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MINI REVIEW

Review of essential oils of *Curcuma aeruginosa*: chemical composition and pharmacological activities

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Abstract

Curcuma aeruginosa is a plant from the Zingiberaceae family that is widely used in traditional medicine to treat coughs, asthma, diarrhea, dysmenorrhea, fever, colic, and others. The essential oil in *C. aeruginosa* is mainly dominated by oxygenated sesquiterpenes, followed by sesquiterpene hydrocarbons, oxygenated monoterpenes, and monoterpene hydrocarbons. The three main components are curzerenone, 1,8-cineole, germacrone, camphor, and curcumenol. *Curcuma aeruginosa* essential oil has several pharmacological properties, including antibacterial, antifungal, antioxidant, anticancer, and antiandrogenic activities. This review aimed to provide information regarding several studies regarding *C. aeruginosa* rhizome essential oil, which focuses on its chemical composition and pharmacological activity. The method to review articles was to search several databases to identify and download abstracts, original articles, and research papers related to *C. aeruginosa*. The inclusion criteria used were articles from 2003-2022. Of the 104 articles found, they were then extracted into 42 articles that focused on *C. aeruginosa* rhizome essential oil, chemical composition, and pharmacological activity as keywords. The many volatile compounds contained in *C. aeruginosa* essential oil have the potential to be developed as medicines, food preservatives, and natural therapies. However, phytochemical and pharmacological studies need to be explored further to isolate bioactive compounds and study their mechanisms of action.

1. Introduction

The genus *Curcuma* is considered one of the most important genera in the *Zingiberaceae* family. It is gaining popularity worldwide as a potential source of new medicines for preventing and combating a variety of diseases. This is due to the presence of several phytochemicals and secondary metabolites, including phenolics and essential oils. The *Zingiberaceae* family includes important rhizomatous medicinal and aromatic plants that are known for their high-value oleoresins and volatile oils (Atun *et al*., 2016). The genus comprises almost 110 species that are widely distributed in the tropics and subtropics of Southeast Asia, including Indonesia (Kamazeri *et al*., 2012; Atun *et al*., 2016), Malaysia (Kamazeri *et al*., 2012), Thailand (Kitamura *et al*., 2007; Jose and Thomas, 2014), Myanmar (Jose and Thomas, 2014), China (Kitamura *et al*., 2007), Bangladesh (Hossain *et al*., 2015) and India (Paramita *et al*., 2018; Jena *et al*., 2020). However, the exact number of species is still controversial among botanists (Jena *et al*., 2020). Numerous species of *Curcuma* can be found in Indonesia, such as *Curcuma longa* (turmeric), *C. xanthorrhiza, C. hyneana, C. zedoaria, C. mangga,* and *C. aeruginosa.* These species are commonly used for various applications. Although *C. aeruginosa* is native to Indonesia, it has not been extensively studied (Paramita et al., 2018).

The Latin name for this plant is *C. aeruginosa*, and it is also referred to as *C. aeruginosa* Roxb. in various articles (Sari and Supratman, 2022). One part of the *C. aeruginosa* that is widely used is the rhizome. *Curcuma aeruginosa* rhizome extract has been used in traditional

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medicine to treat gastrointestinal disorders, including diarrhea and colic. It has also been used by women for postpartum care, uterine involution, treatment of uterine pain, and uterine inflammation (Thaina *et al*., 2009). Several studies have shown that the extract of *C. aeruginosa* can be used as an anthelmintic (Vanda *et al*., 2020), antioxidant (Waras *et al*., 2015), and antibacterial agent (Jose and Thomas, 2014). Rhizome isolates of *C. aeruginosa* have various potential uses. Germacrone has been found to have antiandrogenic properties (Suphrom *et al*., 2012), promote hair growth (Srivilai *et al*., 2018), and exhibit antinociceptive effects (Hossain *et al*., 2015). Additionally, n-hexane and chloroform fractions have shown cytotoxic activity against MCF-7 and Ca-ski cells (Atun *et al*., 2016).

