on ethnomedicinal use and meet preliminary criteria for safety,

thus significantly increased the chances of discovery and reduce the cost and time involved in the process (Novaes & Leite 2011).

The ethnopharmacological approach has proven as a successful strategy in discovery of the natural products derived from medicinal plants, many of which have been the basis for the development of new lead chemicals for pharmaceuticals. For example, the isolation of artemisinin, the compound isolated from sweet wormwood (*Artemisia annua*), an effective antimalarial agent. Professor Youyou Tu successfully discovered it in 1960s (Liu 2017). She has screened over 2000 traditional Chinese herbal preparation, which were found in ancient texts of traditional Chinese medicine (Tu 2011). She later found that the ether extract of *A. annua* inhibiting the growth of *Plasmodium falciparum*, a parasite causing malaria, in clinical studies, and then isolated the pure compound artemisinin and synthesized the derivatives, dihydroartemisinin. Currently, artemisinin-based combination therapy is recommended by the WHO for the treatment of malaria and is being used worldwide (WHO 2015b).

The roots and rhizomes of *Coptidis* spp. (Ranunculaceae) has been used in traditional Chinese medicine for treatment of *damp-heat* syndrome such as diarrhoea and dysentery. According to the Chinese Pharmacopoeia, "Huanglian" may contain three *Coptis* species: *Coptis chinensis*, *Coptis deltoidea*, and *Coptis teeta*. Berberine, an isoquinoline alkaloid, is a major active compound in Huanglian and responsible for anti-diarrheal effects (Tang et al. 2009). The MIC of berberine have been described by several researchers against bacteria associated with diarrhoea such as *S. aureus*, MRSA, *Vibrio cholerae*, *Bacillus cereus*, and *E. coli*, in a range of 12.5 - 469 μg/mL (Yu et al. 2005; Cernákova & Kostálová 2002; Amin et al. 1969). Several clinical trials of berberine and its derivatives (berberine tannate, berberine hydrochloride, berberine sulphate) have been performed on the children and found to be effective in the treatment of diarrhoea. Chauhan et al. (1970) reported the effectiveness of berberine tannate and its combination with sulfadimidine and neomycin were found to be effective in the treatment of acute infective diarrhoea in experiments. The effectiveness of berberine tannate has also been proven in clinical trials with children suffering from gastroenteritis (Sharda 1970). Berberine hydrochloride and berberine sulphate also has been reported its effectiveness as anti-diarrheal drugs compare to conventional antibiotics (e.g. chloramphenicol, streptomycin), significantly reduced the volume of diarrheal stools and frequency of the

urgent need for defecation (Rabbani et al. 1987; Khin-Maung-U et al. 1985; Lahiri & Dutta 1967). Several pharmaceutical companies have sold berberine as a pharmaceutical drug for the treatment of intestinal infection diseases and diarrhoea, either in pure form (Berberine Hydrochloride Tablets, Northeast Pharmaceutical Group, Shenyang, China) or the extracts (*e.g.* Huang Lian Su Tablets (*Coptis chinensis* extract).

Another successful example is the isolation of essential oil of *Melaleuca alternifolia* (Myrtaceae), which the research also based on the ethnopharmacological approach. The tree is a paper bark shrub whose native habitat is swampy coastal regions of northern New South Wales. The Bundjalung Aborigines tribe inhaled the vapours emitted from heated leaves for the treatment of coughs and colds, and additionally applied a poultice from the leaves topically for the treatment of wounds and infections (Carson et al. 2006). Several studies on the antimicrobial potential revealed that tea tree oil exhibited antimicrobial effect against *S. aureus* and MRSA, both *in vitro* as well as in clinical trials (Hammer et al. 2012; Dryden et al. 2004; Caelli et al. 2000). The inhibitory effect of this plant could be described by the presence of several monoterpenes, mainly terpinen-4-ol, γ-terpinene, α-terpinene, and 1,8-cineole (Carson et al. 2006). Currently, tea tree oil has been widely commercialized and used for inhalation therapies of bacterial and fungal pneumonia (Li et al. 2016).

It is estimated among 250,000-500,000 known higher plant species, only 1-10% of plants used by humans, 6% of them were tested for at least one biological activity, in which only a small fraction have been investigated for the presence of antimicrobial compounds, and just 15% has been studied phytochemically (Verpoorte 2000; Cowan 1999; Borris 1996). After the post-antibiotic era, there is a rising threat of resistant microorganism and no major classes of novel antibiotics were introduced for the treatment of the infection (Silver 2011). Based on these facts, the research on the screening, identification, and characterisation of plant-derived components are still considered potential for discovery of novel phytochemicals, especially with antimicrobial properties.

2.4. Traditional Indonesian medicine (Jamu)

Indonesia, one of the world's richest nations in terms of its biodiversity, is an archipelago country consist of 17000 islands throughout, expected to possess 10% of the world’s flowering species (estimated 25,000 flowering plants, 55% endemic). Around

6000-7000 species have been used for medicinal purposes. Up to now, it is estimated only one-third of these species have been identified with relative complete information on their biological properties (Zuhud 2009; Riswan and Yamada 2006; Berwanie 2004). Based on the richness of local phytocoenoses, the country's early inhabitants learned how to distinguish useful plants with beneficial effects. This knowledge of traditional folk medicine, familiarly known as "jamu", has been passed down from generation to generation, contributing the use for treatment of diseases, maintaining health, and wellness (Stevensen 1999).

Although the first written records, namely 'serat kawruh' and 'serat centhini' date from the eighteenth century, the earliest evidence for this folk medicinal system dates back to the eighth century, as it is illustrated by the image of a man grinding 'kalpataru' leaf with other ingredients to make a mixture for a woman's health and beauty in a stone relief on the wall of Borobudur temple in Central Java (Riswan and Sangat 2002). Jamu medicines are usually prepared traditionally in form of infusions or decoctions in water by mixing different plant parts such as leaves, bark, roots, fruits, and flowers. Species belonging to the family Zingiberaceae are the most frequently used jamu ingredients. For example, "jamu kunir asam", which mainly consists of turmeric (*Curcuma longa*) and tamarind (*Tamarindus indica*), has been used to cure several diseases associated with pathogenic microorganisms such as diarrhoea and dysentery (Beers, 2001). This jamu has currently available in the market in form of the powder and liquid extract (e.g. Kunyit asam, Sidomuncul, Indonesia). Several species used in jamu such as candlenut (*Aleurites moluccanus*), ilang-ilang (*Cananga odorata*), nutmeg (*Myristica fragrans*), God's crown (*Phaleria macrocarpa*), Java pepper (*Piper cubeba*), and clove (*Syzygium aromaticum*), are economically important crops native to Indonesia.

There are numerous ethnobotanical study of traditional herbal preparation in Indonesia. Sundanese community in West Java has been known to consume medicinal plants (*Allium cepa*, *Citrus maxima*, *Clinacanthus nutans*, *Durio zibethinus*, *Melastoma malabathricum*, and *Zingiber cassumunar*) to treat several illnesses such as diarrhoea, dysentery, stomachache, and toothache (Roosita et al. 2008). The leaf extract of *C. nutans* have previously been reported the *in vitro* antimicrobial effect against Gram-postive, Gram-negative, and *C. albicans* in high concentration of MIC (> 1 mg/mL) (Arullappan et al. 2014; Yang et al. 2013). Several researchers (Bakht et al. 2014; Hannan et al. 2010;

Santas et al. 2010; Benkeblia 2004) have extensively described the antimicrobial activity of *A. cepa*. The polysaccharide gel extract from *D. zibethinus* rand has been reported as growth-inhibitor of oral pathogens at the MIC of 100 mg/mL (Thunyakipisal et al. 2010). Diris et al. (2017) and Grosvenor et al. (1995) described the antimicrobial activity of *Melastoma malabathricum*. Phenolic compound found in the extract of this plant (1-4, the flavonols, ellagic acid) could be responsible for the anti-infective effect, as previously described (Wong et al. 2012). The fruit extract *C. maxima* has been reported to have antibacterial effect against *S. aureus*, *Bacillus subtilis*, and *Micrococcus luteus in vitro*. The fraction collected from this fruit such as caffeic acid and *p*-coumaric acid has been acted as antimicrobials (Mokbel & Suganuma 2006; Proestos et al. 2006). Many researchers have described the antimicrobial effect of essential oil from *Z. cassumunar* against Gram-positive, Gram-negative, and dermatophyte yeast (Boonyanugomol et al. 2017; Pithayanukul et al. 2007).

The knowledge of using folk medicinal plant is not only thriving in Java, but also in other part of Indonesia. Study conducted by (Sujarwo et al. 2015) revealed the traditional herbal preparation in Bali Island called "Loloh", which are generally prepared as decoction and commonly used for treatment of several ailments caused by microorganism such as diarrhoea and dysentery, such as *Andrographis paniculata*, *Blumea balsamifera*, *Jatropha curcas*, and *Lablab purpureus*. Some plants are well studied on their biological activity and their chemical profiles. The *in vitro* antimicrobial effect of *A. paniculata* against Gram-positive and Gram-negative bacteria have been described by several researchers (Al-Shanafi 2017; Malahubban et al. 2013; Mishra et al. 2013; Thalikunnil et al. 2011; Akbar 2011; Roy et al. 2010). Andrographolide, the main active constituent isolated from the leaf part, is responsible on the antimicrobial effect of the plant and has been clinically proved as effective anti-diarrheal drug (Banerjee et al. 2017; Jayakumar et al. 2013). Records in literature indicate that *B. balsamifera*, *J. curcas*, and *L. purpureus* exhibit antimicrobial activity (Rampadarath et al. 2016; Sakee et al. 2011, Priya et al. 2013). "Bakera", a traditional herbal steam bath in Minahasa, North Sulawesi. As a part of thermotherapy, aromatherapy, and recuperation purpose, the women usually used it after childbirth. Zumsteg & Weckerle (2007) reported that 31 plants used in "Bakera" are known as plant-producing essential oil such as *Citrus hystrix*, *Curcuma xanthorrhiza*, *Cymbopogon citratus*, *Cymbopogon nardus, M. fragrans, and S. aromaticum.* Healing properties from these plants could be attributed to the presence of

volatile oil compounds (eugenol, α-pinene, sabinene, zingiberene) (Jantan et al. 2003; Dubey et al. 2000). Eugenol, isolated from *S. aromaticum* has been reported to exhibit antibacterial activity against *S. aureus* ATCC 29213 and MRSA clinical isolates *in vitro* and reduce the colonization of *S. aureus in vivo* by inhibition of biofilm formation. It can effectively eradicate pre-established biofilms, decreases the expression of biofilm- and enterotoxin-related genes, damages the cell membrane, and causes the leakage of the cell contents (Yadav et al. 2015). Several reports also revealed the antifungal activity of eugenol (de Oliveira Pereira et al. 2013; Campaniello et al. 2010; Schmidt et al. 2007) and inhibitory activity against oral bacteria associated with dental caries and periodontal disease (Thosar et al. 2013). Currently, eugenol has been widely sold as a topical antiseptic, a counter-irritant and in dental preparations with zinc oxide for root canal sealing and pain control (DenTek Eugenol, Den Tek Oral Care. Inc., USA).

Although a review of the antimicrobial properties of Indonesian medicinal plants have previously been published by Nugraha and Keller (2011) and other specific investigations targeting anti-acne (Batubara et al. 2009), anti-candidal (Kusuma et al. 2014), anti-biofilm (Pratiwi et al. 2015) and resistant isolates inhibition (Wikaningtyas and Sukandar 2016) have been carried out, many of the jamu species are still wanted for the screening of its antimicrobial effect.

2.4.1. Characteristics of selected studied plant species

Following section provides data on botanical characteristics, geographical distribution, uses in traditional medicine and antimicrobial action of several plant species tested in this study. They were selected based on their well-reported traditional use in Jamu medicine for treatment of diseases likely to be associated with infections caused by pathogenic microorganisms.

***Aleurites moluccanus* (L.) Willd. (Euphorbiaceae)**

Synonym: *Aleurites javanica* Gand., *Aleurites remyi* Sherff, *Aleurites triloba* Forster & Forster f., *Camirium moluccanum* (L.) Ktze., *Croton moluccanus* L., *Jatropha moluccana* L.

Vernacular name: Buwa kare, kembiri, kemili, kemiling, kereh, madang ijo, tanoan, kamere, kemiri.

Botanical description: *A. molucannus* (Figure 1) is a medium-sized tree with a large

spreading crown that can reach 20 m in height and 0.9 m trunk diameter, although it typically grows to 10-15 m in open areas. Crooked trunks and irregular, wide, spreading or pendulous side branches are typical. In narrow valleys, *A. moluccana* usually has a branchless trunk and achieves its greatest height. The bark is grey-brown in colour, and fairly smooth with fine vertical lines. It has very distinctive leaves, which are 3- to 5-nerved from the base, alternate and simple, with entire, wavy margins. The leaf blades are 10-20 cm long with 2 glands at the junction of the leaf base and petiole that secrete a sweetish sap. Younger leaves are usually simple and deltoid to ovate in shape. The upper surface of young leaves is whitish with a silvery gloss, becoming dark green with age. The underside is rusty stellate-pubescent when young. The flower is monoecious, that is, it has both male and female flowers on the same tree. The flowers are greenish-white and fragrant and are arranged in a 10-15 cm terminal panicled cyme, with many small male flowers surrounding the female flower. The corolla is whitish with 5 free petals, dingy white to creamy in colour, oblong in shape and up to 1.3 cm in length. The fruit is green to brownish in colour and is a laterally compressed, ovoid to globose. Indehiscent drupe 5-6 cm long by 5-7 cm wide. Each fruit usually contains 2 or 3 seeds, but 1 seed may be found in male fruit. These seeds are edible when roasted. The seeds are contained within a hard, black, rough shell that is elliptical in shape and about 2.5-3.5 cm long (Krisnawati et al. 2011).

