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Phytochemical screening and antibacterial activity test of *Curcuma zedoaria*, *C. aeruginosa*, and *C. mangga* extracts against *Aeromonas hydrophila*

ADE DWI SASANTI[1,2], WIDANARNI[1,♥], SUKENDA[1], DINAMELLA WAHJUNINGRUM[1], MUNTI YUHANA[1], MIA SETIAWATI[1]

[1]Department of Aquaculture, Faculty of Fisheries and Marine Sciences, Institut Pertanian Bogor. Jl. Agatis, Bogor 16128, West Java, Indonesia. Tel.: +62-251-8622642, ♥email: widanarni@apps.ipb.ac.id

[2]Department of Aquaculture, Faculty of Agriculture, Universitas Sriwijaya. Jl. Palembang-Prabumulih Km. 32, Indralaya, Ogan Ilir 30662, South Sumatra, Indonesia

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Abstract. *Sasanti AD, Widanarni, Sukenda, Wahjuningrum D, Yuhana M, Setiawati M. 2025. Phytochemical screening and antibacterial activity test of* Curcuma zedoaria, C. aeruginosa, *and* C. mangga *extracts against* Aeromonas hydrophila. *Biodiversitas 26: 1574-1581.* The chemical compounds found in plants, with their potent antibacterial properties, hold significant potential as alternatives to antibiotics. This study aimed to identify the compounds present in the extracts of *C. zedoaria* or zedoary, *C. aeruginosa*, pink and blue ginger, and *C. mangga* or mango turmeric and to test their antibacterial activity against *A. hydrophila*. The extract of *C. zedoaria*, *C. aeruginosa*, and *C. mangga* extracts were obtained by maceration in 70% ethanol at a solvent ratio of 1:5 (w/v). The compounds in the extracts were identified by phytochemical analysis, followed by Gas Chromatography-Mass Spectrometry (GC-MS) analysis. The extraction results showed extract yields of *C. zedoaria* 9.87% (w/w), *C. aeruginosa* 9.89% (w/w), and *C. mangga* 9.56% (w/w), respectively. The phytochemical analysis revealed that *C. zedoaria* extract contained the highest levels of flavonoids as quercetin and curcumin, compared to other extracts. The highest total flavonoids, tannins, saponins, and total phenolic compound contents were found in the *C. aeruginosa* extract, whereas the highest antioxidant content was found in the *C. mangga* extract. GC-MS analysis detected the compound 1,2,3-propanetriol with the highest percentage area in *C. zedoaria* extract; in *C. aeruginosa* extract, the highest percentage area was found in the neocurdione compound. In comparison, in *C. mangga* extract, the highest percentage area was found in (E)-Labda-8(17), 12-dien-15, 16-dial compound. All three extracts showed significant antibacterial activity against *A. hydrophila*, as indicated by the inhibition zone of 9.37 to 14.40 mm. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against *A. hydrophila* of the three extracts were 6.25 mg mL^{-1} and 50 mg mL^{-1}, respectively, suggesting their potential as new antibacterial treatments.

Keywords: *Aeromonas hydrophila*, bactericidal, bacteriostatic, *Curcuma*, germacrone

INTRODUCTION

Plant phytochemistry plays an important role in the understanding of the chemical composition and therapeutic potential of various plant species. Plant phytochemical screening aims to identify and characterize the secondary metabolites found in plants, such as alkaloids, flavonoids, steroids, saponins, and tannins. These compounds often function as bioactive agents with health benefits, including antioxidant, anti-inflammatory, antifungal, and antibacterial activities (Rabizadeh et al. 2022). The family Zingiberaceae, genus *Curcuma*, is a plant that holds a significant place in traditional medicine and is often used as a medicinal material to overcome health problems. *Curcuma* sp. is distributed from India to southern China, Southeast Asia, Papua New Guinea, northern Australia, and Indonesia (Sirirugsa et al. 2007; Setiadi et al. 2017). The genus *Curcuma* contains a diverse range of species that are known for their extensive medicinal properties and phytochemical content (Rajkumari and Sanatombi 2018). Phytochemical screening of *Curcuma* sp. has revealed a wide range of bioactive compounds, including curcuminoids, flavonoids, terpenoids, and glycosides, which contribute to its pharmacological activity (Yuandani et al. 2021). Suryani et al. (2022) reported that the pharmacological activities of *Curcuma* sp. include antimicrobial, anti-inflammatory, anticancer, antidiabetic, and antioxidant activities. Anjusha and Gangaprasad (2014) revealed that tannins, saponins, terpenoids, and curcumin found in *Curcuma* sp. acted as antimicrobial agents. The most commonly used parts of *Curcuma* sp. are rhizomes and leaves, both in fresh form and after processing (Mishra et al. 2018). Commonly used *Curcuma* sp., including *Curcuma zedoaria*, *C. aeruginosa*, and *C. mangga*.