Steam distillation or hydrodistillation can be used to extract the essential oil of *C. aeruginosa* from fresh or dried rhizome samples (Dosoky and Setzer, 2018). Two methods are also available for obtaining *C. aeruginosa* rhizome essential oil: two-phase. The essential oils (EOs) of *Curcuma* species are complex and heterogeneous mixtures of several compounds from different chemical classes, including monoterpenes, sesquiterpenes, and other aromatic compounds. Currently, several studies on essential oils of the *Curcuma* genus are gaining attention due to their potential use as natural products. These oils are being investigated for their therapeutic properties in the treatment of some specific diseases. Some factors are responsible for the chemical variation of EOs in plants, including methods of collection, soil and nutrient conditions, environmental conditions such as humidity, temperature, luminosity, altitude, circadian cycle, ultraviolet radiation, seasonality, drying, and the specific part of the plant. Because of these factors, the relative composition of EOs can vary considerably depending on the geographical origin, different agro-climatic zones, and maturity of *C. aeruginosa*. As the plant ages, the concentrations of monoterpenes decline in the rhizomes, while the levels of sesquiterpenes increase (Guimarãesa *et al*., 2020).

Methanol/chloroform solvent extraction and methyl tert-butyl ether (MTBE) solvent extraction. Both solvent extraction methods produce terpenoid chemicals (Simoh and Zainal, 2015). To produce high yields of high-quality essential oils, new extraction techniques are now being developed. One such technique is microwave-assisted extraction (MAE), which can help reduce chemical dangers, is ecologically benign, requires less time for extraction, and uses few solvents (Rassem *et al*., 2016). The objective of this review was to provide information about the essential oils of *C. aeruginosa* and their beneficial biological activities for human health.

2. Methodology

In preparing this review, we searched some online databases and search engines, including Web of Science, Scopus, DOAJ (Directory of Open Access Journals), Science Direct, Research Gate PubMed, and the Indonesian database of SINTA to access relevant abstracts and articles. The keywords used are *Curcuma aeruginosa* or (*Curcuma aeruginosa* Roxb.) + chemical phytochemicals + rhizome or composition + pharmacological or biological activities. Then, retrieved materials were assessed, and those that appeared repeatedly in the databases were excluded. The chosen papers were assessed against the inclusion and exclusion criteria as well as critical evaluation before being used for a review that followed the journal's rules for a logical framework. The inclusion criteria for the publications that were chosen were: (1) investigations on the chemical compositions and pharmacological activities of *C. aeruginosa*, and (2) years of publication between 2003 and 2022, while the exclusion criteria used were all papers written in non-English.

3. *Curcuma aeruginosa* Roxb.

Curcuma aeruginosa Roxb. with local names including temu ireng (Indonesia), temu hitam (Malaysia), Waan-Ma-Haa-Mek or Kajeawdang (Thailand), wild arrowroot or east Indian arrowroot (India) (Azila *et al*., 2017), and kathali holud (Bangladesh) (Hossain *et al*., 2015). *Curcuma aeruginosa* is a medicinal plant used in Indonesian traditional medicine to relieve stomach pain, asthma, enteritis, and rheumatic problems, increase appetite, and prevent obesity (Thaina *et al*., 2009; Nurcholis *et al*., 2016). *Curcuma aeruginosa* is a perennial plant that has tubers which are oblong with leafy shoots of approximately 45-60 cm high; a purplish patch along either side of the midrib on the upper side has the typical burgundy mid-stripe on the leaves (Thaina *et al*., 2009). Rhizome is used in medicine to treat mental disorders, scurvy, asthma, and cough. Additionally, it is used to help discharge lochia and lessen uterine pain and inflammation in labor. This rhizome can also be applied externally as a poultice for itching (Subhadhirasakul, 2006). Figure 1 shows the plant and rhizome of *C. aeruginosa*.

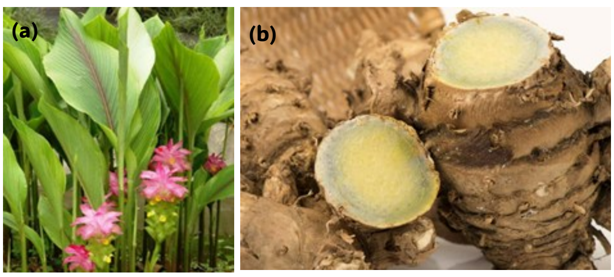

Figure 1. (a) Habit of *C. aeruginosa*; (b) Rhizome of *C. aeruginosa*.