Natural habitat and geographic distribution: This species is extensively found in areas associated with the tropical monsoon climate, which have rather dry conditions in the dry season. It thrives in moist tropical regions up to 1200 m above sea level. It is native to Indo-Malaysia and distributed from India and China, throughout South-East Asia, to Polynesia and New Zealand. It has been introduced for cultivation in many tropical countries all over the world (Krisnawati et al. 2011, PROSEA 2016a).

Traditional uses: The seed is used as a laxative. Pulped kernels are used in poultices to treat headache, fevers, ulcers, swollen joints and constipation. The bark is used to treat stomachache, diarrhoea, and dysentery. The bark sap (mixed with coconut milk) to treat sprue. A decoction of young leaves to treat scrophulosis and boiled leaves are applied externally to treat headache and gonorrhoea (PROSEA 2016a, Ulung 2016).

Antimicrobial activity: The methanol nut extract displayed the most potent bacterial growth inhibitory activity against the *Proteus mirabilis* standard and clinical strains

(MICs of 438 and 215 µg/mL), *K. pneumoniae,* and *S. pyogenes* growth with MICs < 1000 µg/mL (Mpala et al. 2017). Locher et al. (1995) reported growth-inhibitory effect of methanol bark extract of *A. moluccanus* against *S. aureus* and *P. aeruginosa* using disk diffusion method.

***Curcuma mangga* Valeton and van Zijp (Zingiberaceae)**

Synonym: none

Vernacular name: Temu mangga.

Botanical description: *C. mangga* (Figure 2) is an erect, tillering, herbaceous perennial, 50-200 cm high with subterranean rhizome, yellowish-brown outside, white at the top and citron yellow inside and smelled like mango when sliced or crushed. Main rhizome is hard, globose or ellipsoid, while lateral rhizomes are cylindrical or clavate, smaller and copiously branched. Spurious stems are robust, erect with 2-4 scales or sheaths at the base and with 3-7 leaves higher up. Leaf sheaths are conduplicate and 30-65 cm long. Leaves are 2 seriate, blades are elliptical oblong to oblong lanceolate, 15-95 cm by 5-23 cm, and green with light or dark purple-brown zone along the midrib. Inflorescence is lateral, flowering shoot terminal arises from lateral rhizome, peduncle is slightly hairy, spike is cylindrical and 10-20 cm long with numerous leafy green bracts, flowers are between the bracts in the axils of thinly membranous bracteoles, and coma bract is white at base and purple towards the broad apex. Flower is slender with narrow throat; calyx is with 3 broad, obtuse teeth; corolla is 3-4 cm and white; labellum is obovate and 15-25 mm by 14-128 mm wide with a yellow median band; staminodes are white subelliptical; and another is with long narrow spur (Lim 2016).

Natural habitat and geographic distribution: The species occurs wild in the teak forest. It thrives in fertile, moist soils rich in organic matter, in full sun or partial shade, from near sea level to 1000 m. The species is indigenous to Malesia region and widely cultivated in Malaysia, Thailand, and Indonesia (Lim 2016).

Traditional uses: Relieving stomach complaints, gastric ulcer, liver diseases, malaria, vaginal infection, chest pain, fever, parasites, and general debility. Besides, it has also been used in postpartum care, specifically to aid womb healing (Wahab et al. 2011; Ulung 2014).

Antimicrobial activity: The ethyl acetate, hexane, and methanol extract of *C. mangga*

showed growth-inhibitory effect against *Bacillus subtilis*, *P. aeruginosa*, and *S. aureus* at concentration 500 mg/mL by agar disk diffusion (Koshi et al. 2009). The EO isolated from rhizome of *C. mangga* exhibited the antimicrobial effect against *S. aureus* and *C. albicans* at the MIC of 1.2 µL/mL and 3.7 µL/mL, respectively (Kamazeri et al. 2012).

***Orthosiphon aristatus* (Blume) Miq. (Lamiaceae)**

Synonym: *Orthosiphon stamineus* Benth., *Orthosiphon grandiflorum*, *Orthosiphon spicatus*

Vernacular name: Kumis kucing

Botanical description: *O. aristatus* (Figure 3) is a perennial herb, 25-200 cm tall, with quadrangular, poorly ramified, ascending stem. Leaves decussately opposite, ovate or rhombic, 2-9(-12) cm × 1.5-5 cm, cuneate at base, acute or acuminate at apex, serrate, glabrous or minutely pubescent, glandular-punctate; petiole 0.5-2(-4.5) cm long; stipules absent. Inflorescence an opposed cyme arranged in terminal racemes, 7-29 cm long. Flowers pedicellate; calyx 2.5-4.5 mm long (up to 12 mm in fruit), bilabiate, gland-dotted; corolla 10-20 mm long, tubular, bilabiate, white or (pale) lilac; stamens 4, long-protruding from the corolla tube; ovary superior, style long-protruding, slender, with enlarged, club-shaped and shallowly cleft stigma. Fruit splitting into 4 oblong-ovoid nutlets, 1.5-2 mm long, brownish, rugose (PROSEA 2016b).

Natural habitat and geographic distribution: The plant is distributed from India, Indo-China and Thailand, through Malesia to tropical Australia. As a wild plant, it occurs throughout Malesia, but is apparently rare in Borneo, Sulawesi and the Moluccas. It is now grown in South-East Asia (in Java since 1928), Africa, Georgia (Caucasus) and Cuba. It occurs in the wild in thickets, regrowths, grasslands, and along forest borders and roadsides, often in shaded not too dry localities, but also in sunny places, up to 1000 m altitude (PROSEA 2016b).

Traditional uses: It is used to treat jaundice in a mixture with leaves of *Blumea balsamifera* (L.) DC., *Phyllanthus fraternus* Webster, and rhizomes of *Curcuma xanthorrhiza* Roxb., and to treat diabetes together with the leaves of *Andrographis paniculata* (Burm.f.) Nees. In mixtures with leaves of other plants, it is also used against gout, rheumatism and arteriosclerosis. The decoction of a mixture with the leaves of *Hedyotis diffusa* Willd. and *Plantago major* L. for the treatment of vaginal infection

(Ulung 2014, PROSEA 2016b).

Antimicrobial activity: Ho et al. (2010) reported the antimicrobial activity of methanol leaf extract against *S. aureus* by disk diffusion method. Same method has been applied to determine antimicrobial activity against *B. cereus* and *S. aureus* at concentration tested 200 mg/mL (Malahubban et al. 2013). Research done by Alshawsh et al. (2012) revealed the antibacterial activity of leaves aqueous extract against *S. aureus* at an MIC of 1.56 mg/mL, but no inhibition was observed on the ethanol extract. It has been proposed that the activity of antimicrobial activity of this species due to the presences of phenolic compound, namely rosmarinic acid.

***Rotheca serrata* (L.) Steane & Mabb (Lamiaceae)**

Synonym: *Clerodendrum serratum* (L.) Moon, *Volkameria serrata* L *Volkameria serrata* L., *Cyclonema serratum* (L.) Hassk.

Vernacular name: Senggugu

Botanical description: *R. serrata* (Figure 4) is a perennial woody shrub or treelet up to 4 m tall, or rarely seemingly herbaceous, stems relatively stout, mostly unbranched, nodes not annulate; leaves elliptical to obovate, 7-22 cm × 3-8 cm, base acute to subcuneate, apex acute or short acuminate, margin serrate, glabrous on both surfaces, petiole 0.3-1.2 cm long; axillary cymes 3-5 cm long, terminal racemose panicle slender, 6-45 cm long; calyx campanulate, tube 4-7 mm long, deeply dentate to truncate, bluish turning green, corolla zygomorhic, tube swollen, 5-9 mm long, posterior lobes 0.9 cm long, usually dark blue, lateral lobes usually pale blue, lower (interior) lobe deflexed, 1.5 cm long, dark purple or dark violet, showy, not fragrant, stamens long exserted, blue, fruiting calyx somewhat accrescent; drupe subglobose or broadly obovoid, 6-9 mm long, glossy emerald green turning dark purple or black, not splitting (PROSEA 2016c).

Natural habitat and geographic distribution: Commonly found in grasslands, thickets and secondary forest from sea level up to 1700 m altitude. It is distributed from Pakistan and India eastward to central and southern China, Indo-China, Thailand, Malaysia and Indonesia. Also in Mauritius, Madagascar and South Africa, possibly introduced. (Sharma et al. 2009; PROSEA 2016c).

Traditional uses: The leaves are consumed during labour. Pounded leaves are externally applied in various prescriptions for rheumatism and painful joints. Ripe and unripe fruits

are chewed with the leaves of *Piper bettle* L. for coughs. It is also used for poulticing skin diseases, yaws, headache, leprosy, diarrhoea, dysentery, worm infection, and persistent fever. The shoots, raw or toasted, are consumed as a bitter seasoning (Dalimartha 1999; Ulung 2014; PROSEA 2016c).

Antimicrobial activity: Narayanan et al. (2004) reported the antimicrobial activity of ethanol root extract of *R. serrata* against *Streptococcus pyogenes* A and *Proteus mirabilis* at the MIC of 1.91 and 3.90 µg/mL, respectively. The ethanol root extract at concentration 7.5 mg/disk has also been reported to have antimicrobial activity against broad-spectrum Gram-positive and Gram-negative bacteria (Vidya et al. 2010). The aqueous leaf extract of *R. serrata* possess antibacterial activity against *E. coli* at MIC 6.74 mg/mL (Rashid et al. 2013).

***Woodfordia floribunda* Salisbury (Lythraceae)**

Synonym: *Grislea tomentosa* Roxb., *Lythrum punctatum* Span.

Vernacular name: Sidawayah

Botanical description: *W. floribunda* (Figure 5) is an evergreen shrub up to 5 m tall, with diffuse, irregular branching. Leaves opposite, distichous, ovate-lanceolate to narrowly lanceolate, 2-10(-14) cm × 1-3(-4) cm, base rounded to subcordate, apex attenuate to acuminate, entire, sparsely patently pubescent above, densely greyish tomentose and black punctate below; petiole 0-3 mm long; stipules absent. Inflorescence a 1-17-flowered cluster on a much condensed, axillary shoot. Flowers bisexual, slightly zygomorphic, 6-merous, pedicellate, with 2 bracteoles, protandrous; calyx with 1-1.5 cm long tube, greenish at base, pale to dark red distally, lobes 2-3 mm × 1.5-2 mm; petals inserted near the mouth of the calyx tube, narrowly lanceolate, 1-5 mm long, red, pale pink or white; stamens 12, inserted at one-third of the calyx tube and far exserted; ovary superior, incompletely 2-celled, style slender. Fruit a thin-walled, ellipsoid capsule 8-10 mm long, enclosed by the calyx tube, many-seeded. Seeds minute, narrowly obpyramidal, slightly compressed, smooth (PROSEA 2016d).

Natural habitat and geographic distribution: *W. floribunda*, growth in the rocky localities and exposed slopes, but it can be found in a wide range of habitats, including riverbanks, rain forest, semi-deciduous forest, montane grassland and open anthropogenic habitats, in Java at 30-1000 m altitude (PROSEA 2016d).

Traditional uses: The roasted and ground flowers are used externally to wounds and to the cut umbilical cord of a newborn baby to cause it to dry. A decoction of the seeds is recommended as a diuretic for patients with fever, or to treat rheumatism. Flowers, fruiting twigs and seeds are ingredients of complex prescriptions for dysentery and sprue, apparently because of their astringent properties (Ulung 2014).

Antimicrobial activity: No previous reports observed on *W. floribunda*.

3. HYPOTHESIS

The prevalence of communicable diseases remains high in developing countries like Indonesia. Moreover, there is an increasing tendency of the microorganism causing diseases to build resistance towards the common antibiotic used. One option of overcoming this problem is exploring of novel anti-infective products from the plant sources. In Indonesia, there are numerous traditional herbal-based preparations consumed for the curative purposes against infectious diseases. Despite several studies revealing the antimicrobial properties of Indonesian medicinal plants, a systematic screening for antimicrobial potentials following the standard methodological approaches remains limited. Since the medicinal plants have demonstrated great efficacy as antimicrobial remedies in the past, there is a high probability that systematic assessment of the plant products such as extracts and essential oil can lead to the development of novel agents with potent antimicrobial activity.