Curcuma zedoaria, commonly known as zedoary, is used in traditional medicine owing to its anti-inflammatory and antioxidant properties. Zhang et al. (2020) demonstrated the use of *C. zedoaria* to inhibit cancer cell proliferation, and its potential as an anti-H1N1 virus (Li et al. 2020). Essential oils from the leaf and rhizome extracts of *C. zedoaria* have antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, and *Staphylococcus aureus* (Thin et al. 2022). *C. aeruginosa* is known to have antioxidant activity (Suryani et al. 2022) and has potential as an anticancer agent (Wei et al. 2019; Zohmachhuana et al. (2022). Simoh and Zainal

(2015) stated that the *C. aeruginosa* extract contains many terpenoid compounds, sterols, organic acids, fatty acids, and sugars. The research by Kamazeri et al. (2012) showed that the essential oil of *C. aeruginosa* extract has antifungal activity against *Cryptococcus neoformans* and *Candida albicans*.

Syafi'i et al. (2018) reported that demethoxycurcumin was one of the bioactive compounds found in *C. mangga*. Demethoxycurcumin can act as an antimalarial, anti-inflammatory, antifungal, vasodilatory, and antimicrobial agent (Hatamipour et al. 2019). The dominant compound in *C. mangga* leaf extract is ae-curcumene, whereas in the rhizome, it is α-zingiberene and β-sesquiphellandrene (Bikindou et al. 2020). The results of research by Urbanova et al. (2024) showed that essential oil from *C. mangga* extract has antistaphylococcal activity.

Considering the widespread use and pharmacological potential of *C. zedoaria*, *C. aeruginosa*, and *C. mangga*, as well as the increasing interest in natural products as alternatives to synthetic drugs, research was initiated to investigate the antibacterial activity of the three *Curcuma* species against the pathogenic bacterium *Aeromonas hydrophila*. *Aeromonas hydrophila* is known to cause various infections in aquatic organisms (Le et al. 2018; Hoai et al. 2019). The increasing bacterial resistance to conventional antibiotics has made the exploration of natural resources to identify new antibacterial compounds very important. Therefore, the use of plant extracts as antibacterial agents is a promising approach. This is because plants have a very high biodiversity. The high biodiversity of plants has an impact on the abundance of various secondary metabolites that can be used as antibacterials. Secondary metabolites are chemicals that are not directly required for plant survival, but are synthesized to increase the plant's fitness in surviving and interacting with its environment, including pathogens, herbivorous insects, and symbiotes. Active compounds in plants tend to be less toxic than synthetic compounds, making drug development safer for humans and animals. Therefore, this study aimed to identify the compounds present in the extracts of *C. zedoaria*, *C. aeruginosa*, and *C. mangga* and to test their antibacterial activity against *A. hydrophila*, thereby contributing to the development of safer antibacterial drugs.

MATERIALS AND METHODS

Plant collection and extraction

Curcuma zedoaria, *C. aeruginosa*, and *C. mangga* were collected from the Research Center for Medicinal Plants and Aromatics (Balitro), Bogor, West Java, Indonesia (6°34'37"S 106°47'10"E). The rhizomes of *C. zedoaria*, *C. aeruginosa*, and *C. mangga* were washed, thinly sliced, and oven-dried (60°C, 48 hours). After drying, the samples were crushed until smooth and sieved. The resulting powder was extracted using 70% ethanol solvent (Tanvir et al. 2017) at a ratio of 1:5 (b/v), then macerated for 72 hours and stirred using a magnetic stirrer at room temperature. The maceration products were filtered with Whatman No. 1 filter paper and then concentrated by a rotary evaporator (100 psi pressure, 40°C temperature). The resulting dried extracts were stored at -20°C for further processing.