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Generally, essential oils (EOs) of *Curcuma* species are extracted from the rhizome and leaves. The main active components of the rhizome are the nonvolatile curcuminoids and the volatile oil. Aziz *et al*. (2021) have compared the extraction yield of EOs from *C. aeruginosa*, and the results showed that the highest yield of EOs was obtained from the dried rhizome of *C. aeruginosa* (0.63% w/v), followed by dried leaves 0.46% (w/w), fresh rhizomes 0.26% (w/w), and fresh leaves 0.20% (w/w). The rhizomes of *C. aeruginosa* are the aromatic parts containing the volatile EOs and non-volatile EOs, which are typically used as dyes, tonics, flavoring agents, tropical ornamentals, and medicines. EOs are essential ingredients in cosmetics preparations and perfumes (Dosoky and Setzer, 2018). In addition, in vivo studies also found that EOs have anti-inflammatory, antioxidant, anti-nociceptive, and anti-atherosclerotic activities (Liju *et al*., 2011).

4. Phytochemical composition

The rhizomes of *C. aeruginosa* contain a significant amount of essential oils (Ministry of Health of the Republic of Indonesia, 2017), which are lipophilic, and the fresh rhizomes can be extracted using hydro-distillation or steam distillation (Kamazeri *et al*., 2012; Angel *et al*., 2014). Hydro-distillation and steam distillation are commonly used for extracting essential oils from plant materials. The principle of the distillation method is based on the difference in boiling points for the separation of liquid from a mixture, allowing substances with lower boiling points to evaporate. In one distillation cycle, after the mixture evaporates, it is cooled in a condenser, which turns it into liquid. The liquid with a lower boiling point will evaporate first, followed by that with a higher boiling point. Several studies have used hydro-distillation to isolate essential oils from *C. aeruginosa* plants which are originated from Selangor, Malaysia (Jantan *et al*., 2003), Kerala, India (Angel *et al*., 2014; George and Britto, 2015), Chiang Mai, Thailand (Akarchariya *et al*., 2017), Phetchabun, Thailand (Srivilai *et al*., 2018), Ratchaburi, Thailand (Theanphong et al., 2015), and Tuyeng Quan, Vietnam (Oanh *et al*., 2018). Other studies have also used steam distillation to isolate essential oils from *C. aeruginosa* plants, which originate from Bogor, Indonesia (Fitria *et al*., 2019), Kuantan, Malaysia (Kamazeri *et al*., 2012), and Thailand (Aromdee, 2011).

Curcuma aeruginosa EOs are usually composed of relatively equal amounts of monoterpenes and sesquiterpenes. Theanphong *et al*. (2015) reported that EOs in *C. aeruginosa* were mainly dominated by oxygenated sesquiterpenes (42.85%), followed by sesquiterpene hydrocarbons (30.80%), oxygenated monoterpene (10.92%) and monoterpene hydrocarbons (10.82%). The results of hydro-distillation showed that some important pharmacological effects of *C. aeruginosa* were 1,8-Cineole (17.7%), camphor (7.5%), curzerenone (10.5%), and furanogermenone (7.8%) (Pandey *et al*., 2021). Table 1 shows the content of terpenoid compounds of *C. aeruginosa* EOs from several regions, and Figure 2 shows chemical structures of terpenoids of *C. aeruginosa* EOs.

