ANTIMICROBIAL ACTIVITIES OF CURCUMIN EXTRACTED FROM SELECTED ZINGIBERACEAE SPECIES AS POTENTIAL HALAL ACTIVE PHARMACEUTICAL INGREDIENT

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ABSTRACT

The ginger family (Zingiberaceae) includes plants with a distinct smell and taste that are commonly used as spices in the kitchen, but also in a variety of industries (pharmaceutical, medical, and cosmetic) due to their demonstrated biological activity. This study describes the antibacterial activity of curcumin extracts from selected species of the Zingiberaceae family namely "temu emas" (Curcuma zeodoria). "temu kunci" (Curcuma manga), "temu pauh" (Curcuma amada), "lempoyang" (Zingiber zerumbit*)* and "cekur" (Kaempferia galangal). Extracts of these compounds were studied on *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 35218), *Salmonella typhimurium* (ATCC 14028), *Candida albicans* (ATCC 10231) and *Aspergilus brasiliensis* (ATCC 16404). Turbidity is taken as an indication of growth, thus the lowest concentration, which remains clear after macroscopic evaluation is taken as the minimum inhibitory concentration (MIC). In conclusion, amongst the studied members of Zingiberaceae, *temu emas* curcumin natural pigment extracts showed the best antibacterial activities against *E. coli* ATCC 35218 (2 µg / µL), *S. typhimurium* ATCC 14028 (2 µg / µL), *C. albicans* ATCC 10231 (2 µg / µL), *A. brasiliensis* ATCC 16404 (2 µg / µL) and *S. aureus* ATCC 25923 (3 µg / µL). The outcome of this research will be contributing towards new natural carotenoid pigment sources as potential active pharmaceutical ingredients which can be beneficial to halal health-promoting products industry.

Keywords: *Zingiberaceae, Curcumin, Antimicrobial activity, Halal ingredient, Natural Pigment*

1. Introduction

Curcumin [1, 7-bis (4-hydroxy-3-methoxyphenyl)-1, 6-heptadiene-3, 5-dione] is the principal curcuminoid in turmeric which is the cause of its bright yellow colour. It is a carotenoid that can be extracted from the rhizomes of the plant Curcuma longa that changes colour in alkaline conditions or high pH solution (Oglah et al., 2020). Curcumin is an effective scavenger of many reactive oxygen species (ROS) including hydroxyl radicals and superoxide anions which explains its antioxidant properties (Hewlings & Kalman, 2017). Curcumin also shows promising potential in correcting cystic fibrosis. Although the test was only observed in animals, specifically baby hamsters' kidney cells, the research showed that through oral administration, curcumin induced the functional appearance of AF508 CTFR protein which is the affected protein in cystic fibrosis (Egan et al., 2013). Curcumin is industrially produced using oleoresin of turmeric as the raw material. Even the by-products that are produced from the isolation of curcumin have been tested and proven to have antibacterial activities against a range of pathogenic and spoilage bacteria which include *Bacillus cereus, Bacillus coagulans, Bacillus subtilis, Staphylococcus aureus, Escherichia coli*, and *Pseudomonas aeruginosa* (Nelson et al., 2017). A study using size exclusion

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chromatography and dynamic light scattering has shown it to be a dimer at pH 7 but changes its form to be a monomer when it reaches pH 2. The dimer at pH 7 is more stable than the monomer at pH 2 (Biswas & Chattopadhyaya, 2014). Delgado et al. (2016) observed that turmeric in its amorphous state was stable under storage conditions with temperatures ranging below 65.35ºC whereas Tonnesen & Karlsen (1986) found that curcumin exhibited photodecomposition upon exposure to ultraviolet (UV) / visible radiation. This photodecomposition was seen in both solution and the solid-state, as a thin film. The main product was formed by a loss of a couple of hydrogen atoms from the curcumin molecule, resulting in its cyclization of it. Curcumin could have a destabilizing effect in a mixture of other compounds due to its sensitizing properties. Curcumin, a diphenol compound with a low molecular weight that is nontoxic and hydrophobic, is insoluble in water and ether but soluble in methanol, ethanol, and dimethyl sulfoxide. It is drawn to the world of composite film fabrication for its packaging applications because it offers additional functional features such as increased mechanical strength, UV protection, antioxidant properties, and antibacterial activity (Ezati et al., 2021). Curcumin also demonstrated a noticeable pH-responsive colour change. This is because its predominant structure changed under different pH conditions. Curcumin has an ordered crystal structure that consists of a seven carbon chain that includes an, -unsaturated -diketone moiety that is attached to two aromatic rings that have ortho-methoxy phenolic-OH groups (Sahne et al., 2017). Curcumin's molecular configuration is affected by the pH of the solution, polarity, and temperature. At neutral and alkaline pH levels, the α, β-unsaturated β-diketone moiety acts as a hydrogen donor site, resulting in curcumin hydrolysis and degradation. The β -diketone chain, which occurs in the curcumin structure, survives as a ketoenol tautomer form, which depends on the solvent properties. It is formed in the acidic or neutral pH states that exist in the bis-keto, and it will form in the basic pH state that the enolic is formed in. The pH solution ranged from 1-7, indicating the dominant bis-keto form of curcumin, which is yellow in colour and has very low water solubility. As a result, as alkalinity increases, curcumin solubility and stability increase. The enolic form functions as based on the donation of H atoms from C-H bonds from the central carbon atom to neighbouring oxygen atoms, which is due to the weak delocalization of unpaired electrons (Typek et al., 2019).

