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Characteristics of bioactive compounds, antioxidant, and anti-microbial of ginger (*Zingiber officinale var.*) sachets against *Staphylococcus aureus* and *Escherichia coli*

[Karakteristik senyawa bioaktif, antioksidan, dan antimikroba jahe (Zingiber officinale var.) sachet terhadap Staphylococcus aureus dan Escherichia coli]

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ABSTRACT

Ginger has potential as a natural antimicrobial. Phenolic compounds such as zingerone, as well as bioactive components gingerol and shogaol, are known to contribute to ginger's biological activity. During peak harvest seasons, ginger availability increased, leading to spoilage and price declines; therefore, ginger is processed into ginger powder in sachet packaging. However, changes in the bioactive compound profile and the antioxidant and antimicrobial activities resulting from processing into powder are not well understood. This study aimed to identify bioactive compounds and to evaluate the antioxidant and antimicrobial activities of ginger powder products. The test microorganisms were Staphylococcus aureus and Escherichia coli, and the ginger powder samples were obtained from South Lampung. Bioactive compounds were identified using GC–MS, while FTIR was used to characterize functional groups. Antioxidant activity was evaluated using the DPPH assay, and antimicrobial activity was determined by the disc diffusion method. The FTIR spectrum showed absorption bands at 3853.61, 3743.68, and 3285.40 cm^{-1} (O–H), 2925.00 cm^{-1} (C–H), 1633.52 cm^{-1} (C=C), and 1005.09 cm^{-1} (NO_2), confirming functional groups characteristic of ginger. The radical scavenging activity (DPPH value) of ginger was 15.63%. The inhibition zones were classified as medium, measuring 8.53 mm against E. coli and 8.02 mm against S. aureus. GC–MS reveals major compounds suspected to contribute to antimicrobial activity included 4-(3-hydroxy-2-methoxyphenyl)butan-2-one (28.17%), n-hexadecanoic acid (24.90%), and oleic acid (13.70%).

Keywords: GC-MS, FTIR, natural antimicrobe, Ginger

ABSTRAK

Jahe berpotensi sebagai antimikroba alami. Senyawa fenolik seperti zingerone, serta komponen bioaktif gingerol dan shogaol, diketahui berkontribusi terhadap aktivitas biologis jahe. Pada musim panen raya, ketersediaan jahe melimpah sehingga sebagian rusak dan harga menurun; karena itu jahe dapat diolah menjadi bubuk jahe dalam kemasan sachet. Namun, perubahan profil senyawa bioaktif serta aktivitas antioksidan dan antimikroba akibat pengolahan menjadi bubuk belum diketahui. Penelitian ini bertujuan mengidentifikasi senyawa bioaktif, aktivitas antioksidan, dan aktivitas antimikroba pada produk bubuk jahe. Mikroba uji yang digunakan adalah *Staphylococcus aureus* dan *Escherichia coli*, dengan sampel bubuk jahe yang berasal dari Lampung Selatan. Identifikasi senyawa bioaktif dilakukan menggunakan GC-MS, sedangkan FTIR digunakan untuk karakterisasi gugus fungsional. Aktivitas antioksidan diuji menggunakan DPPH dan aktivitas antimikroba ditentukan melalui uji difusi cakram. Spektrum FTIR menunjukkan pita serapan pada 3853,61; 3743,68; 3285,40 cm^{-1} (O–H), 2925,00 cm^{-1} (C–H), 1633,52 cm^{-1} (C=C), dan 1005,09 cm^{-1} (NO_2) yang mengonfirmasi gugus fungsional khas jahe. Nilai DPPH dari aktivitas antioksidan jahe sebesar 15,63%. Zona hambat kategori sedang adalah 8,53 mm terhadap *E. coli* dan 8,02 mm terhadap *S. aureus*. Senyawa dominan hasil GC-MS yang diduga berkontribusi sebagai antimikroba meliputi 4-(3-hidroksi-2-metoksifenil)butan-2-on (28,17%), asam *n*-heksadekanoat (24,90%), dan asam oleat (13,70%).