Phytochemical screening

Flavonoids

The spectrophotometric method was used to analyze the flavonoid content. Briefly, 0.5 mL of sample (extract) containing 1.25 mL of distilled water was taken and 5% sodium nitrite solution (0.075 mL) was added. It was allowed to stand for 5 minutes. Then 10% aluminum chloride (0.15 mL) was added. After 6 minutes, 1.0 M sodium hydroxide solution (0.5 mL) was added. The mixture was then diluted with distilled water (0.275 mL). The mixture absorbance was measured using a spectrophotometer immediately. The flavonoid compounds detected were expressed as Quercetin Equivalents (QE) in mg g^{-1} dry extract (Kamazeri et al. 2012).

Tannins

The tannin content was analyzed using the Folin-Ciocalteu method with tannic acid as a standard (Tanvir et al. 2017). A total of 0.1 mL of the solution containing 1 mg of the extract was mixed with distilled water (7.5 mL), and Folin-Ciocalteu reagent (0.5 mL) was added. Next, 1 mL of 35% sodium carbonate was added to 0.9 mL of distilled water, mixed and then incubated for 30 min. The intensity of the blue complex was measured at 725 nm wavelength. The results are expressed as grams of Tannic acid Equivalent (TE) per 100 g of sample extract.

Saponins

The saponin content was determined by the gravimetric method. A total of 1.25 g of extract was refluxed with light petroleum (50 mL) at 60-80°C for 30 minutes. After cooling, the remaining residue was dissolved with ethyl acetate (50 mL) and the light petroleum solution was discarded. The solution was further extracted by transferring it to a separating funnel to separate the ethyl acetate solution. The remaining residue was then dissolved 3 times with n-butanol (50 mL). The n-butanol solution was mixed and evaporated using a water bath. The evaporation residue was dissolved with methanol (10 mL) and then dripped into ether (50 mL) while stirring. The resulting precipitate was transferred to a filter paper of known weight. It was then dried and weighed until the weight remained. Saponin content is the difference in weight of the filter paper before and after filtration (Noviyanty et al. 2020).

Curcumin

Curcumin content was determined using the method described by Geethanjali et al. (2016). Next, 1 g of the sample was refluxed with 75 mL acetone for 1 h, after which it was filtered and made up to 200 mL. Then, 1 mL was collected and made up to 100 mL in a standard flask. The flasks were wrapped with dark-colored paper and stored in a dark room. The solution's UV spectral reading of the solution was recorded at 420 nm. The UV spectra were recorded using a curcumin standard. The sample's

absorption of the samples was compared with the standard value.

Phenol

The Folin-Ciocalteu colorimetric method was used to measure total phenolic compounds. Folin-Ciocalteu reagent (0.5 mL) was mixed with the extracted sample (0.5 mL). The mixture was then shaken manually for 15-20 seconds. After standing for 3 minutes, a saturated sodium carbonate solution (0.50 mL) was added. The mixture was then diluted with deionized water to a volume of 5 mL. The mixture was incubated in the dark at room temperature for 2 hours. The absorbance level was measured at 765 nm against deionized water using a dual-beam UV-Vis spectrophotometer. In order to determine the value of the total phenolic compounds, the gallic acid standard calibration curve was used as a reference. The values obtained were expressed as mg Gallic Acid Equivalent (GAE) relative to gallic acid standards. The final results were expressed as milligrams GAE per gram dry weight of residue (Babbar et al. 2011).

Antioxidant

The DPPH radical scavenging activity method was used to measure the antioxidant activity. Extract samples (1 mL) were mixed with 0.0003% DPPH (1.2 mL) in methanol at various concentrations (2.5-80.0 µg/mL). Antioxidant activity was expressed as the sample concentration required to reduce the absorbance of DPPH by 50% (IC_{50}) (Tanvir et al. 2017).