4. OBJECTIVES

The objective of this study is the comprehensive investigation of *in vitro* antimicrobial activity and chemical composition of plant-derived products from species used in traditional Indonesian medicine for treatment of infectious diseases against the panel of potentially pathogenic microorganisms. The specific aims of the study are as follow:

1. Evaluation of the antimicrobial effect of ethanol plant extracts using the broth microdilution method.
2. Assessment of antimicrobial and antistaphylococcal effects of essential oil of *C. mangga*, a typical jamu preparation, by broth microdilution volatilisation and modified broth microdilution methods.
3. Characterisation of the chemical composition of antimicrobially active essential oil of *C. mangga* using GC-MS and GC-QTOF-MS.

5. MATERIAL AND METHODS

5.1. Plant materials

The plants were obtained from the Biopharmaca collection garden, Bogor Agricultural University (IPB) in Dramaga, Bogor (West Java, ID) in July 2015 and August 2016. Specimens were then authenticated by Ervizal Amir Muhammad Zuhud and deposited in the Herbarium of the Department of Forest Resources Conservation and Ecotourism, the Faculty of Forestry, IPB. The scientific names of the plant species were verified using online sources (The Plant List 2013). The botanical names, families, common names, voucher specimen numbers, traditional uses and preparation of the tested parts are given in Table 1.

5.2. Preparation of plant extract

Plant materials were dried and finely ground into powder using an electric mill GM100 (Retsch, Haan, DE). Each powdered sample (15 g) was extracted with 450 mL of 80% ethanol (Penta, Prague, CZ) and placed on a rotary shaker (GFL3005, Burgwedel, DE) for 24 h at room temperature. Ethanol has been chosen as a solvent because of its traditional use for in jamu medicines (Indonesian Pharmacopoeia 2014). Extracts were filtered, concentrated *in vacuo* using a rotary vacuum evaporator R-200 (Buchi, CH) at 40°C. Dried residues were dissolved in 100% dimethylsulfoxide (DMSO) (Sigma-Aldrich, Prague, CZ) to obtain extract stock solution at a concentration of 51.2 mg/mL, which was kept at -80°C until tested.

5.3. Essential oil isolation

Plant materials were dried and finely ground into powder using an electric mill GM100 (Retsch, Haan, DE). Fifty grams of powdered sample was subjected to hydrodistillation in 1 L of distilled H_2O for 3 h using Clevenger apparatus (Merci, Brno, CZ). The EO was then collected, dried using anhydrous sodium sulphate (Merck, Darmstadt, DE) and stored at 4°C in airtight glass vials.

5.4. Microorganisms and media

The following American Type Culture Collection (ATCC) in the form of Culti-Loops standard strains (Oxoid, Basingstoke, UK) were used: *Candida albicans* ATCC 10231, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922,

Pseudomonas aeruginosa ATCC 27853, *Staphylococcus aureus* ATCC 29213, *S. aureus* ATCC 33591, *S. aureus* ATCC 33592, and *S. aureus* ATCC BAA-976.

Table 1. Ethnobotanical data on Indonesian medicinal plants tested

Botany name [Family]	Local name	Voucher specimen	Traditional use	Preparations (Administrations)*	Part used	Yield (%)
Aerva sanguinolenta (L.) Blume [Amaranthaceae]	Sambang colok	Ar-0017	Vaginal infection	Decoction (I)	Leaf	24.12
Agathis macrophylla (Lindl.) Mast. [Araucariaceae]	Agatis	Ar-0001	Oral disease, pharyngitis	Decoction (I)	Bark Leaf Wood	20.57 27.60 2.98
Aleurites moluccanus (L.) Willd. [Euphorbiaceae]	Kemiri	Ar-0013	Diarrhoea	Decoction (I)	Bark	12.83
Amomum compactum Sol. ex Maton [Zingiberaceae]	Kapulaga	Ar-0012	Acne treatment, oral disease	Decoction (I)	Seed	6.85
Amorphophallus muelleri Blume [Araceae]	Iles-iles	Ar-0011	Dysentery	Decoction (I)	Tuber	6.77
Barleria prionitis L. [Acanthaceae]	Landep	Ar-0074	Diarrhoea abscess, pharyngitis	Fresh (E), Decoction (I)	Leaf Stem	31.87 17.90
Bryophyllum pinnatum (Lam.) Oken. [Crassulaceae]	Sosor bebek	Ar-0077	Ear infection,	Decoction (I)	Leaf	25.22
Clerodendrum × *speciosum* Dombrain [Lamiaceae]	Nona makan sirih	Ar-0020	Dysentery	Decoction (I)	Flower Leaf	18.05 18.55
Curcuma mangga Valeton & Zijp. [Zingiberaceae]	Temu mangga	Ar-0069	Liver disease, anti-malarial, anti-viral, parasites	Decoction (I)	Rhizome	19.04
Dracaena angustifolia (Medik.) Roxb. [Asparagaceae]	Suji	Ar-0080	Dysentery	Decoction (I)	Leaf	29.39
Eleutherine bulbosa (Mill.) Urb. [Iridaceae]	Bawang dayak	Ar-0003	Diarrhoea, vaginismus	Fresh (I), Decoction (I)	Bulb Leaf	11.16 19.33
Evodia hortensis J.R.Forst. & G.Forst. [Rutaceae]	Zodia	Ar-0022	Dermatophytosis	Decoction (I)	Leaf	25.55
Fibraurea tinctoria Lour. [Acanthaceae]	Akar kuning	Ar-0075	Diarrhoea, skin diseases	Decoction (I)	Leaf Root	26.89 12.07

Table 1. Ethnobotanical data on Indonesian medicinal plants tested *(continued)*

Botany name [Family]	Local name	Voucher specimen	Traditional use	Preparations (Administrations)*	Part used	Yield (%)
Gardenia jasminioides J. Ellis [Rubiaceae]	Kaca piring	Ar-0079	Diarrhoea, dysentery, vaginal infection	Decoction (I), Macerate (I)	Leaf	25.02
Ipomoea quamoclit L. [Convolvulaceae]	Rincik bumi	Ar-0018	Diarrhoea	Decoction (I)	Leaf	20.01
Ixora paludosa (Blume) Kurz [Rubiaceae]	Asoka	Ar-0002	Diarrhoea	Decoction (I)	Bark	22.16
Lagerstroemia speciosa (L.) Pers. [Lythraceae]	Bungur kecil	Ar-0005	Dysentery, diarrhoea diphtheria, tuberculosis	Decoction (I)	Leaf Wood	11.82 4.57
Mussaenda frondosa L. [Rubiaceae]	Bunga nusa indah	Ar-0004	Acne treatment	Decoction (I), Fresh (E)	Flower	21.59
Oldenlandia corymbosa L. [Rubiaceae]	Rumput mutiara	Ar-0019	Urinary tract infection, abscess	Decoction (I)	Leaf	16.29
Orthosiphon aristatus (Blume) Miq. [Lamiaceae]	Kumis kucing	Ar-0076	Vaginal infection	Decoction (I)	Leaf	22.32
Paederia foetida L. [Rubiaceae]	Daun kentut	Ar-0007	Dermatophytosis, ear infection	Decoction (I), Fresh (E)	Leaf	25.18
Phaleria macrocarpa (Scheff.) Boerl. [Thymelaeaceae]	Mahkota dewa	Ar-0015	Oral disease, pharyngitis diarrhoea	Decoction (I)	Fruit Leaf Root	28.08 25.99 10.11
Phyllanthus buxifolius (Blume) Müll.Arg. [Euphorbiaceae]	Seligi	Ar-0024	Skin infection	Decoction (I)	Leaf	20.52
Plantago major L. [Plantaginaceae]	Daun sendok	Ar-0008	Dysentery	Decoction (I)	Leaf	23.00
Plectranthus scutellarioides (L.) R.Br. [Lamiaceae]	Iler	Ar-0010	Dysentery, tuberculosis	Decoction (I)	Leaf Root	17.76 3.07
Polyscias scutellaria (Burm.f.) Fosberg [Araliaceae]	Mangkokan	Ar-0016	Dysentery	Decoction (I), Fresh (I)	Leaf	30.96

Table 1. Ethnobotanical data on Indonesian medicinal plants tested *(continued)*

Botany name [Family]	Local name	Voucher specimen	Traditional use	Preparations (Administrations)*	Part used	Yield (%)
Premna oblongifolia Merr. [Menispermaceae]	Cincau hijau	Ar-0006	Abscess, pharyngitis, pneumonia,	Decoction (I)	Leaf Root	14.83 5.02
Pyrrosia piloselloides (L.) M.G. Price [Polypodiaceae]	Sisik naga	Ar-0025	Diarrhoea, dysentery	Decoction (I)	Leaf Stem	31.39 9.18
Rotheca serrata (L.) Steane & Mabb. [Lamiaceae]	Senggugu	Ar-0021	Dysentery	Decoction (I)	Leaf	31.49
Salacca zalacca (Gaertn.) Voss [Arecaceae]	Salak	Ar-0026	Diarrhoea	Fresh (I)	Fruit	77.89
Sericocalyx crispus (L.) Bremek. [Acanthaceae]	Keji beling	Ar-0072	Diarrhoea	Decoction (I)	Leaf	19.35
Sida rhombifolia L. [Malvaceae]	Sidaguri	Ar-0070	Skin infections	Decoction (I)	Leaf	12.84
Spermacoce neohispida Govaerts [Rubiaceae]	Gempur batu	Ar-0009	Diarrhoea, pneumoniae	Decoction (I), Fresh (E)	Leaf Root	15.91 26.82
Stelechocarpus burahol (Blume) Hook.f. & Thomson [Annonaceae]	Kepel	Ar-0014	Oral disease, pharyngitis	Decoction (I)	Leaf	17.84
Talinum paniculatum (Jacq.) Gaertn [Talinaceae]	Som jawa	Ar-0023	Skin infections	Decoction (I)	Root	13.79
Woodfordia floribunda Salisb. [Lythraceae]	Sidawayah	Ar-0078	Dysentery	Decoction (I)	Leaf	30.34

***)** Way of administration: (E) external use; (I) internal use.

Six clinical isolates (methicillin-resistant *S. aureus,* MRSA 1, MRSA 2, MRSA 3, and MRSA 4; tetracycline-resistant *S. aureus*, TRSA 1 and TRSA 2) were provided on agar plates from the Motol University Hospital (Prague, CZ). The identification of clinical isolates was performed by matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry as it is described in Rondevaldova et al. (2017). Microorganism cultures were maintained at Mueller-Hinton agar (MHA) (Oxoid, Basingstoke, UK) and stored at 4°C until use. Microorganism cultures were maintained in Mueller-Hinton broth (MHB) (Oxoid, Basingstoke, UK) at 4°C until use. The pH of the medium was equilibrated to final value of 7.6 using Trizma base (Sigma-Aldrich, Prague, CZ) (for *E. faecalis*, the MHB was enriched with 1% of glucose). For inoculum standardisation, the turbidity of the microorganism suspension was adjusted to a 0.5 McFarland standard (1.5×10^8 CFU/mL) using a Densi-La-Meter II (Lachema, Brno, CZ) spectrophotometric device.

5.5. Broth microdilution assay

MICs were determined by the broth microdilution method using 96-well microplates modified according to previous recommendations for the effective assessment of the anti-infective potential of natural products (Cos et al. 2006; Clinical Laboratory Standard Institute [CLSI] 2008). Assay microplate preparation and serial dilution were performed using the automated pipetting platform Freedom EVO 100 (Tecan, Mannedorf, CH). Serial dilutions (100 μL) of each extract were distributed into the plate and diluted in the MHB making concentrations ranging from 4 to 512 μg/mL. Thereafter, the plates were inoculated with the respective microorganism suspension to make a final density 5×10^5 CFU/mL for bacteria and 1.5×10^3 CFU/mL for yeast, respectively. Plates were then incubated at 37°C for 24 h (48 h for *C. albicans*). Microorganism growth was measured in terms of turbidity recorded at 405 nm (Cos et al. 2006) by a Cytation 3 microplate reader (Biotech, Winooski, USA). The MIC was expressed as the lowest concentration that showed ≥ 80% inhibition of microbial growth compared to an extract-free growth control. The antibiotics tetracycline and tioconazole were dissolved in ethanol (Sigma-Aldrich, Prague, CZ) and used as positive controls. The DMSO at the concentration of 1% did not inhibit any of the strains tested. The results were expressed as the median/mode of MICs and obtained from three independent experiments that were assayed in triplicate.

5.6. Modified broth microdilution assay

The antimicrobial activity of EO was determined using standard broth microdilution method using 96-well microplates modified for testing of volatile antimicrobial agents (Rondevaldova et al. 2015). First, the EO was dissolved in 100% DMSO at maximum concentration 1%, and diluted in MHB. Serial dilutions (100 µL) of essential oil were distributed into the plate and diluted in the MHB making concentrations starting from 1024 µg/mL. The plates were inoculated with the bacterial suspensions and immediately covered using EVA Capmat (Micronic, Aston, USA). Then, the plates were incubated at 37 °C for 24 h. MICs were evaluated by visual assessment of bacterial growth after colouring of metabolically active bacterial colony with MTT dye (Sigma-Aldrich, Prague, CZ) when the interface of colour change from yellow to purple (relative to that of colours in control wells) was recorded. The antibiotics tetracycline and tioconazole were dissolved in ethanol (Sigma-Aldrich, Prague, CZ) and used as positive controls. The DMSO at the concentration of 1% did not inhibit any of the strains tested. All experiments were carried out in triplicate in three independent experiments and results were expressed as median/mode of MICs.