Kata Kunci: GC-MS, FTIR, antimikroba alami, Jahe

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Introduction

Ginger is a spice plant commonly consumed in Indonesia. Ginger is very popular as a kitchen spice and brewed beverage. Ginger rhizomes provide a range of nutrients, including 79 kcal/100 g energy, 3.60 g/100 g fiber, 3.57 g/100 g protein, 1.15 g/100 g iron, 14 mg/100 g sodium, 7.7 mg/100 g vitamin C, 17.86 g/100 g carbohydrates, and 33 mg/100 g potassium (Rembet & Wowor, 2024). Ginger contains bioactive compounds such as phenolic and terpene compounds (Tang et al, 2021; Supardan et al., 2011). The phenolic compounds are mainly gingerols, shogaols, and paradols, which account for the bioactivities such as antioxidant and antimicrobial (Mao et al 2019; Sandy & Susilawati, 2021). Other compounds, alkanoid groups, have potential as antioxidants and antifungals, namely diarylheptanoids, phenylbutenoids, flavonoids, diterpenoids, and sesquiterpenoids.

In addition to its antioxidant and antimicrobial activity, the benefits of ginger are many, namely as a salient therapy (Menon et al., 2021) such as anti-inflammatory and immunomodulatory agents; anti-viral (Ulfah and Mutakin, 2017), and anti-cancer (Jagatap et al., 2022). Although it can cause kidney problems if consumed excessively (Adekunle et al., 2018), ginger can prevent obesity and metabolic syndrome, such as dyslipidemia, cardiovascular disease, and diabetes mellitus, and improve immunity (Ahmadifar et al., 2019). Ginger also give protective effect against chemotherapy-induced nausea and vomiting, as well as preventing the acute effects of anticancer drugs (Ajith et al., 2008). Ginger enhances immunity and prevents organ toxicity (An et al., 2019); prevents the effects of postoperative nausea and vomiting (PONV); exhibits antiproliferative, antitumor, antiinvasive, and antimetastatic activities (Menon et al., 2021).

Adding fresh ginger or crushing ginger and then adding it to dishes or drinks is the common way for consumption. Dried sachet ginger is very useful because it is easy to carry around and durable. Currently, the availability of ginger is very abundant and has a low price, to anticipate the abundance of ginger at harvest time, further processing is needed in the form of powder to extend the shelf life and increase the added value of ginger. The quality of ginger in each region and harvest season has different constituent components, which can be caused by environmental factors such as climate, rainfall, soil, and the type of ginger variety used (Setyawan et al., 2024; Rizky, A., & Setyawan, A. (2024).

The processing of ginger is done by drying, pulverizing, and packaging using sachet packaging. The drying process that occurs in ginger will affect the bioactive components, antioxidants, and antimicrobials in ginger. It is necessary to study in depth the effect of drying on the characteristics of dried ginger sachets. According to Sartika et al., (2022), the drying technique on ginger skin at 40–50℃ still detected bioactive components such as 2,6-dihexadecanoic acid (26.56%), oleic acid (24.08%), 9Z,12Z-octadecadienoic acid (8.27%), estra1,3,4(10)trien-17-betaol (9.63%), as well as other organic acids, phenols, flavonoids, and esters (31.46%). Therefore, drying at 40-50℃ can be applied to make dried ginger spice sachets. This study was conducted to characterize sachet-packaged ginger powder by identifying bioactive compounds using gas chromatography-mass spectrometry (GC–MS) and characterizing functional groups using Fourier-transform infrared (FTIR). Furthermore, antioxidant activity was measured via the DPPH assay, and natural antimicrobial potential was evaluated against *Escherichia coli* and *Staphylococcus aureus*.

Material and methods

Materials

The ingredients used in this research were ginger obtained from Pringsewu Market, 96% ethanol, Mac Conkey Agar (MCA), Nutrient Broth (NB), Buffered Peptone Water (BPW), Nutrient Agar (NA), DPPH, distilled water, 70% alcohol, aluminum foil, cotton, and paper discs.