Analysis by Gas Chromatography-Mass Spectrometry (GC-MS)

The extract was dissolved in ethanol at a 1:1 (b/v). A Pyrolysis Gas Chromatography-Mass Spectrometry (GC-MS) 5973 Agilent Technology instrument was used. The sample injection volume was 2 µL. The column used was HP-5MS. Helium gas (99.99%) was used as the carrier gas at a total flow rate of 104 µL/min, for a run time of 30 min at an oven temperature of 50°C, injector temperature of 290°C and auxiliary temperature of 290°C. The number of peaks on the chromatogram indicates the number of compounds obtained. The names of the compounds obtained were interpreted based on the mass spectra data of each peak matched with the Pyrolysis GC-MS database (Munaeni et al. 2019).

Antibacterial activity

The disc-diffusion method was used to analyze the antibacterial activity of the extracts. Briefly, a suspension of *A. hydrophila* (100 µL of 10^8 CFU mL^{-1}) was uniformly swabbed on Tryptic Soy Agar (TSA) plates using sterile cotton swabs. Sterile blank discs (6 mm diameter) were individually impregnated with 15 µL of the extracts at various concentrations and placed on the inoculated agar plates, incubated at 37°C for 24 h. Antibacterial activity was calculated by measuring the clear zone produced in millimeters. Oxytetracycline (30 µg mL^{-1}) served as a positive control, and PBS solution (phosphate buffered saline, 8 g of NaCl, 1.5 g of Na_2HPO_4, 0.2 g of KH_2PO_4, 1000 mL of distilled water) was used as a negative control (Kamazeri et al. 2012).

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The plate count method was used to determine the MIC and MBC. The stock extract at a concentration of 50 mg mL^{-1} was serially diluted to different concentrations at a dilution factor of 1:1 in test tubes containing Tryptic Soy Broth (TSB). PBS was used as a negative control. Oxytetracycline 30 µg mL^{-1} was used as a positive control. Briefly, 100 µL of the *A. hydrophila* isolate at a density of 10^8 CFU mL^{-1} was inoculated into each tube, and the tubes were incubated in a water bath shaker at 28-29°C for 24 h at 140 rpm. The viable cell numbers were counted after 24 h by inoculating 100 µL of each treatment suspension into TSA medium and incubating for 24 h. The MIC value was the lowest concentration that could inhibit bacterial growth (bacteriostatic) using the negative control prior to incubation (C*) for comparison and the lowest concentration that can kill 99% of bacteria or bactericidal is designated as MBC (Munaeni et al. 2019).

Data analysis

The data were analyzed qualitatively and quantitatively, and expressed as mean±Standard Deviation (SD). Data were analyzed by one way Analysis of Variance (ANOVA) using Statistical Program Software System (SPSS) version 25. If the results were found to be significantly different, followed by Duncan's further test. Significant differences were those where $p<0.05$ or $P<0.01$.

RESULTS AND DISCUSSION

Extraction and identification of phytochemicals

Curcuma zedoaria, *C. aeruginosa*, and *C. mangga* extracts had yields of 9.87% (w/w), 9.89% (w/w), and 9.56% (w/w), respectively (Table 1). Phytochemical analysis revealed the presence of flavonoid compounds such as quercetin, total flavonoids, tannins, saponins, curcumin, and phenolic compounds in the *C. zedoaria*, *C. aeruginosa*, and *C. mangga* extracts. The highest flavonoid compounds, such as quercetin and curcumin content, were found in *C. zedoaria* extract. The *C. aeruginosa* extract had the highest total flavonoids, tannin, saponin, and total phenolic contents compared to the other extracts. In comparison, the highest antioxidant activity was found in the *C. mangga* extract (Table 2).

Analysis of active ingredients GC-MS method

Phytochemical identification can only detect the large groups of compounds present in plants. Further testing is required to determine other compounds found in the *C. zedoaria*, *C. aeruginosa*, and *C. mangga* extracts. Compound identification by GC-MS may reveal the presence of other compounds that were not detected by phytochemical analysis. The results of GC-MS analysis of *C. zedoaria*, *C. aeruginosa*, and *C. mangga* are presented in Table 3. The largest percentage area in the extract of *C.*

zedoaria was 15.49%, with a retention time of 2.896 min, which was attributed to compound 1,2,3-Propanetriol. The *C. aeruginosa* extract had the highest percentage area of the neocurdione compound at 12.20% with a retention time of 11.851 min, and *C. mangga* extract showed the highest percentage area of 33.07% on compound (E)-Labda-8(17),12-dien-15,16-dial with a retention time of 17.841 min. Most of the compounds found in the three extracts belonged to the sesquiterpenoid group. In addition, there are also those belonging to the group of benzothiazoles (2-(benzothiazol-2-ylamino)-3H-imidazol-4-ol), triol (1,2,3-propanetriol), and primary fatty amide (13-docosenamide).