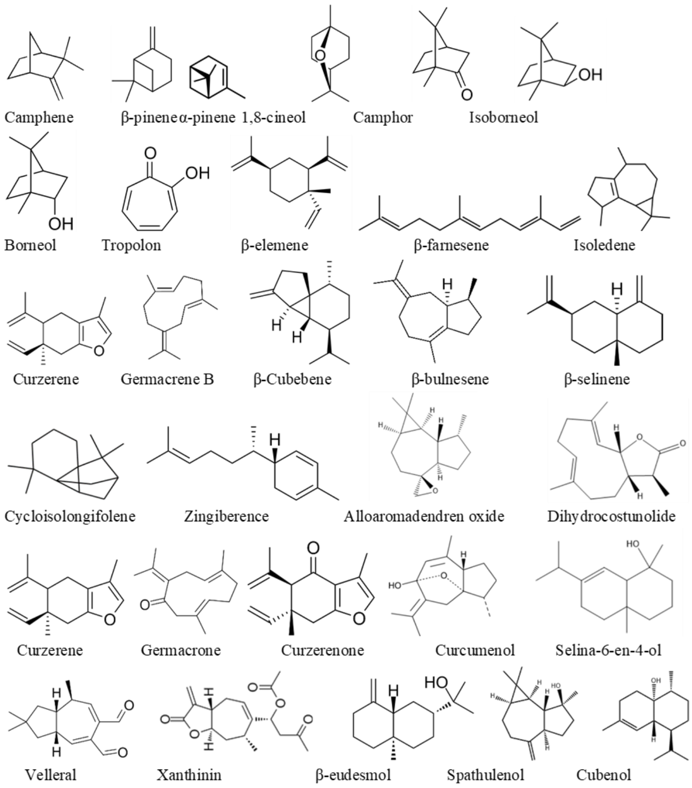

Figure 2. Chemical structures of terpenoids in *C. aeruginosa* EOs.

5. Biological activities of essential oils of *Curcuma aeruginosa*

5.1 Antimicrobial activities

Antimicrobial resistance has been recognized as a major global health issue. The presence of multi-resistant bacteria limits the availability of treatment options for related infections. According to the World Health Organization (WHO), the most common antibiotic-resistant pathogens include *Klebsiella pneumoniae, Escherichia coli, Acinetobacter baumannii, Pseudomonas aeruginosa, Staphylococcus aureus,* and *Enterococcus faecium*. Numerous strategies have been implemented to combat this bacterial resistance, and one of the appealing strategies is the use of secondary metabolites from natural products (Septama *et al*., 2022). Essential oils are potential sources of new antibacterial agents. It has been reported that some essential oils isolated from *C. aeruginosa* exhibited potent antibacterial activities. The essential oils of *C. aeruginosa* were reported to have moderate antibacterial

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Table 1. Content of terpenoids in *C. aeruginosa* EOs from several regions.

Origin	Active Substance (%)			
	Hydrocarbon Monoterpenes	Oxygenated Monoterpenes	Hydrocarbon Sesquiterpenes	Oxygenated Sesquiterpenes
Nongkai, Thailand (Jarikasem *et al*., 2005)	Camphene (0.3) β-pinene (7.7)	1,8-cineole (9.6) Camphor (0.6) Isoborneol (0.5)	β-farnesene (0.2) Curzerene (11) Germacrene B (0.5)	Curzerenone (41.6) Germacrone (1.0)
Ratchaburi, Thailand (Theanphong *et al*., 2015)		1,8-cineole (10.9)		Germacrone (23.5) Curzerenone (11.8)
Chiang Mai, Thailand (Akarchariya *et al*., 2017)	Camphene (1.2) β-pinene (0.4)	1,8-cineole (2.7) Camphor (29.4) Isoborneol (7.3)	β-elemene (1.4) Curzerene (4.8) Germacrene B (5.2)	Germacrone (21.2)
Thailand (Srivilai *et al*., 2017)	β-pinene (8,0)	1,8-cineole (22.7)		Germacrone (17.7) furanodiene (11.4)
Selangor, Malaysia (Jantan *et al*., 2003)	Camphene (1.6) β-pinene (1.6)	1,8-cineole (25.2) Camphor (6.8) Isoborneol (1.5) Borneol (0.5)	β-elemene (4.7) β-farnesene (2.6) β-cubebene (0.9) β-bulnesene (2.1) β-selinene (1.1) Cycloisolongifolene (35.3)	Caryophyllene (1.0) Germacrone (6.5) Velleral (10.0) Xanthinin (0.7) Alloaromadendrene oxide (4.1) Dihydrocostunolide (11.4)
Kuantan, Malaysia (Kamazeri *et al*., 2012)		Camphor (1.3) 1,8-cineole (3.9) Isoborneol (0.6)	β-elemene (1.7) β-farnesene (0.5) Zingiberene (1.2)	Caryophyllene (1.0) Germacrone (6.5) Velleral (10.0) Xanthinin (0.7)
Pahang, Malaysia (Aziz *et al*., 2021)	β-pinene (0.9)	Camphor (6.1) 1,8-cineole (20.5)	Curzerene (1.0) Isoledene (0.9)	Cubenol (0.5) Selina-6-en-4-ol (1.3) Germacrone (1.9) Spathulenol (1.5)
Kerala, India (Angel *et al*., 2014)	Camphene (0.2) β-pinene (27.5)	1,8-cineole (3.6)	β-farnesene (1.5) Zingiberene (1.2)	Curcumenol (38.7) β-eudesmol (3.6)
Nepal (Poudel *et al*., 2022)	Camphene (0.8) α-pinene (0.2) β-pinene (1.3)	Camphor (3.6)	β-elemene (2.6)	Curzerenone (59.6) Curzerene (4.7) Germacrone (5.3) β-eudesmol (1.6)
Bogor, Indonesia (Fitria *et al*., 2019)		1,8-cineole (17.9) Camphor (5.3) Tropolon (18.1)		Curcumol (5.7)