Research procedure

This study used an exploratory method with a descriptive quantitative approach to the dried ginger powder sachet. All analyses were carried out with three repetitions. This study was implemented in two

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sequential phases. In the first phase, ginger was processed into a dried seasoning product packaged in sachets (BJKS), as presented in Figure 1. The preparation process included slicing ginger (approximately 1 × 1 cm), drying at 40–50°C, grinding into powder, packing into sachets, and sterilizing the final product. The second stage aimed to characterize BJKS and evaluate its inhibitory activity against the tested bacteria. Bioactive compounds were identified using GC–MS, and functional groups were characterized using FTIR.

Figure 1. Dried ginger seasoning in sachet packaging (BJKS)

GC-MS analysis

GC–MS was applied to determine the chemical profile of the extract using a Shimadzu QP2010 instrument, and the chromatographic output was reported as a total ion chromatogram (TIC). The sample was introduced into a capillary column (30 m × 0.25 mm i.d., 0.25 µm film thickness) with helium as the carrier gas at a flow rate of 1 mL/min. The injector temperature was maintained at 200°C, and the oven temperature was programmed from 50°C to 250°C at a heating rate of 10°C/min under the selected injection mode. Mass spectra were acquired over an m/z range of 50–600. Unknown constituents were assigned by matching their spectra with reference data in the NIST library (Hartari et al., 2024).

FTIR analysis

FTIR measurements were conducted using an FTIR spectrometer (FT/IR-6800) equipped with a TGS detector and an ATR accessory. Before sample analysis, a background spectrum was recorded. Ginger powder was applied directly onto the ATR crystal and pressed to ensure appropriate contact. Spectra were acquired over the range of 4000–400 cm^{-1} at a resolution of 4 cm^{-1} with 32 scans per sample. After each run, the ATR crystal was cleaned with ethanol and dried. Functional groups in the ginger powder were assigned based on the major absorption bands observed in the spectra (Pancapalaga et al., 2023)

Inhibitory activity

Antibacterial activity against *E. coli* and *S. aureus* was evaluated using the disc diffusion assay. Revived isolates of *E. coli* and *S. aureus* were subcultured on NA and incubated at 37°C for 18–24 h. Several colonies were suspended in sterile 0.9% NaCl, and the suspension turbidity was adjusted to 0.5 McFarland (≈ 1.5 × 10^8 CFU/mL) to obtain the working inoculum. NA plates were inoculated by evenly spreading the standardized suspension across the agar surface. Sterile paper discs (5.5 mm) were immersed in the extract at the designated concentration for 10–15 min, drained to remove excess solution, and then placed onto the inoculated plates. The plates were incubated at 37°C for 24 h. Inhibition zones were identified as clear halos surrounding the discs. Zone diameters were measured using a caliper in two perpendicular directions, averaged, and corrected by subtracting the disc diameter to obtain the final inhibition zone (in mm) (Sartika dkk., 2019).

Antioxidant analysis

Antioxidant capacity was determined using the DPPH radical-scavenging assay with a DPPH working solution, a blank, and the sample extract. After mixing, the solutions were incubated for 30 min, and absorbance was read at 517 nm using a UV–Vis spectrophotometer (Sukardi dkk., 2023). The percentage of inhibition was calculated by dividing the difference between the control absorbance and the sample absorbance by the control absorbance, then multiplying the result by 100%.