Antibacterial activity

The antibacterial activities of the three extracts were determined based on the formation of a clear zone (Table 4). The results showed that all three extracts had potent antibacterial activities against *A. hydrophila*, demonstrating their potential to inhibit this pathogen. The concentration of 6.25 mg mL^{-1} was the lowest concentration that showed an inhibition zone. The higher the concentration of the extract, the larger the formation of the inhibition zone. Based on statistical tests, the inhibition zones formed by the three extracts were not significantly different.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Curcuma zedoaria, *C. aeruginosa*, and *C. mangga* extracts had the same MIC and MBC values. A concentration of 6.25 mg mL^{-1} from *C. zedoaria*, *C. aeruginosa*, and *C. mangga* extracts had the same or lower population density of *A. hydrophila* as the negative control prior to incubation (C*). This indicates that the concentration of 6.25 mg mL^{-1} was the lowest concentration that can inhibit the growth of *A. hydrophila*. Thus, this concentration was the MIC of the three extracts. A plate count at a concentration of 50 mg mL^{-1} showed no colonies of *A. hydrophila*. Therefore, the concentration of 50 mg mL^{-1} was the bactericidal concentration of the three extracts (Figure 1).

Table 1. Physical characteristics and percentage yield of the *Curcuma zedoaria*, *C. aeruginosa*, and *C. mangga* extracts

Characteristics	Ethanol rhizome extracts		
	C. zedoaria	***C. aeruginosa***	***C. mangga***
Color	Brown	Light brown	Blackish-brown
Initial weight (g)	500	500	500
Final weight (g)	49.35	49.45	47.8
Yield (%)	9.87	9.89	9.56

Table 2. Compounds of *Curcuma zedoaria*, *C. aeruginosa*, and *C. mangga* extracts

Characteristics / Compounds	Extracts		
	C. zedoaria	***C. aeruginosa***	***C. mangga***
Flavonoids as quercetin (%)	3.1	1.28	1.21
Total flavonoids (mg/100g)	666	772.22	15.4
Tannins (%)	0.9	1.76	1.2
Saponins (%)	3.4	12.03	5.01
Curcumin (%)	1.48	0.46	0.27
Total phenolic (mg GAE/100g)	1897.13	4175.55	537.38
Antioxidants IC 50% (ppm)	79.14	150.82	195.84

Table 3. Compounds of *Curcuma zedoaria*, *C. aeruginosa*, and *C. mangga* extracts by GC-MS pyrolysis

Retention Time (Min)	Library identification	Area (%)		
		C. zedoaria	***C. aeruginosa***	***C. mangga***
2.896	1,2,3-Propanetriol	15.49	-	-
3.687	3-Isothiazolecarboxmide	0.46	-	-
4.727	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	0.88	-	-
4.765	Camphor	0.51	-	-
9.093	Benzofuran	1.16	2.07	-
10.378	Epicurzerenone	-	-	4.22
10.518	6-Methylxanthotoxin	-	8.21	-
10.606	Curcumol	-	3.60	1.32
11.006	Eudesm-4(14)-en-11-ol	-	2.01	-
11.456	Germacrone	-	2.90	0.81
11.598	5,6,8,9-Tetrahydro-2-isopropyl-benzocyclohepten-7-one	0.69	-	-
11.851	Neocurdione	-	12.20	5.15
11.853	Azulene	0.32	-	-
12.078	Curdione	-	1.97	0.63
12.123	(4aS,6R,8aS)-4,4,7-Trimethyl-4,4a,5,6,8,8a,9-hexahydronaphtho[2,3-b]furan-6-ol	1.01	-	-
12.496	2-(Benzothiazol-2-ylamino)-3H-imidazol-4-ol	6.79	-	-
12.613	Ambrial	-	-	4.43
13.526	Neoprocurcumenol	-	1.03	0.41
14.362	Procucumenol	-	1.28	1.06
14.638	Zederone	-	3.79	-
17.841	(E)-Labda-8 (17), 12_dien-15, 16-dial	-	-	33.07
20.829	13-Docosenamide	2.03	-	0.46