2. Literature Review

2.1 Curcumin properties

As seen from Figure 1, curcumin [1,7- Bis (4-hydroxy-3-methoxyphenyl)-1,6-hep tadiene 3,5-dione] has been recognized as a strong immunomodulator in both humans and animals, which produced an orange-yellow polyphenolic and hydrophobic phytochemical component. It is a predominant curcuminoid uniquely from turmeric rhizomes. For years, curcumin has been considered as a powerful natural antioxidant in traditional Chinese medicine and Indian ayurvedic treatments (Aggarwal et al., 2007; Zheng et al., 2018). From Johannah et al. (2018), they claimed that, the properties of curcumin included with anti-inflammatory, antimicrobial, antioxidant, immunomodulatory, appetite-increasing, and gastro-protective effects on animal health. Apart from that, the limitations effects of curcumin were showed, for example, unstable chemical structure, hydrophobicity, poor absorption in the body (bioavailability of curcumin) which depends on the animal species and sex, and rapid metabolism (Hewlings & Kalman, 2017). Curcumin is one of the effective scavengers of many reactive oxygen species (ROS) which include superoxide anions and hydroxyl radicals which contribute to their antioxidant properties (Ruby et al., 1995). It also

showed a potential in improving cystic fibrosis. Based on research, it was tested on the baby hamster's kidney cells, through oral administration and resulted as curcumin induced the functional appearance of AF508 CTFR protein, which affected the protein in cystic fibrosis (Egan et al., 2013). According to Tonnesen & Karlsen (1986), curcumin is presented as a photodecomposition after being exposed to ultraviolet (UV) / visible radiation, which is possessed in both solutions; liquid and solid (thin film). This is because the couple of hydrogen atoms were loss from the curcumin molecule and resulted in cyclisation. Curcumin also undergone the destabilizing effect in a mixture of the other compounds, due to its stimuli properties.

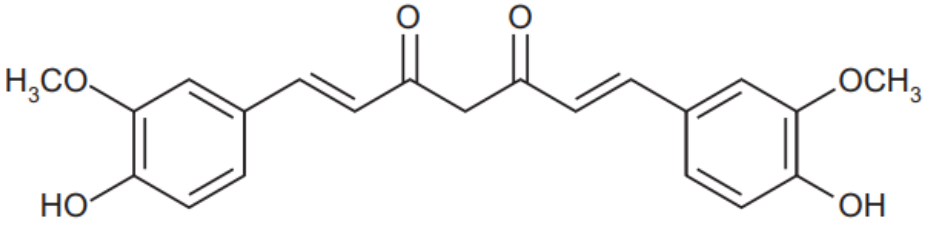

Fig. 1: Chemical structure of Curcumin
Source: Maria L.A.D. Lestari, Gunawan Indrayanto (2014)

Curcumin exhibits ketoenol tautomerism which depends on the solution acidity. In acidic and neutral media, the keto-form is dominant, while in alkaline media it exists in the enol form. The enol form is stabilized by resonance-assisted hydrogen bonding(Anand et al., 2007; Parimita et al., 2007). The melting point of curcumin showed from 176-177 °C (Kharat et al., 2017). The commercial curcumin showed different concentrations, which were; 77% of diferuloylmethane, 17% of dimethoxy-curcumin, and 6% of bisdemethoxycurcumin (Rai et al., 2015). It is recognized as a safe natural product, which the number of substances or chemicals needed in the food were exempted from the food additive regulations insisted in the Federal Food, Drug, and Cosmetics Act of the USFDA (Aggarwal et al., 2007).