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Results and discussion

GC-MS reading of dried ginger sachets

Dried ginger powder sachets (BJKS) were an instant spice product intended for use in foods and beverages without the need for crushing, slicing, or grinding. The chemical constituents of BJKS were characterized using GC–MS. The chromatogram (TIC) showed identifiable peaks, with dominant peaks detected at RT 20.168, 23.899, and 26.911 min. Figure 2 presents the TIC chromatogram of BJKS. Based on Figure 2, the chromatogram shows prominent peaks for ginger constituents at retention times of 6.168, 23.899, and 26.911 min. The most abundant compounds were 4-(3-hydroxy-2-methoxyphenyl)-butan-2-one (28.17%), *n*-hexadecanoic acid (24.90%), and oleic acid (13.70%) (Figure 2; Table 2). Other constituents detected in the BJKS sample, listed according to decreasing percentage, included 1,3-cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl- [S-(R,S)]** (7.50%), cyclohexane, 3-(1,5-dimethyl-4-hexenyl)-6-methylene-, [S-(R,S)]** (6.03%), 1-(1,5-dimethyl-4-hexenyl)-4-methylbenzene (4.12%), beta-bisabolene (3.26%), glyceryl palmitate (2.97%), (E)-1-(4-Hydroxy-3-methoxyphenyl)dec-3-en-5-one (1.83%), 2,5,5,8,11-tetramethyl-4-methylene-6,7,8a-tetrahydro-4*H*,5*H*-chro (1.39%), 4-ethenyl-alpha.,4-trimethyl-3-(1-cyclohexane) methanol (1.30%), 7-epi-cis-sesquisabinene hydrate 9 (1.18%), and (1R,4R)-1-methyl-4-(6-methylhept-5-en-2-yl)cyclohex-2-enol (1.13%). The lowest-percentage compounds were beta-bisabolol (0.92%), 4-(1-hydroxyallyl)-2-methoxyphenol (0.90%), and trans-sesquisabinene hydrate (0.68%). Based on these results, the primary constituents of red ginger peel in BJKS can be grouped into butanone-related compounds, hexadecanoic acid, and oleic acid, with the highest peak area associated with the butanone derivative, indicating its potential relevance to natural antimicrobial properties. In addition, methyl hexadecanoate (methyl palmitate) has been reported to exhibit antibacterial activity, including effects related to disruption of the bacterial cell wall and membrane structure. Such activity may act synergistically with other bioactive constituents to strengthen antibacterial performance (Karunia *et al*., 2017)

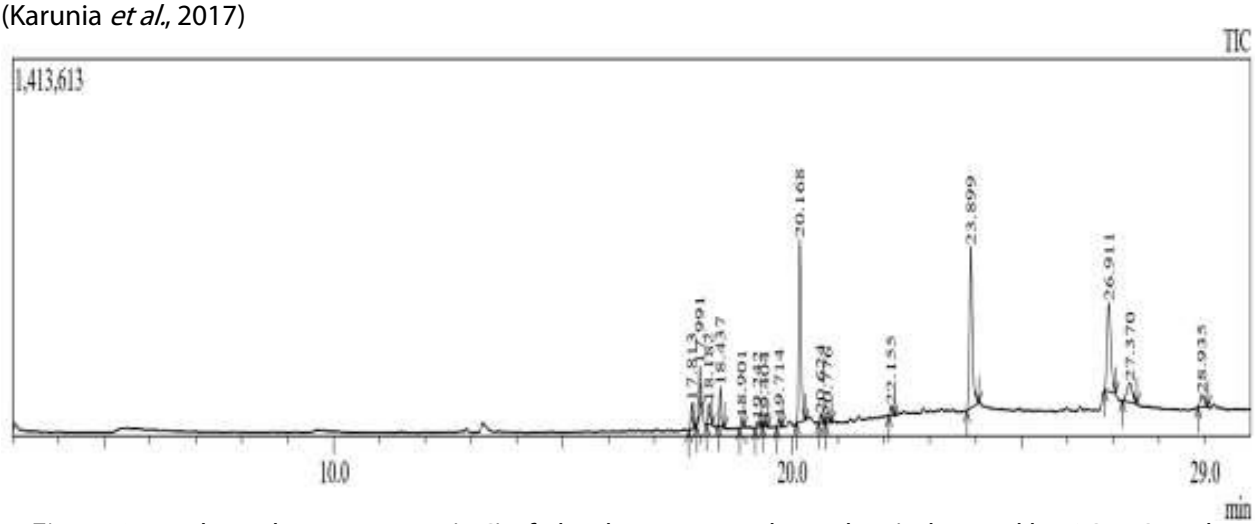

Figure 2. Total ion chromatogram (TIC) of dried ginger powder sachets) obtained by GC–MS analysis

Main compounds from the ginger GC-MS test results

The constituent compounds, formulas, molecular weights, and molecular shapes of jahe (Ginger)were summarized in Table 1. It can be seen that ginger has natural bioactive and anti-microbial components. Compoounds no 2; 5; 6; 12, 14, 18 (Table 2) have properties as anti-oxidants, while compounds number 1; 2; 4; 14; 15;18 have natural anti-microbial properties (An et al., 2019).