activity towards microorganisms using the disc-diffusion method. This includes Gram-positive bacteria of *Staphylococcus aureus* and *Bacillus cereus*, with the zone of inhibition of 7.0±0.0 and 9.3±0.4 mm, respectively (Kamazeri *et al*., 2012). Table 2 showed the antibacterial activity of *C. aeruginosa* EOs.

Antibacterial activity tests in several of these studies used two methods, namely the diffusion and dilution methods. The diffusion method used is the disc diffusion method. The disc diffusion method is the method most often used to determine antibacterial sensitivity to an antibiotic. In this method, a disc is used which functions as a container for antibacterial substances. The disc is then placed on an agar plate that has been inoculated with the test bacteria, then incubated at a certain time and temperature, according to the optimum conditions of the test bacteria. In general, the results obtained can be observed after incubation for 24 h at a temperature of 37°C (Aziz *et al*., 2021). The observation results obtained were whether or not a clear area formed around the disc, indicating an inhibitory zone for bacterial growth. Meanwhile, the dilution method referred to as the microdilution method is the development of a dilution method which is tested using a 96-well plate filled with media, bacterial inoculum and test compounds in various concentrations in small amounts. The substance to be tested for bacterial activity is serially diluted in liquid media, then inoculated with bacteria and incubated at a time and temperature appropriate to the bacteria being tested. Antibacterial activity is determined as the minimum inhibitory concentration (MIC). The advantage of this microdilution method is that it has better sensitivity than other antibacterial testing methods (Golus *et al*., 2016).

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Table 2. Antibacterial activities of essential oils of *C. aeruginosa*.

Analyzed samples	Methods	Results	References
EOs obtained from hydro-distillation	Broth microdilution assay in Mueller-Hinton Broth	The essential oils in rhizome oil of *C. aeruginosa* are responsible for potent antimicrobial activity against *S. aureus* with an inhibition zone (IZ) of 21.94±0.24 mm at MIC 125 mg/mL, against B. cereus with an IZ of 20.83±0.36 mm at MIC 125 mg/mL.	Akarchariya *et al*. (2017)
EOs obtained from water distillation	Broth dilution method	EOs were shown to modulate activity against *E. faecalis* with an MIC value of 6.25 mg/mL, weak activity against *B. subtilis* with an MIC value of 50 mg/mL, and no activity against *E. coli* and *S. aureus*.	Theanphong *et al*. (2015)
	Green Fluorescent Protein Microplate Assay (GFPMA)	EOs from fresh rhizome of *C. aeruginosa* possessed inhibitory activity against *M. tuberculosis* with an MIC value of 2,500 mg/mL. The positive control isoniazid had an MIC value of 0.023-0.046 mg/mL.	Theanphong *et al*. (2015)
Hydro-distillation of 20 genotypes of *C. aeruginosa* rhizomes	Disc-diffusion, using broth dilution process to determine MIC and MBC	EOs of *C. aeruginosa* exhibited good antibacterial activity against *S. aureus*. Values of MIC and MBC of 11 EOs against *S. aureus* ranged from 7.81 to 2,000 µg/mL and 250 to 2,000 µg/mL, respectively.	Nurcholis *et al*. (2021)
Steam distillation of *C. aeruginosa*	Disc-diffusion method using agar plates, Mueller Hinton Agar (MHA)	Antimicrobial activity of *C. aeruginosa* EOs was determined by disc-diffusion method against *S. aureus, B. cereus,* and *P. aeruginosa.* The zone of inhibition was 7.0±0.0, 9.3±0.4, and 7.5±0.0 mm, and no zone of inhibition for *E. coli.*	Aziz *et al*. (2021)
C. aeruginosa rhizomes were distilled for 6 h using steam distillation. Distillation was carried out at 100-105°C.	*Streptococcus mutans* was grown in Tryptic Soy Broth (TSB) at 37°C. Antibacterial activity of EOs of *C. aeruginosa* against *S. mutans* was determined using the micro -dilution method.	EOs of *C. aeruginosa* rhizome had good antibacterial activity against *S. mutans*. The MIC value of rhizome oil was 15.63 g/mL.	Wahyuni *et al*. (2017)
MeOH extract of *C. aeruginosa* rhizome has been used to isolate curcumenol (sesquiterpene).	The inhibition zone was measured by comparing with paper disks (6 mm in diameter).	Curcumenol had moderate antibacterial activity at a concentration of 50 ppm. It had an inhibition zone of 8 mm for *S.* Typhi (1.33 inhibition index), while *E. coli* showed weak activity at a concentration of 50 ppm, which had an inhibition zone of 4 mm (0.67	Rahayu *et al*. (2018)