2.1.1 Chemical Structures

The absorption spectra of carotenoids depend largely on the number of conjugated double bonds (c.d.b.) in their molecules. The longer the chromophore, the higher the wavelengths of maximum absorption. Acyclic carotenoids absorb maximally at longer wavelengths than cyclic carotenoids with the same number of c.d.b. in which conjugation extends into rings, because, in the latter, there are steric strains. The UV/Vis spectrum of carotenoids is of great importance for analysts because it provides valuable information about their structure. The spectrum is due to the presence of the long chromophore of conjugated double bonds (c.d.b.). At least 7 c.d.b. are needed for a carotenoid to have perceptible colour. Thus, a carotenoid with 7 c.d.b., has a slight yellowish colour, whereas carotenoids with 3 and 5 c.d.b. are colourless (He et al., 2018).

2.1.2 Functional Groups

Functional groups are incorporated into polymer molecules for several reasons. Their presence may modify the physical properties, such as adhesion to a substrate or solubility in selected solvent composition, or the functional group may be essential for the polymer-forming reaction or to enable subsequent chemical interaction with other entities. The effectiveness of the functional group in satisfying these requirements will be related to its availability as well as a paradoxical situation such as chemical structures.

2.2 Biological activity of curcumin

2.2.1 Antioxidant activity

In both in vitro and in vivo settings, various assays have been used to determine the antioxidant activity of curcumin. The antioxidant effect is due to its hydroxyl groups, which are directly proportional in between functional groups and to the free radical scavenging activity of this polyphenolic compounds. The presence of one or more hydroxyl groups showed a significant superior of antioxidant activity due to the curcumin derivative bis (3,4-dihydroxycinnamoyl)-methane (Sharma, 1976). In line with this, a role for the –OH group in free radical scavenging has been proposed in other compounds for example carboxymethyl cellulose (Ezati et al., 2021). From the previous studies, the researchers have suggested that, the main role of -OH in the free-radical scavenging effect of curcumin (Sun et al., 2002). In the curcumin, the position of the hydroxyl group plays a major role. It has been mentioned by Rukkumani et al. (2004), the 2-hydroxyphenyl group which was found in the curcumin derivative bis(2-hydroxycinnamoyl)-methane showed a better antioxidant activity as compared to the 4-hydroxyphenyl group which present in curcumin.

2.2.2 Antimicrobial activity

From the previous study mentioned by Chen et al. (2010), the antiviral activity of curcumin can oppose to the influenza virus. They claimed that, over 90% of the compound can help in reducing the viral population in cell culture at a treatment concentration of 30 µM. It has been claimed that curcumin can prevent the spread of the influenza virus, which can interfere with cellular virus adhesion. The effect of curcumin and curcuminoids on fungal strain *Candida albicans* (Zhang et al., 2012). The antifungal effect of curcumin was powerful as compared to *demethoxycurcumin*. It was claimed that, in curcumin, the methoxyl group makes it more lipophilic, which resulted in uninterrupted entry into the fungal cell membrane, which can prevent its growth. In the previous study conducted by Adamczak, A. et al. (2020), they experimented on the antimicrobial activity of turmeric against more than 100 pathogens that are belonging to 19 species. It is included with moulds and moulds and a lot of Gram-positive and Gram-negative bacteria. It is proven that; curcumin has a higher antimicrobial tendency against Gram-positive bacteria as compared to Gram-negative bacteria.

3. Materials and Method

3.1 Sample preparation

The raw material of 5 selected ginger species has been stored in the freezer at a temperature of -20 °C prior to further processing, before 3kg of them has been dried using a freeze dryer ((FDU-1100, EYELA, Tokyo Rikakikai Co., Japan). This has been done at a temperature of -50 °C and a vacuum level of 6.5 Pa for three days. Then, the sample has been ground into a fine powder using a heavy-duty blender (LB20E, Laboratory Blender). The grounded sample was conveniently stored under appropriate conditions at a temperature of -20 °C to minimize enzymatic reactions (Othman, 2009).