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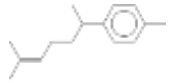
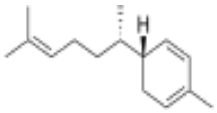
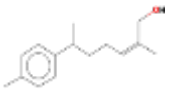
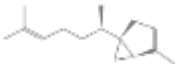
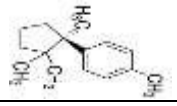
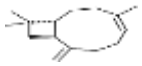
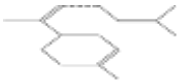
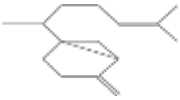
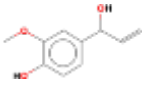
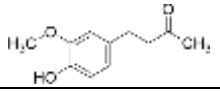
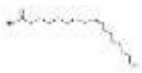
Table 1. Main Compounds from the ginger sachet

No	Compounds	Formula	Molecular weights (g/mol)	Molecular structure
1	1-(1,5-dimethyl-4-hexenyl)-4-methylbenzene	$C_{15}H_{22}$	202	
2	-1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)- 2-methyl-, [S-(R*,S*)]- - (-)-Zingiberene - l-Zingiberene - Zingiberene -.alpha.-Zingiberene	$C_{15}H_{24}$ CAS 495-61-4	204	
3	beta.-Bisabolene-Cyclohexene, 1-methyl-4-(5-methyl-1-methylene- 4-hexenyl)-, (S)- -1,5-Heptadiene, - - 6-methyl-2-(4-methyl-3-cyclohexen-	$C_{15}H_{24}$ CAS 118-655-0	204	
4	(1S,5S)-2-Methyl-5-((R)-6-methylhept-5-en-2-yl) -bicyclo[3.1.0]hex-2-ene -7-epi-Sesquithujene	$C_{15}H_{24}$ CAS 159407 359	204	
5	Cuparene	$C_{15}H_{22}$	202	
6	Isocaryophylene	$C_{15}H_{24}$	204	
7	cis-.alpha.-Bisabolene $$ 4-[(1Z)-1,5-Dimethyl-1,4- hexadienyl]-1-methyl-1-cyclohexene	$C_{15}H_{24}$	204	
8	Bicyclo[7.2.0]undec-4-ene, 4,11,11-trimethyl-8- methylene-,[1R-(1R*,4Z,9S*)]-	$C_{15}H_{24}$	204	
9	(1S,5S)-4-Methylene-1-((R)-6-methylhept-5-en-2- yl)bicyclo[3.1.0]hexa	$C_{15}H_{24}$	204	
10	Cyclohexanemethanol, 4-ethenyl-.alpha.,.alpha.,4-trimethyl-3-(1-methylethenyl)-, [1R-(1.alpha.,3.alpha.,4.beta.)]-	C15H26O C AS:639-99-6	222	
11	Formic acid, 3,7,11-trimethyl-1,6,10-dodecatrien-3- yl ester	$C_{16}H_{26}O_2$	250	
12	Nerolidyl acetate	$C_{17}H_{28}O_2$ CAS:2306-78-7	264	
13	4-(1-Hydroxyallyl)-2-methoxyphenol	$C_{10}H_{12}O_3$ CAS:112465-50-6	180	
14	Vanillylacetone /Zingerone	$C_{11}H_{14}O_3$ CAS:122-48-5	194	
15	Oleic Acid/emersol	$C_{18}H_{34}O_2$ CAS:112-80-1	282	
16	2,5,5,8a-Tetramethyl-4-methylene-6,7,8,8a- tetrahydro-4H,5H-chromen-4a-yl hydroperoxide	$C_{12}H_{22}$	238	