The results showed that several studies of temu ireng essential oil have different antibacterial MIC values and different inhibition zones. This is because the terpene compounds contained in temu ireng essential oil are involved in the breakdown of membranes by lipophilic components. Differences in bacterial sensitivity to essential oils are caused by the constituent factors or content of the essential oils; this is also influenced by their ability to penetrate the cell walls and membrane structures of bacterial cells. Monoterpenes and sesquiterpenes are the main constituent components of ginger oil. oxygenated monoterpenes such as camphor and 1,8-cineole, while oxygenated sesquiterpenes such as germacrone have previously been studied as compounds with high potential for antibacterial properties (Akarchariya *et al*., 2017). The high content of oxygenated monoterpenes and sesquiterpenes in temu ireng will provide stronger antibacterial capabilities. These geographical factors determine the quality of temu ireng that will be used as a medicinal ingredient, especially its ability to inhibit bacterial growth.

5.2 Antifungal activities

Fresh rhizomes were washed, comminuted, and 150 g of each sample was hydrodistilled in a Clevenger-type apparatus for 8 h. The oily layers obtained were separated and dried with anhydrous sodium sulfate. Broth microdilution method for determining MIC and disc agar diffusion technique for measuring the zone of inhibition. EO from the rhizome of *C. aeruginosa* showed good antibacterial activity against *Microsporum nanum*. The MIC value of rhizome oil was 40.0 µg/µL, while *Trichophyton mentagrophtes, Trichophyton rubrum, Microsporum canis, Epidermophyton floccosum, Mucor* sp., *Aspergillus niger,* and *Aspergillus fumigatus* did not exhibit antifungal activity (Jantan *et al*., 2003). Strong antifungal efficacy against *T. mentagrophytes, A. flavus, A. fumigatus, C. albicans*, and other pathogenic fungi were demonstrated by essential oils with high camphor and camphene content (Tirillini

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et al., 1996). Meanwhile, the camphor and camphene content in *C. aeruginosa* EOs is very small so that the antifungal activity of this fungus cannot be detected in the MIC value. The EOs of *Boesenbergia pandurata* and *C. xanthorrhiza* have antifungal activity on *T. mentagrophtes, T. rubrum, M. nanum, M. canis, E. floccosum, Mucor* sp. *and A. niger* showed in MIC value (Jantan *et al*. 2003). This was possibly because in *B. pandurata* there are camphor, geraniol and 1,8-cineole compounds, and in *C. xanthorrizha there are* compounds in the sesquiterpene group, namely xanthorrhizol (Mary et al., 2012). Even though xanthorrhizol's route of antimicrobial action is still unclear, it may be able to decrease microbially-induced nuclear factor kappa B (NF -kB) and mitogen-activated protein kinase (MAPK) (Oon et al., 2015).

Inhibitory zone testing of *Cryptococcus neoformans* and *Candida albicans* was carried out on the EOs of *C. Mangga, C. aeruginosa,* and *Zingiber cassumunar* using the disc-diffusion method, showing that the EOs of *C. mangga* had a larger inhibitory zone, namely 14.8±0.4 and 10.3±0.4 mm, compared to *C. aeruginosa* EOs with an inhibition zone of 8.8±0.4 and 7.0±0.0 mm (Kamazeri *et al*., 2012). Meanwhile, *Z. cassumunar* EOs does not have an inhibitory zone for these two fungi. This can be explained by the fact that the active components of the oil constitute only a small part of the oil used. Therefore, the concentration of the active component can be much lower compared to the standard antibiotic (Matasyoh *et al*., 2009), namely tetracycline. If the active components are isolated and purified, they may exhibit higher antimicrobial activity. It should also be considered that the relatively weak inhibition of *C. aeruginosa* EOs may be influenced by the ability of the antifungal compound to diffuse uniformly through the agar.