5.7. Broth microdilution volatilisation assay

Antimicrobial potential of the essential oil in liquid and vapour phase was determined using broth microdilution volatilisation method (Houdkova et al. 2017). The experiments were performed in white 96-well immunoplates (total well volume = 400 µL) covered by tight-fitting lids with flanges designed to reduce evaporation (SPL Life Sciences, Naechon-Myeon, KR). Initially, 30 µL of MHA was pipetted into every flange on the lid and inoculated with five µL of bacterial suspension after agar solidification. Then, the EO was dissolved in 100% DMSO at maximum concentration 1%, and diluted in MHB. 100 µL two-fold serial dilution of sample concentration starting from 1024 µg/mL were distributed into the plates. The plates were then inoculated with bacterial suspensions. Finally, the clamps (Lux Tool, Prague, CZ) with the handmade wooden pads (size 8.5 × 13 × 2 mm) were used for fastening plate and lid together. Then, the plates were incubated at 37 °C for 24 h. MICs were evaluated by visual assessment of bacterial growth after colouring of metabolically active bacterial colony with MTT dye (Sigma-Aldrich, Prague, CZ) when the interface of colour change from yellow to purple (relative to that of colours in control wells) was recorded. The antibiotics tetracycline and tioconazole were

dissolved in ethanol (Sigma-Aldrich, Prague, CZ) and used as positive controls. The DMSO at the concentration of 1% did not inhibit any of the strains tested either in broth or agar media. All experiments were carried out in triplicate in three independent experiments and results were expressed as median/mode of MICs.

5.8. GC and GC-MS analysis

For determination of main components of essential oil, the analysis was carried out using the dual-column/dual-detector GC system Agilent GC-7890B equipped with autosampler Agilent 7693, two columns, a fused-silica HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm, Agilent 19091s-433) and a DB-17MS column (30 m × 0.25 mm, film thickness 0.25 µm, Agilent 122-473), and a flame ionisation detector (FID) coupled with single quadrupole mass selective detector (MSD) Agilent MSD-5977B (Agilent Technologies, Santa Clara, CA, USA). The operational parameters were set as follow: the sample was diluted in *n*-hexane (Merck, Darmstadt, DE) to concentration of 5 µg/mL and 1 µL of the solution was injected in split mode (1:20) into inlet preheated to 250°C, carrier gas He (1 mL/min), oven temperature started at 60 °C for 3 minutes to 231ºC at a rate of 3 °C/min and held isothermal for 10 minutes. A mass detector was set to the ionisation energy 70 eV, ion source temperature 200 °C; scan time 1 s; mass range 30-600 m/z. Following the same operational parameter, the determination of essential oil compounds was also carried out using gas chromatograph with quadrupole time-of-flight mass spectrometer (GC-QTOF-MS, Agilent GC 7890B/QTOF 7200B, Agilent Technologies, Santa Clara, CA, USA) and a fused-silica HP-5MS column (30 m × 0.25 mm, film thickness 0.25 µm, Agilent 19091s-433).

The data were processed using qualitative analysis software version B.07.00 (Agilent Technologies, USA). The identification of chemical constituents was based on the co-injection of authentic standard compounds, comparison of their retention indices (RI) and retention time, and matching of mass spectra with those listed in National Institute of Standards and Technology Library ver. 2.0.f (NIST, USA) and Adams (2007). The retention indices were calculated for compounds separated by HP-5MS column using the retention times of a series of *n*-alkanes ranging from C_9 to C_{29} (Sigma-Aldrich, Prague, CZ). The final number of compounds was calculated as the sum of components simultaneously identified using both columns and remaining constituents identified by individual columns only. The relative percentage contents of essential oils components were determined by FID, which was indicated on both columns.

6. RESULTS AND DISCUSSION

6.1. Screening of *in vitro* antimicrobial activity of plant extracts

In this study, a total of 49 ethanol extracts from 37 different Indonesian medicinal plant species were investigated for their *in vitro* antimicrobial activity. The MIC values determined by means of the broth microdilution method are shown in Table 2. There were 21 plant extracts, namely: *Aerva sanguinolenta*, *Agathis macrophylla*, *Aleurites moluccanus*, *Amorphophallus muelleri*, *Bryophyllum pinnatum*, *Clerodendrum* x *speciosum*, *C. mangga*, *Eleutherine bulbosa*, *Fibraurea tinctoria*, *Ixora paludosa*, *Lagerstroemia speciosa*, *Orthosiphon aristatus*, *Phaleria macrocarpa*, *Phyllanthus buxifolius*, *Plectranthus scutellarioides*, *Premna oblongifolia*, *Rotheca serrata*, *Sericocalyx crispus*, *Spermacoce neohispida*, *Talinum paniculatum*, and *Woodfordia floribunda*, exhibited growth-inhibitory effect against at least one out of five microorganisms tested at a concentration ranging from 128 to 512 μg/mL.

Among 21 active plant extracts tested, the extract of *O. aristatus* leaf produced the strongest antimicrobial effect, inhibiting the growth of *C. albicans* and three bacteria (*S. aureus*, *E. faecalis*, and *P. aeruginosa*) at MICs of 128 and 256 μg/mL, respectively. The result is in correspondence with previously published results showing the antimicrobial effect of methanol and ethanol leaf extract of *O. aristatus* against *S. aureus, P. aeruginosa,* and *C. albicans,* determined by disk and agar diffusion methods (Ho et al. 2013, Neharkar & Laware 2013; Vijayan et al. 2013). In addition, the essential oil of *O. aristatus* leaf has also been reported as exhibiting antifungal properties against several plant pathogens such as *Botrytis cinerea*, *Colletotricum capsici*, *Fusarium solani*, *Phytophthora capsici* and *Rhizoctonia solani* (Hossain et al. 2008). Several compounds which have been isolated from ethanol leaf extract of *O. aristatus* such as diterpenoids orthoarisins, polymethoxylated flavonoids, and rosmarinic acid, could be responsible for broad-spectrum antimicrobial effect, as they have been previously reported to exert growth-inhibitory effects (Olah et al. 2003; Gohari et al. 2010; Salawu et al. 2011; Di et al. 2013, Veneziani et al. 2017).

The leaf extract of *W. floribunda* also exhibited strong anti-fungal effect against *C. albicans* and moderate inhibition activity against two bacteria (*S. aureus* and *E. faecalis*) at respective MICs of 128 and 256 μg/mL. Up to now, there are no previous studies reporting any antimicrobial effect for *W. floribunda*. However, the leaf extract of related

species *Woodfordia fruticosa* has been reported to show inhibitory activity against methicillin-resistant *S. aureus* and the phytochemical screening of the *n*-butanol fraction of its leaf by GC-MS revealed the presence of secondary metabolites such as diethyl phthalate and thymol (Dubey et al. 2014). Both of these compounds were previously described as exhibiting several antimicrobial properties (Mujeeb et al. 2014). Yoshida et al. (1990) reported the isolation and characterisation of a hydrolysable tannin dimer, woodfordin C, from the methanol leaf extract of *W. fruticosa*. This compound has been reported as exhibiting anti-tumour and antimicrobial effects via the inhibition of DNA topoisomerase enzyme II that is important for DNA replication (Akiko et al. 1992; Mitscher 2005). As one of the chemotaxonomic markers found in the Lythraceae family, it is assumable that the chemical compounds found in *W. fruticosa* may also contribute to the antimicrobial effect in *W. floribunda*. Nevertheless, it should be considered that incomplete data on the metabolite profiles of these plants limits the interpretation of any chemotaxonomic markers as some species within the same genus might produce different compounds (Liu et al. 2017).

The rhizome extract of *C. mangga* exhibited moderate antimicrobial activity against *S. aureus* and *E. faecalis* at MIC of 256 μg/mL, respectively. Our results can be supported by findings of Renisheya et al. (2011) who determined an antimicrobial effect of ethanol extract of *C. mangga* against clinical isolates strains of *S. aureus* and *P. aeruginosa* via the disk diffusion technique. Philip et al. (2009) used agar the diffusion method and reported inhibitory effects of methanol, ethyl acetate, and hexane extracts of *C. mangga* rhizome on *P. aeruginosa*. In contrast to the results observed in this study, there were no inhibitory activity recorded on the Gram-negative bacteria probably due to the difference in methodology, microbial strains, and the extract concentrations tested.

The bark extract of *A. moluccanus* exhibited the growth-inhibitory activity against *S. aureus, E. faecalis*, and *C. albicans* at the range of MICs 256-512 μg/mL. Locher et al. (1995) have previously reported antimicrobial profile of methanol bark extract of *A. moluccanus* against *S. aureus* and *P. aeruginosa* using disk diffusion method, whereas the results are in agreement with this current study. Earlier work reported the isolation of bioactive compounds from the stem bark of *A. moluccanus* known as 3-acetyl aleuritolic acid and moluccanin, which exhibited antibacterial effects (Alimboyoguen et al. 2014). Thus, above-mentioned compound could be responsible for the antimicrobial activity on

A. moluccanus bark extract.

As previously reported by Rashid et al. (2013), the aqueous leaf extract of *R. serrata* demonstrated an inhibitory effect on *E. coli* in a disk diffusion assay. On the contrary, no inhibitory activity was observed in this research on *E. coli*, probably due to the difference in the solvent used. To the best of my knowledge, there are no previous studies reporting the antimicrobial effect of *R. serrata* leaf extract on *C. albicans* and *S. aureus.*

In general, the susceptibility of Gram-positive bacteria and yeast were higher than Gram-negative bacteria. Only three plant extracts (*O. aristatus*, *B. pinnatum*, and *S. crispus*) inhibited the growth of *P. aeruginosa* at a MIC of 512 μg/mL. None of the plant extracts tested in this study were found to inhibit the growth of *E. coli*. It is necessary to note that the differences of the antimicrobial effect of plant species tested between this research and others on the antimicrobial activity of plant species tested could be affected by several factors, e,g. the extraction techniques used, the type of solvent used, the methods of antimicrobial susceptibility testing, the different strains of microorganisms used, and the geographical origin of plant materials (Price & Morgan 2006; Dai & Mumper 2010).

Table 2. Antimicrobial activity of ethanol extracts from Indonesian medicinal plants

Plant samples	Part used	Microorganisms/Minimum inhibitory concentrations (µg/mL)				
		Staphylococcus aureus	*Enterococcus faecalis*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Candida albicans*
Aerva sanguinolenta	Leaf	-[a]	512	-	-	512
	Bark	512	-	-	-	-
Agathis macrophylla	Leaf	-	-	-	-	-
	Wood	-	-	-	-	-
Aleurites moluccanus	Bark	256	512	-	-	256
Amomum compactum	Seed	-	-	-	-	-
Amorphophallus muelleri	Tuber	-	-	-	-	512
Barleria prionitis	Leaf	-	-	-	-	-
	Stem	-	-	-	-	-
Bryophyllum pinnatum	Leaf	-	-	512	-	-
Clerodendrum x speciosum	Flower	-	-	-	-	-
	Leaf	-	-	-	-	512
Curcuma mangga	Rhizome	256	512	-	-	-
Dracaena angustifolia	Leaf	-	-	-	-	-
Eleutherine bulbosa	Bulb	-	-	-	-	512
	Leaf	-	-	-	-	512
Evodia hortensis	Leaf	-	-	-	-	-
Fibraurea tinctoria	Leaf	-	512	-	-	-
	Root	-	512	-	-	-
Gardenia jasminoides	Leaf	-	-	-	-	-
Ipomoea quamoclit	Leaf	-	-	-	-	-
Ixora paludosa	Leaf	512	-	-	-	512
Lagerstroemia speciosa	Leaf	512	-	-	-	512
	Wood	-	-	-	-	-
Mussaenda frondosa	Flower	-	-	-	-	-

Table 2. Antimicrobial activity of ethanol extracts from Indonesian medicinal plants *(continued)*

Plant samples	Part used	Microorganisms/Minimum inhibitory concentrations (µg/mL)				
		Staphylococcus aureus	*Enterococcus faecalis*	*Pseudomonas aeruginosa*	*Escherichia coli*	*Candida albicans*
Oldenlandia corymbosa	Leaf	-	-	-	-	-
Orthosiphon aristatus	Leaf	256	256	256	-	128
Paederia foetida	Leaf	-	-	-	-	-
	Fruit	-	512	-	-	-
Phaleria macrocarpa	Leaf	-	-	-	-	-
	Root	-	-	-	-	-
Phyllanthus buxifolius	Leaf	-	512	-	-	-
Plantago major	Leaf	-	-	-	-	-
Plectranthus scutellarioides	Leaf	512	-	-	-	-
	Root	-	-	-	-	-
Polyscias scutellaria	Leaf	-	-	-	-	-
Premna oblongifolia	Leaf	-	-	-	-	512
	Root	-	-	-	-	-
Pyrrosia piloselloides	Leaf	-	-	-	-	-
	Stem	-	-	-	-	-
Rotheca serrata	Leaf	256	-	-	-	512
Salacca zalacca	Fruit	-	-	-	-	-
Sericocalyx crispus	Leaf	512	512	512	-	512
Sida rhombifolia	Leaf	-	-	-	-	-
Spermacoce neohispida	Leaf	512	-	-	-	-
	Root	-	-	-	-	-
Stelechocarpus burahol	Leaf	-	-	-	-	-
Talinum paniculatum	Root	-	512	-	-	512
Woodfordia floribunda	Leaf	256	256	-	-	128
Antibiotics[b]		0.5	16	16	1	0.25

Footnotes: [a]) not active (MIC >512 µg/mL) [b]) tetracycline and tioconazole were used as positive controls for bacteria and yeast, respectively.