Table 4. Antibacterial activity of *Curcuma zedoaria*, *C. aeruginosa*, and *C. mangga* extracts at different concentrations against *A. hydrophila*

Treatment	Inhibitory zone diameter (mm)		
	C. zedoaria	***C. aeruginosa***	***C. mangga***
PBS solution	0±0.00[a]	0±0.00[a]	0±0.00[a]
1.563 mg mL^{-1}	0±0.00[a]	0±0.00[a]	0±0.00[a]
3.125 mg mL^{-1}	0±0.00[a]	0±0.00[a]	0±0.00[a]
6.25 mg mL^{-1}	9.73±0.50[b]	11.77±0.68[b]	9.37±0.15[b]
12.5 mg mL^{-1}	10.10±0.10[b]	12.20±0.17[b]	9.53±0.15[b]
25 mg mL^{-1}	12.50±0.50[c]	13.37±0.06[c]	11.93±0.11[c]
50 mg mL^{-1}	14.33±0.12[d]	14.40±0.10[d]	14.33±0.11[d]
Oxytetracycline (30 µg mL^{-1})	14.57±0.40[d]	14.57±0.40[d]	14.57±0.40[d]

Note: Data (mean±SD) with different letters indicate a significant difference (P<0.05)

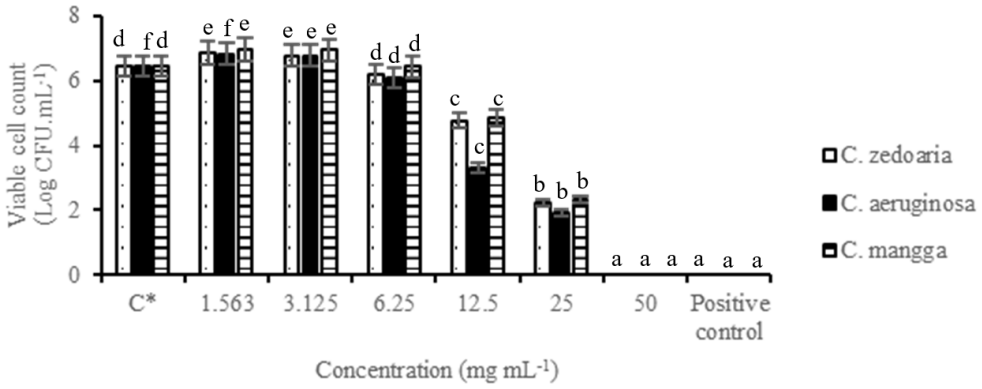

Figure 1. Effects of *Curcuma zedoaria*, *C. aeruginosa*, and *C.mangga* extract on growth of *A. hydrophila* for 0 and 24 h. Data are expressed as mean±SD (P<0.05) when compared with the negative control without the addition of extracts for 0 h (C*). Different superscript in the same extract type indicates a significantly different

Discussion

Phytochemical screening is an important first step in identifying bioactive components in plant extracts, as these compounds have great potential as alternatives to antibiotics (Awad and Awaad 2017; Villanueva et al. 2023). The phytochemical identification of *C. zedoaria*, *C. aeruginosa*, and *C. mangga* extracts showed significant differences in the content of bioactive compounds with potential as therapeutic agents. Herbal materials are known as prominent medicinal sources of polyphenols, particularly flavonoids (Rejab and Ksibi 2019). The highest flavonoids as quercetin, were found in *C. zedoaria* extract with a concentration of 3.1%, while the highest total flavonoids were found in *C. aeruginosa* with 772.22 mg/100g. Quercetin is often used as a reference compound to determine flavonoid content in extracts due to its widespread occurrence in various plants and its significant biological activity. Total flavonoids include various types of flavonoids, including quercetin, kaempferol, catechins, anthocyanins, isoflavones and other flavonoids (Elgendy et al. 2020). Quercetin acts as a powerful natural antioxidant and cytotoxic agent with multiple therapeutic benefits. Quercetin effectively inhibits bacterial activity by damaging cell membranes, preventing biofilm formation, and reducing virulence factors (Baqer et al. 2024). Flavonoids, with their strong antioxidant activity and the ability to inhibit bacterial growth (Kumar et al. 2022; Lo et al. 2022; Semwal et al. 2023) offer reassurance about the potential health benefits. The presence of high concentrations of flavonoids, such as quercetin in *C. zedoaria*, and total flavonoids in *C. aeruginosa*, suggests great potential in the treatment of infections and as antioxidant agents. It shows promise against drug-resistant bacterial strains, and specific structural modifications can enhance its antibacterial efficacy, highlighting its potential as a therapeutic agent.