5.3 Antioxidant activities

Antioxidant activities of EOs extracted from *C. aeruginosa* have been studied in vitro using different systems. An effective concentration of 50% (EC_{50} values) of EOs from fresh rhizome of *C. aeruginosa* in various antioxidant systems was compared using the positive control of vitamin C. The EC_{50} values of EOs using DPPH radical scavenging, OH scavenging, H_2O_2 scavenging, and reducing power assays were 24.32, 244.48, 4031.37, and >5000 µg/mL, while the positive control of vitamin C revealed EC_{50} values of 21.21, 228.24, 346.75 and 453.93 µg/mL, respectively (Theanphong et al., 2015). The EC_{50} value on DPPH and OH radical scavenging did not have a statistically significant difference between essential oils and vitamin C. This was due to the content of the main compounds in essential oils such as germacrone and curzerenone, which can react with DPPH and OH radicals so that they can donate hydrogen atoms. Strong antioxidant activity describes the germacron from *Curcuma zedoaria* (Christm.) Rosc., which was employed to alleviate oxidative stress in NG108-15 cells caused by hydrogen peroxide (Hamdi et al., 2015). Furthermore, Oanh *et al*. (2018) reported that EOs obtained by hydro-distillation separation contained 37 compounds representing 95.6% of the total volatile compounds, including monoterpenes hydrocarbons (32.2%), oxygenated monoterpenes (6.8%), sesquiterpenes hydrocarbons (20.4%), oxygenated sesquiterpenes (33.1%), and non-terpenes (2.1%). The main constituents of EOs were β-pinene (21.9%), neocurdione (16.1%), curcumol (15.2%), β-elemene (6.6%), myrtenyl acetate (6.1%), β-caryophyllene (4.9%), α-pinene (3.4%) and α-selinene (3.3%) (Oanh *et al*., 2018). Essential oils can reduce stable free radicals; this can be correlated with oxygenated sesquiterpene components as potential antioxidants (Zeljković *et al*., 2015).

The fresh rhizomes were hydrodistilled for 3 h using a Clevenger-type apparatus. The obtained essential oil was dried over anhydrous sodium sulfate (Na_2SO_4) and preserved in a sealed vial at 4°C until further analysis. The antioxidant capacity of *C. aeruginosa* was evaluated using the DPPH radical scavenging method by comparing it with the activity of ascorbic acid as a known antioxidant. IC_{50} and EC50 values were respectively 28 µg/mL and 30 µg/mL. The reducing power assay was 2.54 at 50 µg/mL in essential oil, while ascorbic acid was 2.67 at 50 µg/mL. At a maximum concentration of 50 µg/mL, inhibition of nitric oxide was 76.8% for ascorbic acid, which serves as the standard. Inhibition of *C. aeruginosa* essential oil was higher at 72.3% (George and Britto, 2015). The most common main ingredients in *C. aeruginosa* essential oil, based on differences in growing location, are camphor, 1,8-cineole, germacrone, curzerenone, and isocurcumenol (Sari and Supratman, 2022). Zhang *et al*. (2017). The content of terpenoid compounds in *Curcuma phaeocaulis*, namely germacrone, 1,8-cineole, curzerene, 8,9-dehydro-9-formyl-cycloisolongifolene, plays a role in activity. Evaluation of antioxidant activity in plants can result in the discovery of natural antioxidants that have pharmacological and food value. Therefore, the antioxidant properties of essential oils can play a valuable role in food conservation and also in the prevention of oxidative damage associated with the pathophysiology of many diseases. According to the findings, a few essential oils and associated substances may have the ability to function as natural antioxidants (Sun *et al*., 2016).