3.2 Curcumin extraction

3.2.1 Chemical Extraction

10 g of grounded dried of 5 selected ginger species powder has been added into 50 ml tubes and rehydrated with 1 ml of distilled water. Then, 5 ml of a mixture of acetone and methanol (7:3) has been added to each tube. The samples are then stored overnight in a dark condition

at room temperature, whereby each has been prepared in triplicate. The next day, the samples have been vortexed and centrifuged for 2 minutes at 13 500 g (NU-C200R-E, Nuaire, USA), with the resulting supernatant being transferred into 50 ml graduated polypropylene centrifuge tubes. This step is then repeated by adding 5 ml of the same solvent until the supernatant of the sample becomes colourless (normally two or three times). Then, an equal volume of hexane and distilled water (5 ml) has been added to extract the carotenoid from the combined supernatants. The solution is then allowed to separate, with the upper hexane layer containing the carotenoids being collected. This procedure has been repeated with hexane alone until it becomes colourless. After that, the combined upper layer has been dried to completion under a gentle stream of oxygen-free nitrogen. Tubes are then capped and sealed with parafilm to exclude oxygen and immediately stored at -20 °C prior to further analysis. To extract the carotenoid curcumin, an equal volume of hexane and distilled water will be added to the combined supernatants. The solution will then be allowed to separate and the upper layer containing the carotenoids was collected. The combined upper phase will be dried to completion under a gentle stream of oxygen-free nitrogen (Othman et al., 2017).

3.3 High–Performance Liquid Chromatography (HPLC) Analysis

HPLC employing diode-array detection (DAD) is the most common analytical method in determining qualitative and quantitative carotenoid profiles. The HPLC Agilent 1200 series (Agilent Technologies, USA) is being used to analyse the curcumin content extracted from selected species. It is equipped with a binary pump with auto sampler injector, micro vacuum degassers, and thermostatted column compartment, whereas the reverse phase column is a ZORBAX Eclipse XDB-C18 end-capped (5 µm), sized at 4.6 x 150 mm. A HPLC grade of acetonitrile: water (9:1, v/v) has been prepared as eluent A, whereas the HPLC grade of ethyl acetate is designated as eluent B. Meanwhile, Ultrapure water 18.0 MΩ is prepared using a Milipore S.A.S water purification system.

3.4 Antimicrobial activities

3.4.1 Media preparation

All procedures are carried out in a Class 2 biohazard cabinet. The inner surfaces are wiped with 70% ethanol and left under ultraviolet (UV) light for 20 minutes prior use. Five target microbe representing Gram negative and Gram-positive bacteria, yeast and fungus namely: *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 35218, *Salmonella typhimurium* ATCC 14028, *Candida albicans* ATCC 10231 and *Aspergilus brasiliensis* ATCC 16404 were selected for this study. All microbial cultures were purchased from American Type Culture Collection (ATCC).

Media: General and selective media (broth/agar) were prepared. Suitable media were identified and the required amount was weighed according to the manufacturer's instructions. Distilled water was used to dissolve the media in a Duran bottle and autoclaved at 121˚C for 20 min. The resulting media solutions were placed in a 60°C water bath prior to immediate usage or 4°C for storage / future usage.

Inoculation: Circa 5-8 ml broth was aseptically poured into each sterilized test tube using an aseptic technique. Similarly, a microbe-coated-bead was extracted from the cryo-vial and released into the broth containing test tube. Test tubes were labelled accordingly (i.e. inoculum vs control broth). Both (inoculum and control) test tubes were then incubated in a shaking incubator set at (i) 37°C for 18 – 24 hours (*S. aureus*, *E. coli*, *S. typhimurium* and *C.*

albicans) and (ii) 26°C for 48 hrs (*A. brasiliensis*). Turbidity is taken as an indication of growth. Broth in the control test tube however should remain clear, void of growth.

Standardization: Prior to use, an 18-24-hour-old culture will be adjusted to obtain turbidity comparable to that of 0.5 McFarland standard (1.5 x 108 CFU/ml) or 0.05 to 0.1 absorbance value at 600 nm as measured by cell density meter (Biowave C model Co8000).

Sample stock solution: Samples were stored at -20°C and thawed prior use. A specific amount of sample was directly pipetted into wells containing a specific amount of broth (final volume of sample-broth mixture: 50 µl) creating a serial sample dilution ready to be exposed against the targeted microbe.