In addition, the *C. aeruginosa* extract showed the highest tannin content at 1.76% and saponins at 12.03%. Tannins can disrupt bacterial cell wall function and inhibit essential enzymes (Farha et al. 2020). Saponins are known to increase the permeability of bacterial cell membranes, causing cell damage that can inhibit bacterial growth (Sun et al. 2019). The high tannins and saponins content of *C. aeruginosa* strengthens its potential as an effective antibacterial agent. The *C. zedoaria* extract showed the highest curcumin content of 1.48%. Curcumin is a polyphenolic compound with various health benefits, including anti-inflammatory, antioxidant and antibacterial activities (Geethanjali et al. 2016; Teow et al. 2016). The high curcumin content of *C. zedoaria* suggests its potential for wider therapeutic applications, particularly in the treatment of diseases associated with inflammation and infection. The *C. aeruginosa* extract showed the highest total phenolics with 4175.55 mg GEA/100g. Total

phenolics are an important indicator of the antioxidant potential of an extract, which can protect cells from oxidative damage (Babbar et al. 2011). The high antioxidant activity of *C. mangga* (195.84 ppm) indicates its potential to prevent cell damage caused by free radicals, which plays an important role in preventing degenerative diseases.

The chemical components in the plant extracts were further analyzed by Gas Chromatography-Mass Spectrometry (GC-MS), which allows the specific identification, characterization, and quantification of compounds with high accuracy. Retention time in Gas Chromatography-Mass Spectrometry (GCMS) is essential for the identification and characterization of chemical substances. It denotes the duration a chemical requires to traverse the GC column prior to detection. This metric is crucial for differentiating drugs based on their distinct retention durations, which can fluctuate considerably according to the compound's chemical characteristics and the analytical conditions (Hamadi and Varga 2022). Curdione, curcumol, and germacrone are the major compounds found in the rhizomes of *Curcuma* spp. (Xia et al. 2005; Wu et al. 2017). Based on the results of the study, curdione was detected in *C. aeruginosa* and *C. mangga* extracts with a retention time of 12.078 min and at an area percentage of 1.97% and 0.63%, respectively. The *C. aeruginosa* and *C. mangga* extracts also contained curcumol compounds at 3.60% and 1.32%, respectively, with a retention time of 10.606 min. Similarly, germacrone compounds were found in the *C. aeruginosa* extract at 2.90%, and *C. mangga* extracts at 0.81% with a retention time of 11.456 min. The presence of these compounds plays an antibacterial role and shows potential as substitutes for antibiotics (Qu et al. 2009; Wei et al. 2019; Gharge et al. 2021).

Furthermore, *C. zedoaria* extract has the largest compound percentage area in 1,2,3-propanetriol at 15.49% with a retention time 2.896 min. This compound belongs to the triol group, also known as glycerol, which has antibacterial properties by affecting the osmotic pressure and permeability of cell membranes (Durlak et al. 2019; Poblete-Castro et al. 2020). The *C. aeruginosa* extract, showed the highest percentage area in the compound neocurdione of 12.20% with a retention time 11.851 min, which is a part of the sesquiterpenoid group. Sesquiterpenoids, such as neocurdione, are known to have diverse antibacterial activities, including the ability to damage bacterial cell membranes, cause the leakage of essential cellular components, and inhibit bacterial growth (Riyadi et al. 2023). These compounds can also interact with proteins and enzymes in bacterial cells, disrupting their normal function and causing cell death (Wei et al. 2021).