6.2. Assessment of antimicrobial and antistaphylococcal effect of *C. mangga* EO

Initially, the hydrodistilled *C. mangga* EO was evaluated for its antimicrobial effect using broth microdilution volatilisation method. The results (Table 3) showed that *S. aureus* was susceptible to the *C. mangga* EO, exhibiting *in vitro* growth-inhibitory effect in the liquid phase at MIC of 256 µg/mL, but no inhibition activity at vapour phase (MIC > 1024 µg/mL). The EO did not exhibit inhibitory activity against other microorganisms (*E. coli*, *E. faecalis*, *P. aeruginosa*, and *C. albicans*) in both liquid and vapour phase (MIC > 1024 µg/mL, respectively).

With exception of growth-inhibitory effect of the oil against *S. aureus* in liquid phase, there were no positive results observed for remaining microorganisms tested. Therefore, the anti-staphylococcal effect of the oil was subsequently determined more in detail using broth microdilution method modified for testing of volatile antimicrobial agents. The results of modified broth microdilution method (Table 4) showed that *C. mangga* EO exhibited antistaphylococcal activity at the MIC ranging from 128 to 1024 µg/mL. Tetracycline-resistant *S. aureus* (TRSA) 2 was the most sensitive strains to the EO, inhibited at the lowest MIC of 128 µg/mL.

These findings are in correspondence with the results previously reported by Kamazeri et al. (2012), where the *C. mangga* rhizome EO exhibited growth-inhibitory activity against *S. aureus* ATCC 25923 at an MIC of 1.2 µL/mL. These results can also be supported by previous studies conducted by Romulo et al. (2018) and Renisheya et al. (2011), who observed the inhibitory activity of *C. mangga* rhizome extracts against *S. aureus*. However, this is the first study demonstrating the antistaphylococcal effect of its EO against the broad spectrum of standard and clinical strains, including MRSA and TRSA.

Several factors that are influencing the activity of EOs are the composition, functional groups present in active components, and their synergistic interactions between the compounds (Dorman & Deans 2000; Chouhan et al. 2017). It is well known that Gram-positive bacteria are more susceptible to EOs than Gram-negative bacteria. The susceptibility are influenced by the cell structure differences between them. Gram-negative bacteria have a rigid lipopolysaccharide in the outer membrane, which is absent in Gram-positive bacteria, therefore limiting the diffusion of the hydrophobic compounds (Hyldgaard et al. 2012). The

bioactive components present in EOs might attach to the surface of the cell, and thereafter penetrate to the phospholipid bilayer of the cell membrane. The accumulation of the compounds detrimentally influence the structural integrity of cell membrane, disturbing the cell metabolism, and causing cell death (Lv et al. 2011).

Table 3. *In vitro* antimicrobial activity of *Curcuma mangga* rhizome essential oil in liquid and vapour phase

Microorganisms	Minimum Inhibitory Concentration (µg/mL)			
	EO		Tetracycline	
	Broth	Agar	Broth	Agar
Staphylococcus aureus ATCC 29213	256	> 1024	0.5	ND
Enterococcus faecalis ATCC 29212	> 1024	> 1024	16	ND
Pseudomonas aeruginosa ATCC 27853	> 1024	> 1024	16	ND
Escherichia coli ATCC 25922	> 1024	> 1024	1	ND
Candida albicans ATCC 10231	> 1024	> 1024	0.5*	ND

ATCC: American type culture collection. *: Tioconazole for *C. albicans*. ND: Not determined.

Table 4. *In vitro* antistaphylococcal activity of *Curcuma mangga* rhizome essential oil

S. aureus strains	Minimum Inhibitory Concentration (µg/mL)	
	EO	Tetracycline
ATCC 29213	256	0.5
ATCC 33591	1024	128
ATCC 33592	512	128
ATCC BAA-976	512	0.25
MRSA 1	256	0.5
MRSA 2	256	64
MRSA 3	256	32
MRSA 4	256	0.5
TRSA 1	256	16
TRSA 2	128	16

ATCC: American type culture collection; MRSA: methicillin-resistant *S. aureus*; TRSA: tetracycline-resistant *S. aureus*

6.3. Analysis of chemical composition of *C. mangga* EO

The isolation of *C. mangga* EO resulted in the yellow oil with a yield 0.32 % (v/w). There were 106 compounds identified by GC-MS and GC-QTOF-MS, using HP-5MS and DB-17MS columns, representing 95.79/94.99% of their total contents, respectively. The analyses showed that the major constituents of the oil from *C. mangga* rhizome were represented by monoterpenoids, sesquiterpenoids, and diterpenoids with total content 85.63/77.23%, whereas the sesquiterpenoids were the most dominant components (43.36/32.40%). The characterisation of *C. mangga* rhizome EO by GC-MS and GC-QTOFMS revealed several main compounds such as 15,16-dinorlabda-8(17),11-dien-13-one (22.76/13.70%), by ambrial (14.90/9.52%), 13-nor-eremophil-1(10)-en-11-one (6.64/5.39%), 15,16-dinorlabda-8(17),12-dien-14-al (5.71/6.68%), and aromadendrene oxide (5.53/3.27%). There were also significant amounts of caryophyllene oxide (3.60/5.26%), isolongifolol (3.55/1.91%), m-camphorene (3.15/-%), hexadecanoic acid (3.11/10.44%) and β-pinene (1.43/2.98%). Details of EO composition are shown in Table 5. The FID chromatograms of the EO in HP-5MS column and DB-17MS column are shown in Figure 6 and 7. Total ion current (TIC) chromatogram of the EO in HP-5MS obtained from GC-QTOF-MS is shown in Figure 8. The mass spectra of the main compounds of the EO are shown in Figure 9, Figure 10, Figure 11, Figure 12, and Figure 13.

It has been documented that β-pinene was detected as the major compound of the EO from *C. mangga* rhizome collected from the wild population in Indonesia (> 15%) (Lolita, 2012), while it was found as a minor compound in this study (< 3%). On the other hand, Kamazeri et al. (2012) reported sesquiterpenoid caryophyllene oxide (18.71%) as the main compound in *C. mangga* rhizome EO obtained by steam distillation, which was also detected in lower amounts in the current study (< 6%). Ambrial appears as one of the major compounds in the EO of *C. mangga* rhizome from Indonesia, but only small amount (2.3%) of this compound was detected in the literature on plant material obtained in Malaysia (Wahab et al., 2011). The significant variances between chemical compositions of *C. mangga* EOs analysed in this study and above-mentioned reports could be influenced by the differences in geographical origin of plants, growing conditions, and isolation methods (Figueiredo et al. 2008; Kokoska et al. 2012).

Table 5. Chemical composition of *Curcuma mangga* rhizome essential oil

Component	Retention Indices (RI)			Relative Content (%)		Identification[d]
	Published[a]	Observerd[b]	Observed[c]	HP-5	DB-17	
Monoterpenoids						
5,5-Dimethyl-1-vinylbicyclo[2.1.1]hexane	921	919	912	0.05	0.13	MS, QTOF, RI
Cyclene	925	923	914	0.02	-[f]	MS, QTOF, RI
α-Pinene	937	933	926	0.35	0.74	MS, QTOF, RI, Std
Camphene	952	948	940	0.78	1.69	MS, QTOF, RI
β-Pinene	979	977	970	1.43	2.98	MS, QTOF, RI
m-Cymene	1023	1025	1023	0.13	0.37	MS, QTOF, RI, Std
Limonene	1030	1029	1024	0.09	0.24	MS, QTOF, RI, Std
Camphenilone	1083	1085	1080	0.03	0.06	MS, QTOF, RI
Camphenol	1097	-	1095	+[h]	0.06	MS, QTOF, RI
Perillene	1101	1102	1100	0.14	0.18	MS, QTOF, RI
Fenchol	1115	1115	-	0.03	0.05	MS, QTOF, RI
α-Campholenal	1125	1127	1124	0.13	0.19	MS, QTOF, RI
Nopinone	1137	1139	1134	0.13	0.71	MS, QTOF, RI
Pinocarveol	1139	1140	1139	0.11	0.96	MS, QTOF, RI
Camphor	1145	1146	-	0.08	0.05	MS, RI
3-Methylcamphenilanol	1148	[e]	[e]	-	0.05	MS
β-Pinene oxide	1156	[e]	[e]	-	0.16	MS
Pinocarvone	1164	1164	1160	0.17	-	MS, QTOF, RI
Borneol	1167	1167	-	0.03	0.11	MS, RI
Isoneral	1170	-	1173	+	-	QTOF, RI
3,7-Dimethyl-3,6-octadienal	1184	1178	-	0.09	0.11	MS, RI
α-Terpineol	1189	-	1189	+	0.06	MS, QTOF, RI
Myrtenal	1193	1198	1194	0.75	0.80	MS, QTOF, RI
Verbenone	1205	1212	1207	0.09	0.12	MS, QTOF, RI
Myrtenol	1213	-	1227	+	0.49	MS, QTOF, RI
Carveol	1219	1220	1222	0.17	-	MS, QTOF, RI

Table 5. Chemical composition of *Curcuma mangga* rhizome essential oil *(continued)*

Component	Retention Indices (RI)			Relative Content (%)		Identification[d]
	Published[a]	Observerd[b]	Observed[c]	HP-5	DB-17	
Isobornyl formate	1232	[e]	[e]	-	0.08	MS
Myrtenyl acetate	1235	1243	1239	0.11	0.15	MS, QTOF, RI
Pinocarvyl acetate	1258	1255	1251	0.11	-	MS, QTOF, RI
Bornyl acetate	1285	1288	1284	1.10	1.50	MS, QTOF, RI
Limonene dioxide	1294	-	1294	+	-	QTOF, RI
p-Mentha-1,4-dien-7-ol	1296	[e]	[e]	-	0.36	MS
β-Ionone	1491	[e]	[e]	-	0.55	MS
Dihydro-β-ionone	[g]	1530	1521	2.56	-	MS, QTOF, RI
Group sum (%)				**8.68**	**12.95**	
Sesquiterpenoids						
Cryprotene	1345	1355	1351	0.47	-	MS, QTOF, RI
Cyclosativene	1368	1367	1363	0.43	0.84	MS, QTOF, RI
Ylangene	1372	-	1365	+	-	QTOF, RI
Longicyclene	1374	[e]	[e]	-	0.29	MS
Sobrerol	1388	1382	-	0.05	-	MS, RI
Sativene	1396	1393	1387	0.27	0.26	MS, QTOF, RI
α-Himachalene	1449	-	1447	+	-	QTOF, RI
Selinane	1476	1465	-	0.21	-	MS, RI
Caparratriene	1493	1494	-	1.24	-	MS, RI
Epicubebol	1493	-	1493	+	-	QTOF, RI
Dihydro-β-agarofuran	1496	-	1475	+	-	QTOF, RI
Cubebol	1515	1520	1516	0.10	0.03	MS, QTOF, RI
Calamene	1523	1527	-	0.05	-	MS, RI
Diepicedrene-1-oxide	1551	-	1545	+	-	MS, QTOF, RI
Caryophyllene oxide	1581	1588	1580	3.60	5.26	MS, QTOF, RI
Sesquisabinene hydrate	1581	-	1588	+	-	QTOF, RI
Globulol	1583	[e]	[e]	-	0.50	MS

Table 5. Chemical composition of *Curcuma mangga* rhizome essential oil *(continued)*

Component	Retention Indices (RI)			Relative Content (%)		Identification[d]
	Published[a]	Observerd[b]	Observed[c]	HP-5	DB-17	
Humulene oxide	1606	1614	-	0.10	-	MS, RI
13-nor-Eremophil-1(10)-en-11-one	1629	1635	1625	6.64	5.39	MS, QTOF, RI
Caryophylladienol II	1637	[e]	[e]	-	0.35	MS
Cubenol	1642	[e]	[e]	-	0.08	MS
Longifolenaldehyde	1668	1677	1667	2.81	1.41	MS, QTOF, RI
Allohimachalol	1674	[e]	[e]	-	1.63	MS
Cadalene	1674	1684	1673	0.26	-	MS, QTOF, RI
Ylangenal	1675	-	1684	+	-	QTOF, RI
Aromadendrene oxide	1678	1643	-	5.53	3.27	MS, RI
Saussurea lactone	[g]	1688	1679	1.45	-	MS, QTOF, RI
ent-Germacra-4(15),5,10(14)-trien-1β-ol	1695	1696	-	0.08	0.66	MS, RI
Isolongifolol	1738	1754	-	3.55	1.91	MS, RI
Costol	1778	-	1768	+	-	MS, QTOF, RI
Ambrial	1809	1807	1795	14.90	9.52	MS, QTOF, RI
Dehydrosaussurea lactone	1838	1838	1834	0.27	0.32	MS, QTOF, RI
Isolongifolol acetate	1850	1843	-	0.23	-	MS, RI
Eudesma-5,11(13)-dien-8,12-olide	1890	[e]	[e]	-	0.12	MS
Corymbolone	1899	1899	-	0.11	-	MS, RI
β-Cyclocostunolide	1983	1917	-	1.01	0.41	MS, RI
Sclareolide	2089	[e]	[e]	-	0.15	MS
Group sum (%)				**43.36**	**32.40**	
Diterpeneoids						
8,13-epoxy-15,16-Dinorlab-12-ene	1894	1886	-	0.33	1.32	MS, RI
(E)-15,16-Dinorlabda-8(17),12-dien-14-al (isomer I)	[g]	1934	1923	5.71	6.68	MS, QTOF, RI
Cembrene	1939	1939	1944	0.43	-	MS, QTOF, RI