3.5 Sample contamination free (sterility) status

50 µl samples were pipetted onto Nutrient agar (NA) and Sabouraud Dextrose agar (SDA) plates to ensure their sterility status. Samples on NA plates were incubated at 37°C for 24 hours while samples on SDA were incubated at 26°C for 72 hours prior to naked-eye observation to check for the presence of bacterial and fungal colonies respectively. The absence of colonies on both NA and SDA plates indicated sample sterility hence ready for antimicrobial evaluation.

3.6 Minimum inhibitory concentration (MIC) determination

A quantitative in vitro assay to evaluate the sample's inhibitory potential against the selected microbe. A serial dilution of the sample was prepared by pipetting 50 µL, 40 µL, 30 µL, 20 µL and 10 µL samples in a designated column of 96-well microtiter plate. Subsequently, 0 µL, 10 µL, 20 µL, 30 µL and 40 µL of broth were added into each well to reach a final volume of 50 µL (sample-broth mixture) prior to the final addition of 50 µl of standardised microbial culture (a population of 106 cfu/ml). The microtiter plates were then incubated at 37°C (*S. aureus*, *E. coli*, *S. typhimurium* and *C. albicans*) and 26ºC (*A. brasiliensis*) for 24 hours. Turbidity is taken as an indication of growth, thus the lowest concentration, which remains clear after macroscopic evaluation is taken as the minimum inhibitory concentration (MIC).

3.7 Inhibitory status determination assay

The inhibitory activity status of each sample (permanent/cidal) or static/temporary will be determined immediately after the macroscopic MIC evaluation. 20 µL of MTT reagent (1 mg/ml) each was pipetted into the MIC wells (clear wells), further incubated for 20 minutes (bacteria and candida) and 2-3 hours (*A. brasiliensis*). Clear coloured well (containing microbe-sample-media mixture) indicates cidal or permanent inhibitory activity while a dark purple-coloured well (containing microbe-sample-media mixture) indicates static or temporary inhibitory activity). The Minimum Bacterio/Candido/Fungicidal Concentration values are recorded as mean concentration of triplicates.

4. Results and Discussion

4.1 Total curcumin content from Zingiberaceae family

Table 1: Total Curcumin content (µg/g DW) using chemical extraction method

Sample	Curcumin content (µg/g DW)
Kaempferia galangal (Cekur)	0.70 ± 0.02
Curcuma zeodoria (Temu emas)	**12.95 ± 1.07**
Zingiber zerumbit (Lempoyang)	0.10 ± 0.01
Curcuma manga (Temu kunci)	0.05 ± 0.01
Curcuma amada (Temu pauh)	1.20 ± 0.20

Table 1, it showed that the highest curcumin content was detected in Curcuma zeodoria (Temu emas) with a value of 12.95 ± 1.07. Each of the chromatograms detected the presence of the curcumin peak by comparing the peaks' retention times to the curcumin standard. The type and amount of specific carotenoids, like curcumin, will depend on the activity of functional enzymes and candidate enzymes that control carotenoid biosynthesis (Othman et al., 2017).