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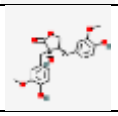
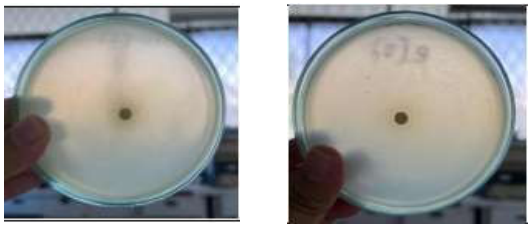
No	Compounds	Formula	Molecular weights (g/mol)	Molecular structure
17	(E)-1-(4-Hydroxy-3-methoxyphenyl)hexadec-3-en- 5-one	$C_{23}H_{36}O_3$	360	
18	Furanon (-)-Nortrachelogenin	$C_{20}H_{22}O_7$	374	

Antioxidant Activity (DPPH)

Antioxidant testing of the ginger powder sachet produced an antioxidant activity value of 15.63%. The DPPH scavenging activity obtained in the present study is consistent with the findings of Al-Baarri et al. (2025), who reported that the addition of ginger extract to a food matrix increased antioxidant activity and total phenolic content, suggesting that ginger-derived ingredients can provide quantifiable antioxidant-related benefits in processed products. The antioxidant activity of ginger is mainly attributed to its phenolic compounds, particularly gingerols and shogaols (Mao et al., 2019).

Microbial inhibitory activity

Figure 3 demonstrates that red ginger peel extract exhibits natural antimicrobial activity against *E. coli*, as indicated by the presence of a clear inhibition zone (bacteria-free area) surrounding the paper disc. As shown in Figure 3, the smallest inhibition zones were observed at the 10% extract concentration, measuring 8.02 mm against *S. aureus* and 8.53 mm against *E. coli*. According to Surjowardojo *et al.* (2015), inhibition zones of <5 mm in the agar diffusion assay are classified as weak activity, whereas zones of 5–10 mm are categorized as moderate (Ginger extract has antimicrobial effects, but not as strong as standard antibiotics).



Staphylococcus aureus
d = 8.02mm

Escherichia coli
d = 8.53 mm

Figure 3. Ginger inhibition zone by the disc method

One of the identified constituents, such as zingerone, may have contributed to the inhibition of *S. aureus* and *E. coli* because it is a phenolic compound containing hydroxyl (–OH) and carbonyl (C=O) functional groups associated with antibacterial activity (Larijanian et al., 2024; .Yit & Zainal-Abidin, 2024) Phenolics can interact with bacterial proteins to form phenol–protein complexes; because these interactions are relatively weak, free phenolic molecules may penetrate the cell and cause protein precipitation and denaturation. At higher concentrations, phenolics may induce protein coagulation and disrupt the cell membrane (Ali et al., 2013). This mechanism was consistent with the disc diffusion results, where clear inhibition zones were formed around the discs (Figure 3).

FT-IR test

FTIR spectroscopy is a rapid, simple, and non-destructive analytical technique that reveals and displays all chemical properties of a sample in the FTIR spectrum. Based on this spectrum, compound identification can be conducted both qualitatively and quantitatively. The FTIR reading results for ginger are summarized in Table 2 and Figure 4.

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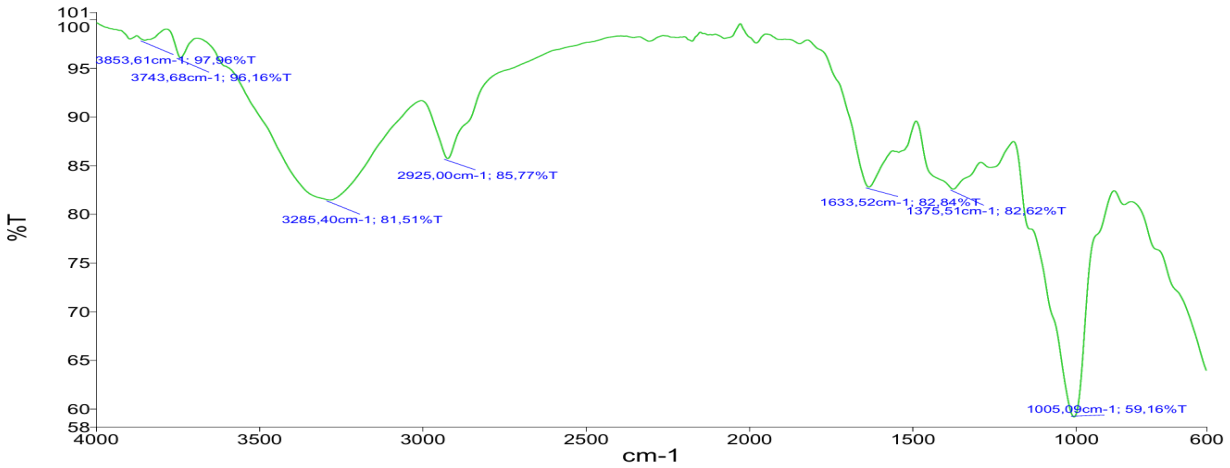