Table 5. Chemical composition of *Curcuma mangga* rhizome essential oil *(continued)*

Component	Retention Indices (RI)			Relative Content (%)		Identification[d]
	Published[a]	Observerd[b]	Observed[c]	HP-5	DB-17	
(E)-15,16-Dinorlabda-8(17),12-dien-14-al (isomer II)	1958	1964	-	0.40	3.36	MS, RI
Sandaracopimaradiene	1960	[e]	[e]	-	2.86	MS
m-Camphorene	1960	1967	1952	3.15	-	MS, QTOF, RI
(E)-15,16-Dinorlabda-8(17),11-dien-13-one	1994	1995	1982	22.76	13.70	MS, QTOF, RI
Epimanool	2056	2044	-	0.47	0.34	MS, RI
N.I., Diterpenoid, MW = 260	[g]	2084	2072	-	-	MS, QTOF, RI
Labda-8(17),12-diene-15,16-dial	2383	2363	2138	0.44	1.91	MS, QTOF, RI
Group sum (%)				**33.69**	**30.17**	
Others						
2,5 Dimethyltetrahydrofuran (isomer I)	694	[e]	[e]	-	0.21	MS
2,5 Dimethyltetrahydrofuran (isomer II)	694	[e]	[e]	-	0.35	MS
3-Ethylbutanal	719	[e]	[e]	-	0.11	MS
2-Nonanone	1092	1094	1090	0.03	0.22	MS, QTOF, RI
2-Nonanol	1101	1105	-	0.01	0.41	MS, RI
3-Decanol	1188	[e]	[e]	-	0.27	MS
3-(2,6,6-Trimethylcyclohexen-1-yl)-prop-2-enal	[g]	-	1275	+	-	MS, QTOF, RI
1,3,3-Trimethyl-2-(2-methylcyclopropyl)-1-cyclohexene	[g]	1281	-	0.45	0.89	MS, RI
2-Undecanone	1294	1296	-	0.07	0.43	MS, RI
Undecanol	1371	[e]	[e]	-	0.11	MS
4,4,7a-Trimethyl-2,4,5,6,7,7a-hexahydro-1H-inden-1-ol	[g]	-	1372	+	-	MS, QTOF, RI

Table 5. Chemical composition of *Curcuma mangga* rhizome essential oil *(continued)*

Component	Retention Indices (RI)			Relative Content (%)		Identification[d]
	Published[a]	Observerd[b]	Observed[c]	HP-5	DB-17	
3-(2,6,6-Trimethyl-1-cyclohexen-1-yl)-2-propen-1-ol	[g]	-	1380	+	-	MS, QTOF, RI
3-(2,6,6-Trimethyl-1-cyclohexen-1-yl)propanal	[g]	1385	-	0.08	0.08	MS, RI
4-(2,2-Dimethyl-6-methylenecyclohexyl)-2-butanone	1407	1400	-	0.09	-	MS, RI
5,5,8a-Trimethyloctahydro-1(2H)-naphthalenone	[g]	-	1532	+	-	MS, QTOF, RI
5,5,8a-Trimethyldecalin-1-one	[g]	1541	-	0.26	0.28	MS, RI
2-Methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)butanal	[g]	1561	1553	1.71	0.91	MS, QTOF, RI
2-Methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2-butenal	1584	1592	1595	0.12	0.28	MS, QTOF, RI
2,4a,8,8-Tetramethyldecahydrocyclopropa[d]naphthalene	[g]	1603	1486	0.53	1.57	MS, QTOF, RI
2-Methyl-4-(2,6,6-trimethylcyclohex-1-enyl)but-2-en-1-ol	[g]	[e]	[e]	-	0.71	MS
Butyl 4,7,10,13,16,19-docosahexaenoate	[g]	1825	-	-	0.19	MS, RI
1-Hydroxy-4a,5-dimethyl-3-(propan-2-ylidene)-4,4a,5,6-tetrahydronaphthalen-2(3H)-one	1895	-	1905	+	-	QTOF, RI
Hexadecanoic acid	1968	1975	-	3.11	10.44	MS, RI
6,6,9a-Trimethyl-4,5,5a,6,7,8,9,9a-octahydronaphtho[1,2-c]furan-1(3H)-one	1997	2028	-	0.60	0.36	MS, RI

Table 5. Chemical composition of *Curcuma mangga* rhizome essential oil *(continued)*

Component	Retention Indices (RI)			Relative Content (%)		Identification[d]
	Published[a]	Observerd[b]	Observed[c]	HP-5	DB-17	
1-(5,5,8a-Trimethyl-2-methylenedecahydro-1-naphthalenyl)-3-methyl-3-pentanol	[g]	2151	-	2.80	-	MS, RI
Group sum (%)				**9.86**	**17.63**	
Total Identified				**95.59**	**93.15**	

[a]) Retention Indices (RI) taken from Adams (2017) and National Institute of Standards and Technology Library ver. 2.0.f (2017). [b]) Retention indices calculated from retention times on a HP-5MS based on C_9-C_{29} alkanes as measured by gas chromatograph with mass selective detector (GC-MSD). [c]) Retention indices calculated from retention times on a HP-5MS and based on C_9-C_{29} alkanes as measured by gas chromatograph with quadrupole time-of-flight mass spectrometer (GC-QTOF-MS). [d]) Identification method: MS = Mass spectrum obtained from MSD was identical to that of National Institute of Standards and Technology Library (ver. 2.0.f), QTOF = Mass spectrum obtained from QTOF-MS was identical to that of National Institute of Standards and Technology Library (ver. 2.0.f), RI = retention indices was consistent with that of the literature database, Std = constituent identify confirmed by co-injection of authentic standards. [e]) Retention indices were not calculated for compounds identified only by DB-17MS column. [f]) Compounds not detected in the sample. [g]) Literature data not available. [h]) Value obtained from GC-QTOF-MS is below 1.55%.

In order to achieve better identification of EO constituents, two different columns (non-polar HP-5MS and DB-17MS of middle polarity) were used. The differences that occur in the relative peak area could be caused by the co-elution of the compounds in the first column. Due to the difference in the polarity, the co-elute compounds may be resolved on the second column (Eyres & Dufour 2009). As a result, the use of DB-17MS column revealed 16 additional compounds identified in *C. mangga* rhizome EO. Not only improving quantification and identification of EO components, the use of dual column/dual detector system can also avoid false-positive identification of compounds (Huang et al., 2014). Additionally, the sample was also analysed using GC-QTOF-MS. This analytical approach provides fast scanning, higher sensitivity, and mass accuracy compared to the common quadrupole MS (GC-MS), as it can deconvolute the overlapping peaks and detect some minor compounds (Rohloff, 2015; Nsuala et al., 2017). Based on this approach, 15 additional minor constituents were found in the *C. mangga* rhizome EO.

Although the antistaphylococcal activity of main constituents of the *C. mangga* rhizome EO and its mechanism was not investigated in the present study, previous literature showed the antimicrobial potential of some detected components. For example, β-pinene which has been reported to possess antimicrobial activity against *S. aureus* and MRSA at the MICs of 20 µL/mL and 6250 µg/mL, respectively (Leite et al., 2007; Rivas da Silva et al., 2012). Several studies have also reported the antimicrobial effect of EO with sesquiterpenoids as their major components (Shafaghat, 2012; Alencar Filho et al., 2017). Previous research demonstrated the antimicrobial activity of caryophyllene oxide against *S. aureus* at an MIC of 10.4 µg/mL (Cote et al., 2017).

There are numerous of diterpenoids possessing a labdane skeleton occur in nature, which called labdane diterpenes. It has become of interest because of the wide range of biological activity, such as such as antibacterial, antifungal, antiprotozoal, enzyme inducing, anti-inflammatory activities and modulation of immune cell functions (Singh et al. 1999). These compounds have been isolated from several plant families, such as Acanthaceae, Annonaceae, Apocynaceae, Asteraceae, Caprifoliaceae, Cistaceae, Cupressaceae, Lamiaceae, Pinaceae, Solanaceae, Taxodiaceae, Verbenaceae and Zingiberaceae (Demetzos & Dimas, 2001). Labdane diterpenes are undoubtly predominant diterpenoids in Zingiberaceae family, specifically in genera *Alpinia* (Sirat et al. 1994; Xu et al. 1996; Ghosh et al. 2013; Ma et al. 2017; Manse et al. 2017), *Hedychium*

(Chimnoi et al. 2008; Cheng et al. 2012; Endringer et al. 2014; Songsri et al. 2016), and *Curcuma* (Abas et al. 2005; Singh et al. 2010; Win et al. 2017).

Many researches showed the potential of labdane diterpenes as antimicrobials. Darias et al. (1990) tested epigomeric acid and gomeric acid that were isolated from *Salvia* and *Sideritis* genera from Canary Island using disk diffusion method. Both of these compounds showed antimicrobial activity against *B. subtilis*, *Micrococcus luteus*, and *S. aureus*. Kalpoutzakis et al. (1998) isolated seven labdane diterpenes from the leaves of *Cistus incanus* subsp *creticus*, which five of them, namely ent-13-epi-manoyl oxide, ent-3β-acetoxy-13-epi-manoyl oxide, ent-3β-hydroxy-13-epi-manoyl oxide, (13-E)-labd-7,13- diene-15-yl malonic acid, and 13(E)-labd-l3-ene, 8a-01-15-yl malonic acid were found active against *S. aureus*, *P. aeruginosa*, and *Klebsiella pneumoniae*. Two other labdane diterpenes are sclareol and manool, have been reported by Ulubelen et al. (1994) for antimicrobial activity by disk diffusion method, as well as broth dilution method, exhibiting a MIC of 48.25 and 13.75 µg/mL, respectively.

The diterpene labda-8(17)-12-diene-15,16-dial, which appeared as minor compound in this study, has been isolated from *C. mangga* previously (Abas et al. 2005). This compound also has been isolated from chloroform extract of the rhizome of *C. amada* and exhibited growth-inhibitory effect against *Mycobacterium tuberculosis* at an MIC of 500 µg/mL (Singh et al. 2010). Liu & Nair (2011) also reported this compound possessing anti-inflammatory and anti-tumour activities. (E)-15,16-dinorlabda-8(17),12-dien-14-al which is widely distributed in the genus *Alpinia*, has been reported previously by Ghosh et al. (2013) to have antimicrobial activity against Gram-positive and Gram-negative bacteria at the MIC ranges of 12.5-25 µg/mL. The antibacterial mechanism of this compound is due to the membrane degradation of cell wall and causing the leakage of the cell. In addition, Morita & Itokawa (1988) reported the antifungal effect of this compound against *Candida* spp. at the MIC ranges of 6.25-25 µg/mL. Above-mentioned compounds could be responsible for the inhibitory acitivy of *C. mangga* EO in this study. Althought there is no previous reports on the antimicrobial effect of (E)-15,16-dinorlabda-8(17),11-dien-13-one, based on the previous findings on the antimicrobial activity of other labdane diterpees, it could be assumed this compound is also responsible for its antistaphylococcal activity.

7. CONCLUSION

The present study describes the potent of *in vitro* antimicrobial effects of medicinal plants used in traditional Indonesian medicine. At the beginning, screening of *in vitro* antimicrobial activity of 49 ethanol extracts from 37 different Indonesian medicinal plant species was conducted against two Gram-positive, two Gram-negative, and one yeast. Based on the evaluation, the leaf extract of *O. aristatus* and *W. floribunda* exhibited the anti-candidal effect at the lowest MIC of 128 µg/mL. The rhizome extract of *C. mangga* exhibited antistaphylococcal activity at an MIC of 256 µg/mL. This species, a typical herbal medicine of jamu belonging to Zingiberaceae family, was chosen for further research focused on evaluation of its antimicrobial effect and characterisation of its chemical composition. The analysis of rhizome of *C. mangga* EO using broth microdilution volatilisation method and modified standard broth microdilution method showed that the EO exhibited growth-inhibitory effect against all strains of *S. aureus* tested (MIC 128-1024 µg/mL), whereas the inhibition activity against tetracycline-resistant *S. aureus* was the strongest (MIC 128 µg/mL). To the best of my knowledge, this is the first study demonstrating the antistaphylococcal effect of *C. mangga* rhizome EO against the broad spectrum of standard and clinical strains of *S. aureus*. The identification of *C. mangga* EO by GC-MS and GC-QTOF-MS revealed 106 compounds, where 15,16-dinorlabda-8(17),11-dien-13-one and ambrial are the most predominant compounds.