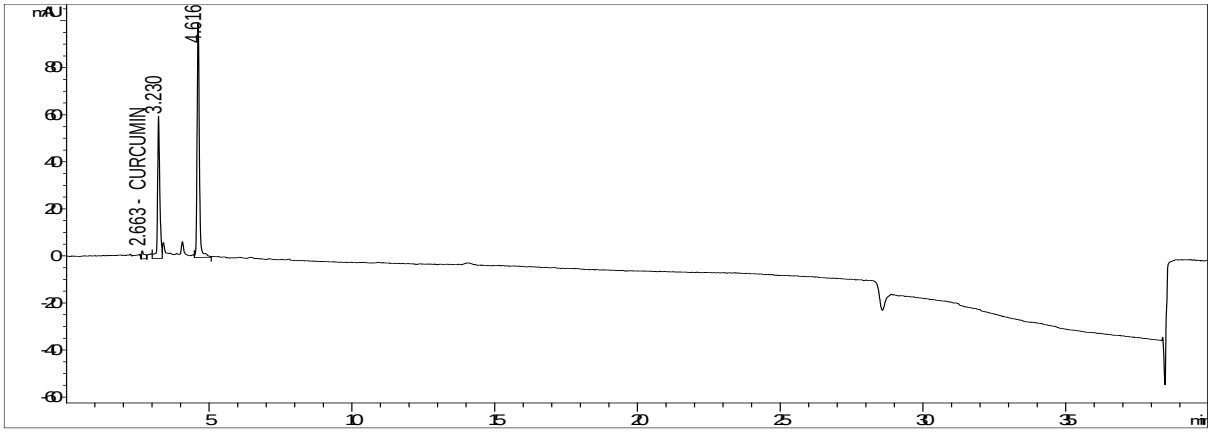

Fig. 2: HPLC Chromatogram for Curcumin Extraction on Cekur

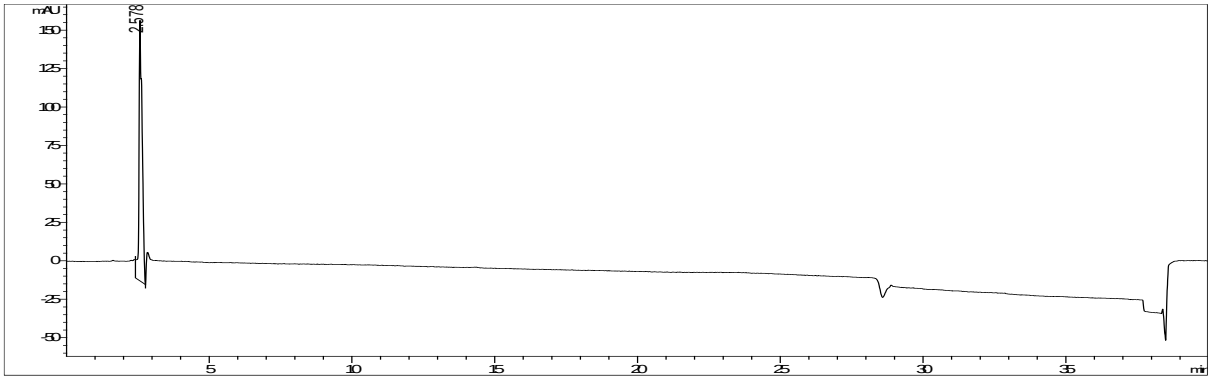

Fig. 3: HPLC chromatogram for curcumin extraction on Lempoyang

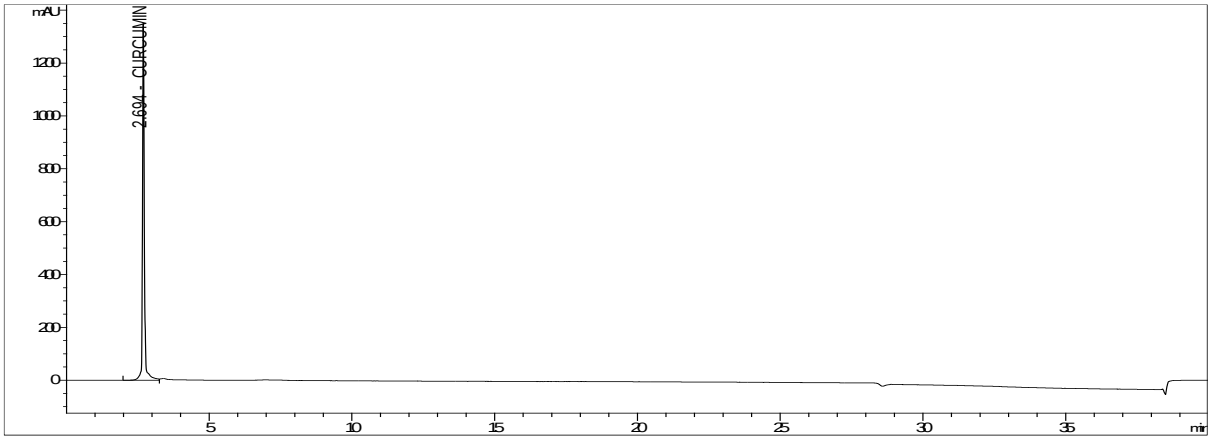

Fig. 4: HPLC chromatogram for curcumin extraction on Temu emas

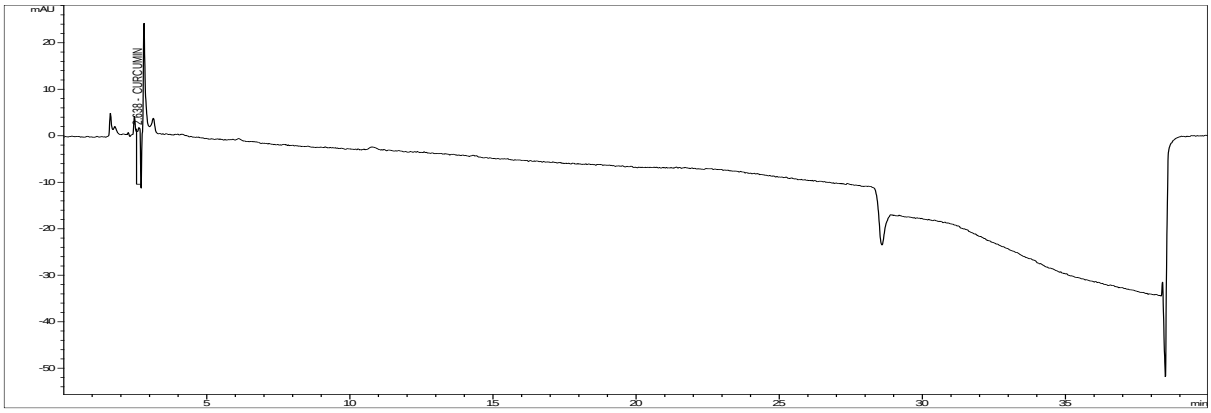

Fig. 5: HPLC chromatogram for curcumin extraction on Temu kunci

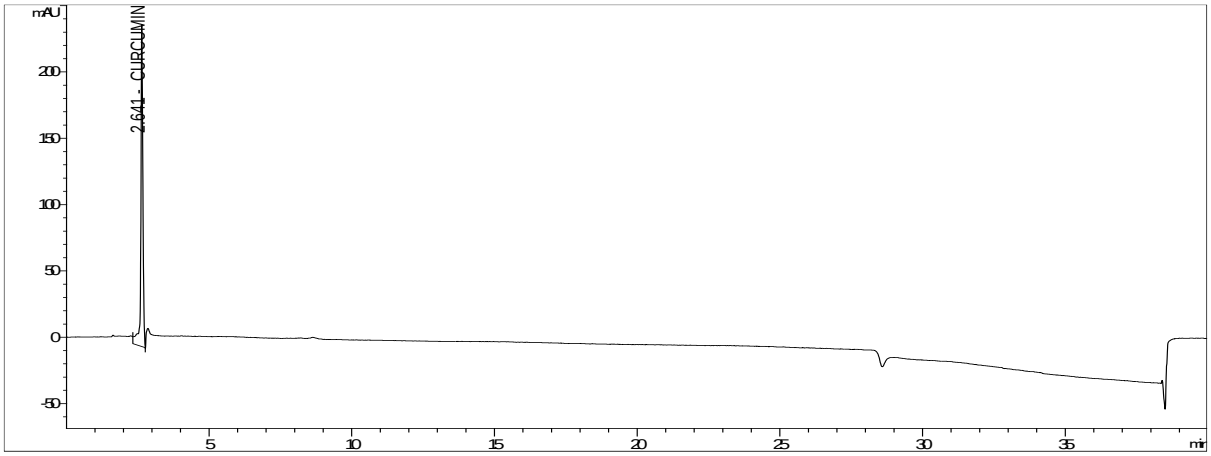

Fig. 6: HPLC chromatogram for curcumin extraction on Temu pauh

The compound extracted from each sample using the alkaline extraction method was represented by a single peak in Figures 2, 3, 4, 5, and 6. This peak was obtained from the HPLC analysis. This indicates that only one compound from these samples was successfully extracted using the method. It was found that each of the chromatograms detected the presence of the curcumin peak by comparing the peaks' retention times to the curcumin standard. The peaks of the elution were also detected at a wavelength of 425 nm, which was consistent with the results of the study by Nabati et al. (2014) on the extraction of curcumin. The type and amount of specific carotenoids, like curcumin, will depend on the activity of functional enzymes and candidate enzymes that control carotenoid biosynthesis, according to Othman et al. (2017).