Figure 4. FT-IR results of ginger extract

Analysis of the results of FTIR testing on ginger shows results at a wavenumber of 3853.61 cm^{-1} (97.96 %); 3743.68 cm^{-1} (96.16 %); and 3285.40 cm^{-1} (81.51 %), which were assigned to O–H stretching vibrations of hydroxyl-containing compounds (phenolics/alcohols) (Table 2). This was consistent with the ginger reference spectrum reported by Styawan et al. (2023), where the O–H stretching band appeared around 3287 cm^{-1}. The broad nature of O–H absorption is commonly associated with hydrogen-bond interactions in hydroxyl groups (Subamia dkk., 2023). The band at 2925.00 cm^{-1} (85.77%) corresponded to aliphatic C–H stretching, which matched the reference band at ~2925 cm^{-1} reported by Styawan et al. (2023).

At wavenumber 1633.52 cm^{-1} (82.84%) detected that ginger has C=C bonds with alkene compound types with varying intensity (Table 2). Peaks at 1375.51 cm^{-1} (82.62 %) and 1005.09 cm^{-1} (59.16%) shows NO_2 group with strong intensity. Styawan et al. (2023) reported bands around ~1371 cm^{-1} (commonly associated with $–CH_3$ bending) and ~998 cm^{-1} (often assigned to C–OH/C–O stretching). According to Subamia dkk (2023), the wavenumber range of approximately 1100 to 1000 cm^{-1} corresponds to monosubstituted aromatic compounds. The functional groups measured by FTIR spectroscopy with each specific wavenumber show that they are compatible with the ginger group, namely having O–H, C–H, C=C, and NO_2. It can be seen that ginger has antioxidant characteristics (O–H and C=C groups) and has the potential to act as a natural anti-microbial (C–H and NO_2 groups).

Table 2. Ginger compound data by FT-IR test

No.	Functional Groups	Results	
		Wavenumber (cm^{-1})	%Transmittance
1	O–H phenol, alcohol bonding hydrogen	3853.61	97.96
2		3743.68	96.16
3		3285.40	81.51
4	C–H alkane (strength)	2925.00	85.77
5	NO_2 Nitro (strength)	1633.52	82.84
6		1005.09	59.16

Conclusion

These findings indicate that ginger powder sachets contain identifiable bioactive compounds. Ginger powder sachets exhibited moderate antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, producing inhibition zones of 8.02 mm and 8.53 mm, respectively. GC–MS analysis revealed that the predominant constituents were 4-(3-hydroxy-2-methoxyphenyl)-butan-2-one (28.17%), *n*-hexadecanoic acid (24.90%), and oleic acid (13.70%), which are considered to contribute to the antimicrobial potential of the product. Antioxidant capacity determined using the DPPH assay yielded 15.6334%. FTIR characterization further supported the presence of functional groups typically associated with ginger compounds, indicated by absorption bands at 3853.61, 3743.68, and 3285.40 cm^{-1} (O–H), 2925.00 cm^{-1} (C–H), 1633.52 cm^{-1} (C=C), and 1005.09 cm^{-1} (NO_2). These results confirm that ginger powder sachets contain identifiable bioactive components with measurable antibacterial and antioxidant activities.

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