The findings suggest that the leaf extracts of *O. aristatus* and *W. floribunda* and the EO of *C. mangga* rhizome as promising agents for development of traditional medicine based anti-infective preparation. However, further researches focused on the isolation and characterisation of antimicrobially effective constituents of *O. aristatus* and *W. floribunda* and the safety and *in vivo* efficacy of *C. mangga* EO, will be needed before its possible use.

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9. APPENDICES

Appendix A. Photographic illustrations of plant used in traditional Indonesian medicine

Figure 1. *Aleurites molucannus*

Figure 2. *Curcuma mangga*

Figure 3. *Orthosiphon aristatus*

Figure 4. *Rotheca serrata*

Figure 5. *Woodfordia floribunda*

All photos are taken by authors in July 2015 and 2016

Appendix B. Chromatograms of *C. mangga* rhizome EO

Figure 6. FID chromatogram *C. mangga* rhizome EO on HP-5MS column.

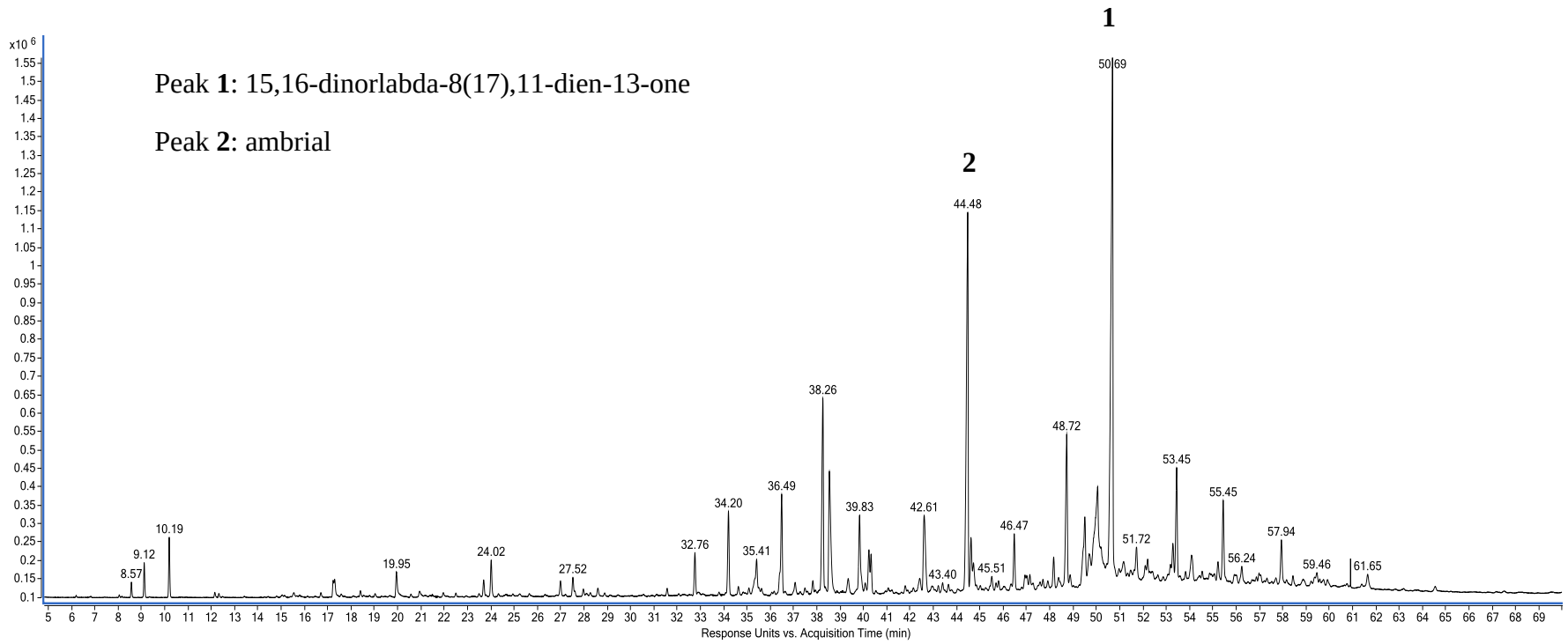


Figure 7. FID chromatogram of *C. mangga* rhizome EO on DB-17MS column.

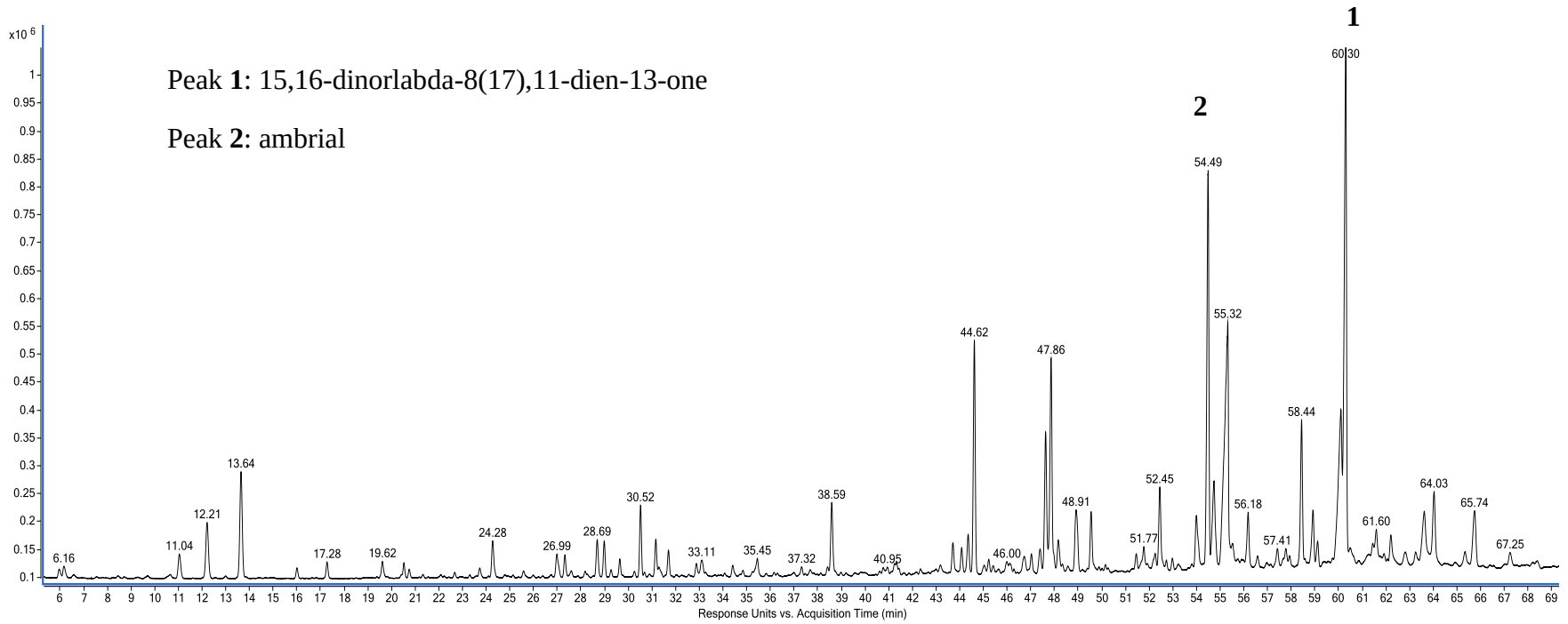
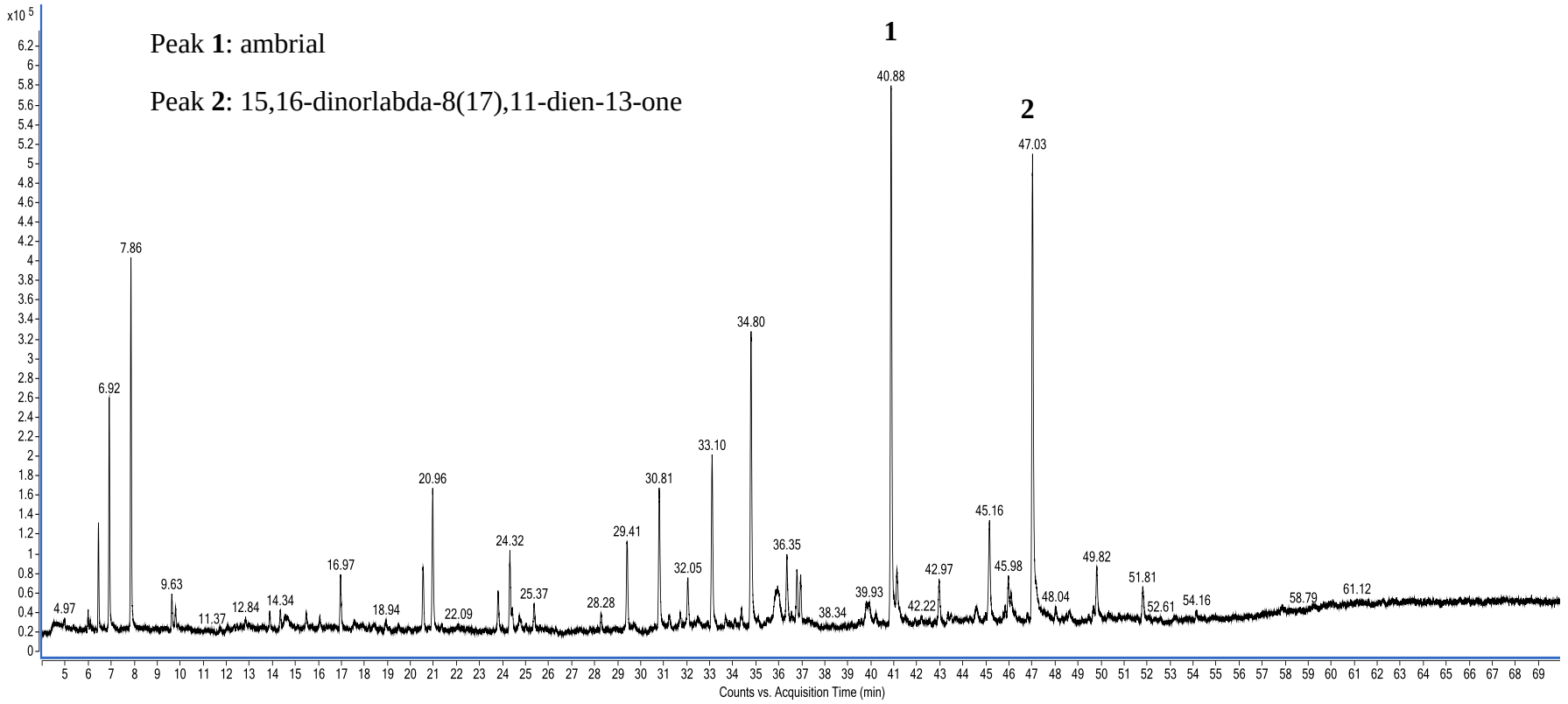


Figure 8. Total ion current (TIC) chromatogram of *C. mangga* rhizome EO on HP-5MS column obtained from GC-QTOF-MS