4.2 Minimum inhibitory concentration (MIC)

Table 2: MIC minimum inhibitory concentrations; minimum bacteriocidal/yeastocidal/fungicidal concentration (µg / µL)

Sample	**Minimum Bacteriocidal/Yeastocidal/ Fungicidal Concentration (µg / µL)**				
	S. aureus (ATCC 25923)	*E. coli* (ATCC 35218)	*S. typhimurium* (ATCC 14028)	*C. albicans* (ATCC 10231)	*A. brasiliensis* (ATCC 16404)
Cekur (20mg/mL)	9	6	6	3	3
Lempoyang (20mg/mL)	9	6	6	6	3
Temu emas (5mg/mL)	**3**	**2**	**2**	**2**	**2**
Temu kunci (20mg/mL)	9	6	6	6	3
Temu pauh (20mg/mL)	9	6	6	6	3

The prevalence of multidrug-resistant organisms is currently rising, endangering the ability to treat an increasing number of infectious diseases. As a result, the creation of novel and potent medications to combat current pathogens that are antibiotic-resistant is urgently required (Otieno et al., 2017). The five Zingiberaceae species examined in this study have demonstrated positive antimicrobial activities against the tested microorganisms. Regarding *E. coli* ATCC 35218, *S. typhimurium* ATCC 14028, *C. albicans* ATCC 10231, and *A. brasiliensis* ATCC 16404, temu emas extracts have demonstrated excellent antibacterial activities. The extract from Temu emas was most effective against these bacteria. The *S. aureus* ATCC 25923 was only moderately inhibited by the extracts of cekur (20 mg/ml), temu kunci (20 mg/ml), and temu pauh (20 mg/ml). The most resistant to all the extracts, however, was *A. brasiliensis* ATCC 16404. By using the agar well diffusion method and broth dilution method, the antimicrobial activity of 5 species from the Zingiberaceae family was examined. In Table 2, the outcomes are displayed. Solvent extracts (5, 10, and 20 mg/ml) demonstrated measurable inhibitory activity in five different species when tested at all the various concentrations. Regarding every bacterial strain, inhibition was seen. The dilution method was used to calculate the MIC values from various species of Zingiberaceae extracts. Table 2 displays the findings. Cekur ranged from 3 to 9 (g / L), lempoyang ranged from 3 to 9 (g / L), temu emas ranged from 2 to 3 (g / L), temu kunci ranged from 3 to 9 (g / L), and temu pauh ranged from 3 to 9 (g / L) were the five species of Zingiberaceae extracts with the highest MIC values. In the case of 5 bacterial strains, the MIC values of the temu emas extracts were comparable to those of the cekur, lempoyang, temu kunci, and temu pauh extracts. Temu emas extracts were found to be more effective when diluted to a concentration of 2 (g/L), which inhibited the growth of the bacteria *E. coli* ATCC 35218, *S. typhimurium* ATCC 14028, *C. albicans* ATCC 10231, and *A. brasiliensis* ATCC 16404. The powerful antimicrobial activity of these extracts was demonstrated by the inhibition of microbial growth at concentrations as low as 2 (g/L). Traditional uses of many Curcuma species include their medicinal qualities. The antifungal, antibacterial, and anti-inflammatory activity has been reported for species like Curcuma longa, Curcuma zedoaria, Curcuma aromatica, and Curcuma amada, according to Apisariyakul et al., 1995; Mujumdar et al., 2000; Negi et al., 1999; Yoshioka et al., 1998). According to Chattopadhyay et al. (2004), curcumin that was extracted using a variety of solvents (hexane, ethanol, methanol, etc.) other than alkaline extraction has been shown to have antibacterial effects on over 24 pathogenic bacteria, including *Streptococcus*, *Staphylococcus*, *Lactobacillus*, and others. The current study supported the claimed applications of rhizomes in conventional systemic medicine for the management of a variety of infectious diseases brought on by microbes. However, more research is required to assess the potential efficacy of the crude extracts more accurately as antimicrobial agents.

5. Conclusion

In conclusion, by using the chemical extraction method, HPLC analysis of five selected Zingiberaceae species revealed that temu emas contained the highest levels of curcumin. All five Zingiberaceae extracts have demonstrated strong antimicrobial activity against the tested organisms. The antimicrobial activities of the extracts against a broader range of human pathogenic microorganisms need to be assessed and confirmed through further research. Temu emas with a concentration of 5 mg/ml demonstrated greater activity than the other extracts and generated inhibition zones that ranged from 3 to 9 (g/L). Temu emas inhibited *S. aureus* ATCC 25923 at a concentration of 5 mg/ml. All four species, cekur, lempoyang, temu kunci, and temu pauh, produced inhibition only at concentrations of 20 mg/ml, which showed inhibitory activity concentrations as high as 9 g/L for *S. aureus* ATCC 25923.

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