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5.4 Anticancer activities

The cytotoxic activities of EOs extracted from *C. aeruginosa* rhizomes have been extensively studied. Some methods have been used for the evaluation of anticancer activities, including brine shrimp lethality test, as well as against Michigan Cancer Foundation-7 (MCF-7) and Vero cells. The EOs of *C. aeruginosa* showed cytotoxic activities to brine shrimp and MCF-7. The highest concentration of *C. aeruginosa* and *C. xanthorrhiza* essential oils was able to inhibit 50.2% and 59.1% of MCF-7 cancer cells in vitro, respectively (Fitria *et al*., 2019). N-hexane fraction of C. aeruginosa showed potential as an antiproliferation against the MCF -7 cancer cells with an IC50 value of 69.5 µg/mL (Atun et al., 2016), proving that *C. aeruginosa* extracts and volatile metabolite compounds could both function as antiproliferative compounds against cancer cells. The major volatile compounds reported to be responsible for this activity were tropolone, eucalyptol, and curcumol (Fitria *et al*., 2019). Histon Deacetylase (HDAC), an enzyme involved in numerous biochemical processes including transcription regulation, tubulin regulation, cytoskeletal function, regulating cardiac growth, thymocyte development, and aiding DNA repair, was thought to be inhibited by tropolone, which in turn seemed to prevent the growth of cancer cells (Ononye *et al*., 2013). Due to the 1,8-cineole compound's abundance in *C. aeruginosa* essential oil, it is also believed to be involved in inhibiting the growth of MCF-7 cancer cells. There has been much coverage of curcumol's potential to inhibit the growth of cancer cells. Applying curcumol to MDA-MB cancer cells (breast cancer) resulted in an IC50 value of 100.2 µg/mL for cell growth inhibition. This was achieved by stopping the cells in the G1 phase and inducing death through the mitochondrial pathway (Huang *et al*., 2017).

The methanol extract of *C. aeruginosa* rhizomes had significant cytotoxicity against lung adenocarcinoma (A-549) cells and human cervical cancer (HeLa), with low IC50 values of 15.42±1.5 µg/mL and 25.40±0.13 µg/mL. This shows an increase in DNA fragmentation, apoptotic and necrotic indices. *C. aeruginosa* methanol extract was able to induce apoptosis in A-549 and HeLa cells by increasing the activity of caspase-8 (initiator) and caspase-3 (executor). The identified phenolic, flavonoid and terpenoid compounds may be responsible for the anticancer activity (Zohmachhuana et al., 2022). Terpenoid compounds that act as anticancer agents are the sesquiterpenes including: α-curcumene (Shin and Lee, 2013), curzerene (Sun *et al*., 2017), curcumenol (Han *et al*., 2019), curzerenone (Rahman *et al*., 2013), epicurzerenone (Cui *et al*., 2019), and caryophyllene oxide (Ortega-Ortega *et al*., 2017).

5.5 Antiandrogenic activities

Essential oils, n-hexane extract, and germacrone were tested using Franz diffusion cells and thick human foreskin as a membrane to increase skin penetration. Minoxidil skin penetration with 0.2% and 2% extracts increased the total flux by ~4-fold, germacrone by ~10-fold, and essential oil by ~20-fold. In brief, the study showed that the absorption of minoxidil through human skin was observed in three samples (Srivilai *et al*., 2018). The test was conducted using hair tonic that was divided into four preparations: 1) placebo, as the hair tonic's main ingredient, 2) 5% *C. aeruginosa* extract in hexane, 3) 5% minoxidil, and 4) a combination of 5% *C. aeruginosa* extract and 5% minoxidil. Men with hair loss over the vertex scalp underwent tests. The findings revealed that there was a rise in the number of hairs in the three groups, which were 5% extract, 5% minoxidil, and a combination of the three, compared to the placebo group. In summary, the combination of 5% minoxidil and 5% hexane extract from *C. aeruginosa* is more effective at reducing hair loss and promoting hair growth (Pumthong *et al*., 2012).

Curcuma aeruginosa grows in tropical and subtropical regions, especially in Asia. Bioactive compounds in *C. aeruginosa* EOs include curzerenone, 1,8-cineole, germacrone, camphor, and curcumenol. These compounds have pharmacological properties including antibacterial, antioxidant, anticancer, antifungal, and antiandrogenic activities. The many volatile compounds contained in *C. aeruginosa* essential oil have the potential to be developed as medicines, food preservatives, and natural therapies. However, phytochemical and pharmacological studies need to be explored further to isolate bioactive compounds and study their mechanisms of action.

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