Appendix C. Mass spectra of the major compounds of *C. mangga* rhizome EO

Figure 9. Mass spectrum of (E)-15,16-dinorlabda-8(17),11-dien-13-one

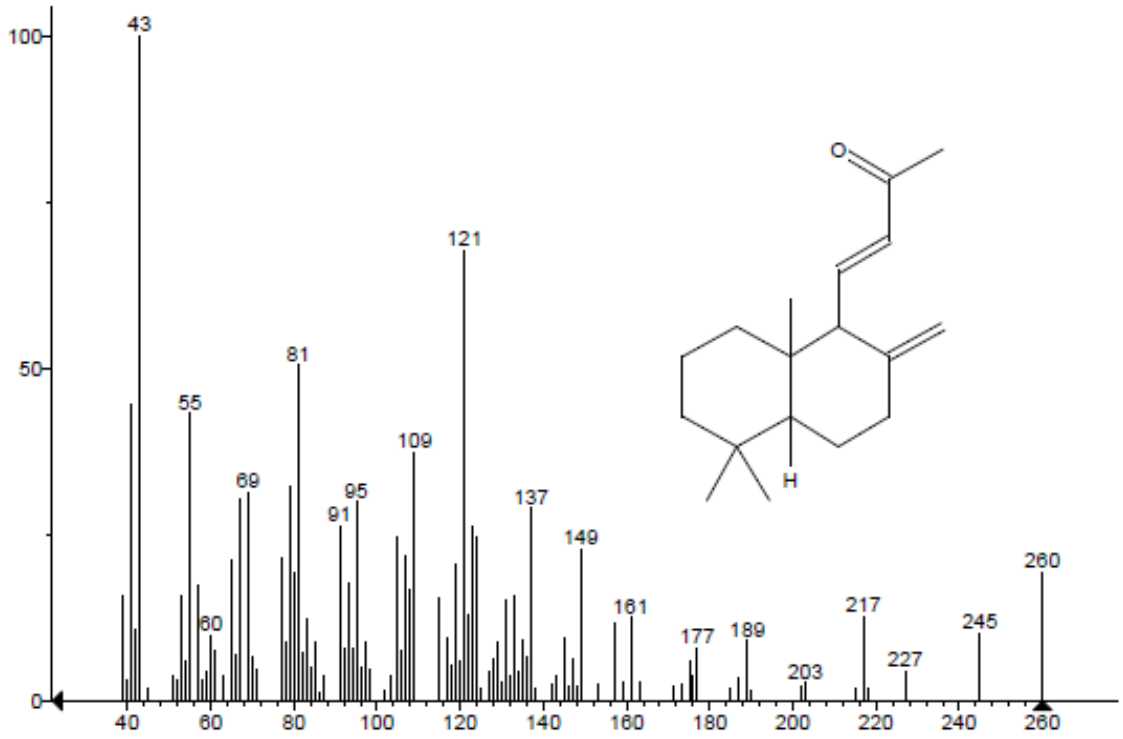


Figure 10. Mass spectrum of ambrial

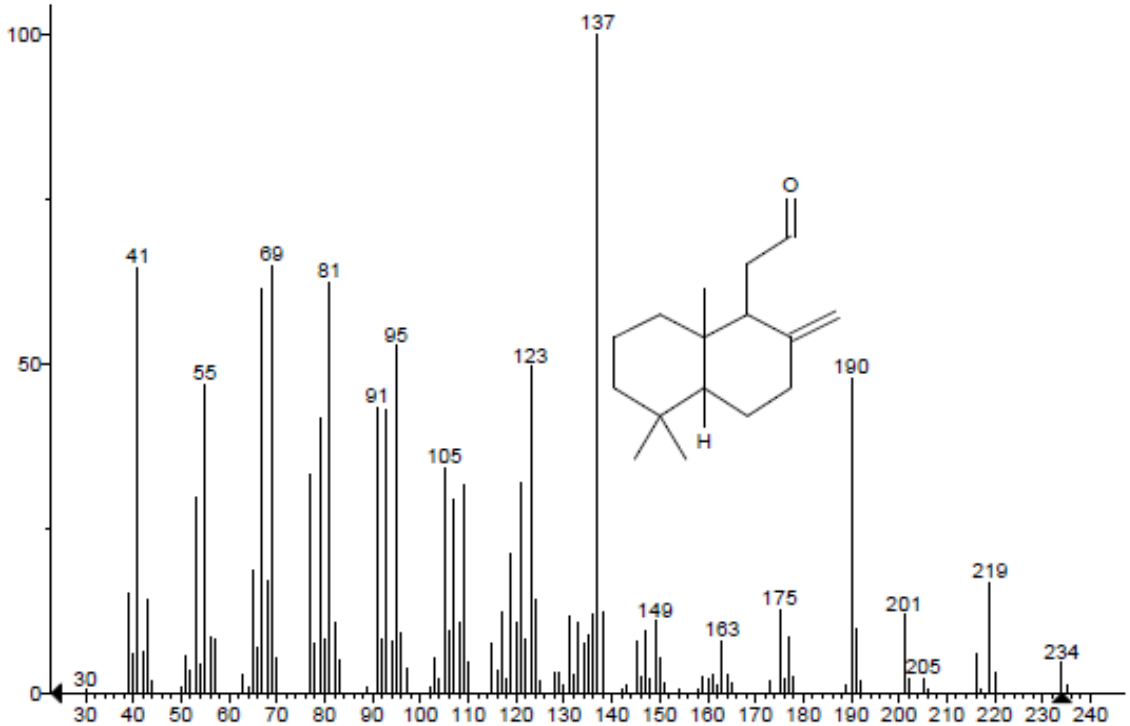


Figure 11. Mass spectrum of 13-nor-eremophil-1(10)-en-11-one

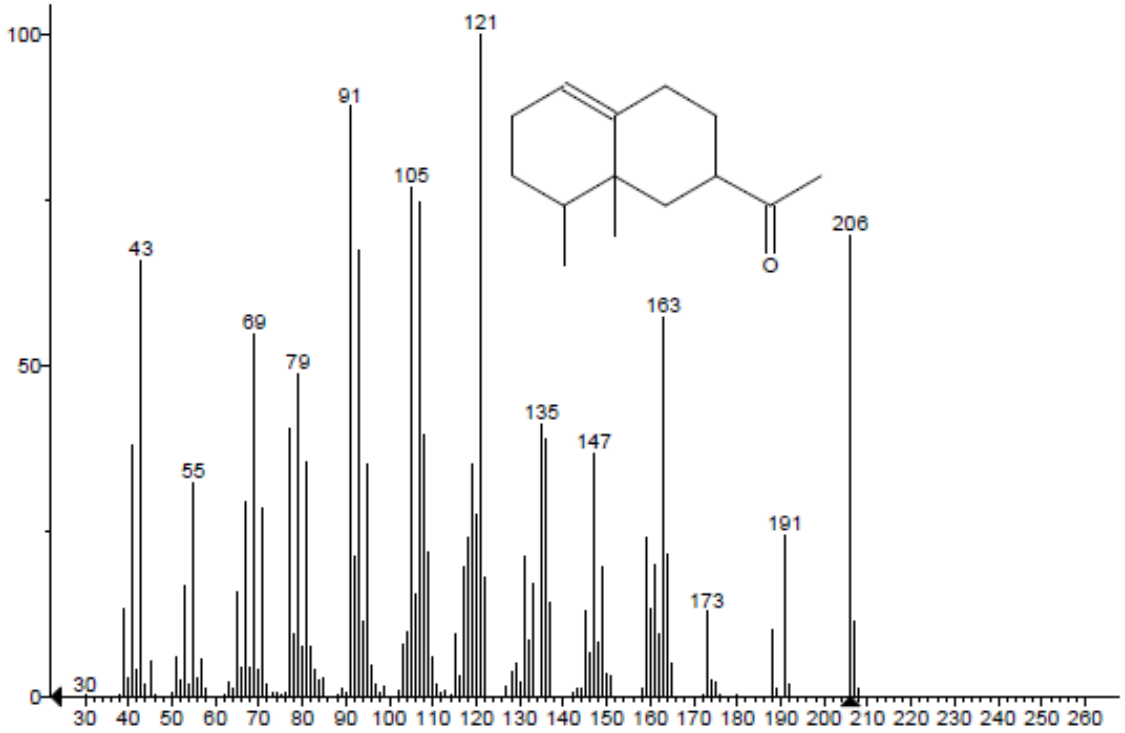


Figure 12. Mass sprectrum of (E)-15,16-dinorlabda-8(17),12-dien-14-al

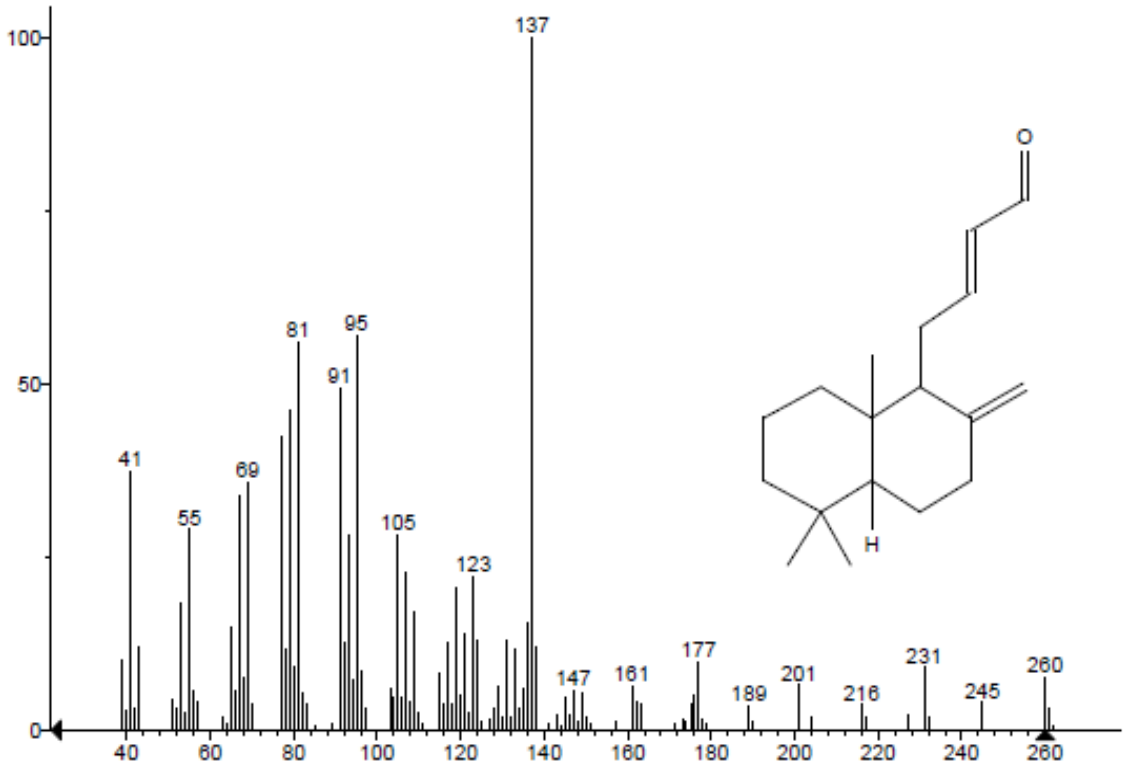
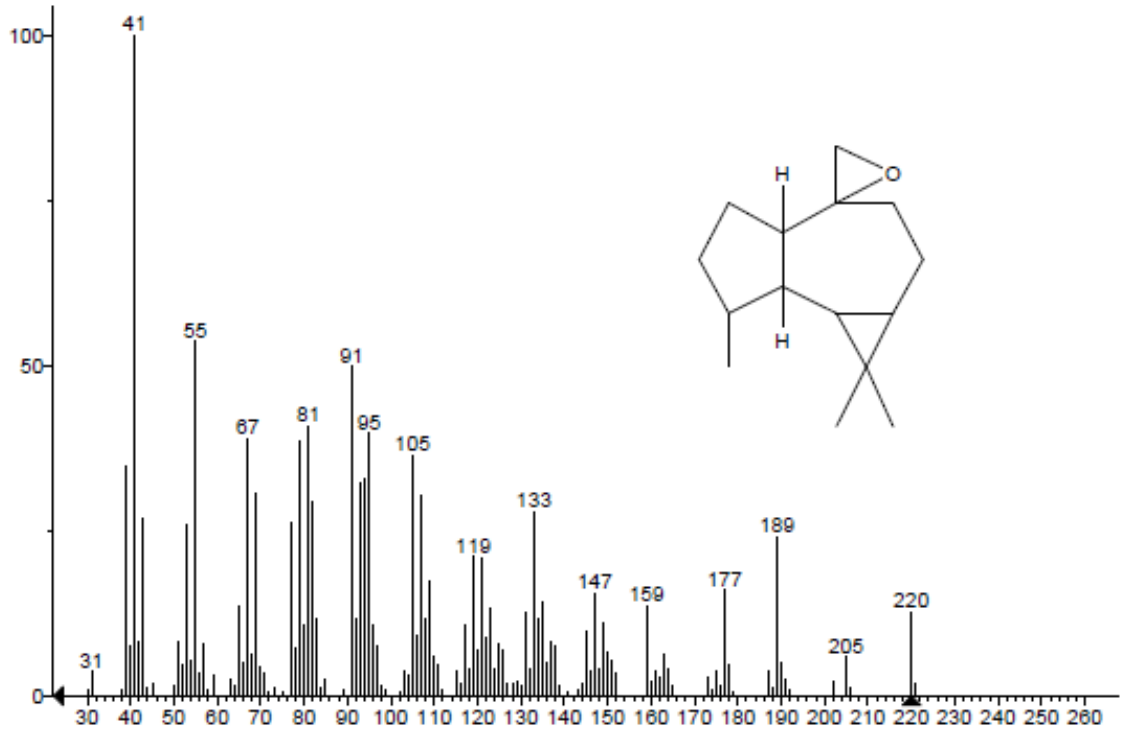


Figure 13. Mass spectrum of aromadendrene oxide



Appendix D. List of author's publications

Publications:

- Romulo A, Zuhud EAM, Rondevaldova J, Kokoska L. 2018. Screening of *in vitro* antimicrobial activity of plants used in traditional Indonesian medicine. Pharmaceutical Biology **56**:287-293 (IF 1.916).

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- Romulo A, Rondevaldova J, Kokoska L. 2017. The study of *in vitro* antimicrobial activity of plants used in traditional Indonesian medicine. Book of abstracts (Abstract no. 60). The 21th International Congress PHYTOPHARM 2017 and 10th Anniversary of the TCM Research Center Graz 2nd July - 5th July 2017, Graz, Austria.
- Romulo A, Rondevaldova J, Kokoska L. 2016. *In vitro* antimicrobial activity of plants used in traditional Indonesian medicine. Book of abstracts (Abstract no. 43). Trends in Natural Product Research: A Young Scientists Meeting of PSE and IUNG-PIB 30th May - 2nd June 2016, Puławy, Poland.