Curcuma mangga extract showed the highest percentage area of (E)-Labda-8(17),12-dien-15,16-dial at 33.07% with aretention time 17.841 min. This diterpenoid is also known to have antibacterial activity (Urbanova et al. 2024). (E)-Labda-8(17),12-dien-15,16-dial works by inhibiting bacterial growth through enzyme inhibition mechanisms and by damaging the structure of the bacterial cell wall, resulting in disruption of cell integrity and bacterial death. Overall, most of the compounds detected in the three extracts belong to the group of sesquiterpenoids, benzothiazoles, triol and primary fatty amides such as 13-docosenamide. These compounds are known to have significant antibacterial activity through various mechanisms such as cell membrane damage, disruption of bacterial enzyme functions, and inhibition of important metabolic processes required for bacterial growth and survival (Gjorgjieva et al. 2018; Durlak et al. 2019; Morsy et al. 2020; Yosief et al. 2020; Li et al. 2022).

Based on the results of phytochemical screening and GC-MS analysis of *C. zedoaria*, *C. aeruginosa*, and *C. mangga* extracts, various bioactive compounds with antibacterial potential were identified. The antibacterial activities of the three extracts against *A. hydrophila* were successfully tested. Based on the test results, the inhibition zone of the three extracts began to form at a concentration of 6.25 mg mL^{-1}. At lower concentrations, no inhibition zones were formed because the concentration of antibacterial compounds in the extracts was not sufficiently high to affect bacterial growth effectively. The number of antibacterial compounds present at low concentrations was insufficient to reach the threshold required to inhibit bacterial growth. The antibacterial activity of compounds is generally dose-dependent, and their effectiveness increases as their concentration increases (Li et al. 2017). This can be observed by the increase in the diameter of the inhibition zone, which increases as the dose of the extract increases. Statistical analysis showed that there was no significant difference in the diameter of the inhibition zone among the three extracts at all concentrations tested. This indicates that although the three extracts have the potential to inhibit the growth of *A. hydrophila*, their efficacies in producing inhibition zones were not statistically different. The clear zone formed at a concentration of 6.25 mg mL^{-1} can be interpreted as evidence that this concentration is sufficient to induce an antibacterial effect.

The minimum concentration that prevents growth of the test microbe is determined by the MIC (Thin et al. 2022). The MIC test results showed that the MIC for the three extracts was at a concentration of 6.25 mg mL^{-1}. This is also supported by the data in Table 4, which shows that no inhibition zone is formed at a concentration below 6.25 mg mL^{-1}. This indicates that the concentration of 6.25 mg mL^{-1} is the lowest effective concentration for inhibiting the growth of *A. hydrophila*. The MBC test showed that the MBC of the three extracts was at a concentration of 50 mg mL^{-1} (Figure 1). The results of phytochemical screening and GC-MS identification supported these findings by detecting antibacterial compounds such as flavonoids, tannins, saponins, curcumin and sesquiterpenoids in the extracts, as well as specific compounds such as neocurdione, 2-(benzothiazol-2-ylamino)-3H-imidazol-4-ol and (E)-Labda-8(17),12-dien-15,16-dial. These compounds are effective at inhibiting bacterial growth at low concentrations while killing bacteria at higher doses (Parvekar et al. 2020). This is a clear demonstration of the dose-dependent nature of the antibacterial activity, a key concept in our field, where the active compound must be present at a sufficiently high concentration to exert a

bactericidal effect. These findings demonstrate the potential of *C. zedoaria*, *C. aeruginosa*, and *C. mangga* extracts as sources of potent antibacterial compounds and suggest that the use of extracts in therapeutic applications requires higher doses to achieve bactericidal effects.

In conclusion, *C. zedoaria* or white turmeric extract had the highest flavonoid as quercetin and curcumin content. *C. aeruginosa* is rich in total flavonoids, tannins, saponins, and phenols, and *C. mangga* has the highest antioxidant activity. GC-MS analysis identified the main compounds in each extract: 1,2,3-Propanetriol in *C. zedoaria*, neocurdione in *C. aeruginosa*, and (E)-Labda-8(17), 12-dien-15, 16-dial in *C. mangga*. The three extracts showed antibacterial activity against *A. hydrophila* with inhibition zones of 9.37-14.40 mm, MIC of 6.25 mg mL^{-1}, and MBC of 50 mg mL^{-1}.

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