

## RESEARCH ARTICLE

# Effect of Methyl Jasmonate and Plant Growth Promoting Rhizobacteria (PGPR) on Antioxidant Potential and Metabolomic Profiles of Zingiberaceae Rhizomes

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**Abstract:** The rhizomes of Zingiberaceae plants are used as cooking spices, medicines, cosmetics and beverage ingredients. Zingiberaceae rhizomes have the potential to be immunostimulants, antioxidants and immunomodulators. Antioxidants are chemical compounds that bind free oxygen radicals and prevent free radicals from damaging healthy cells. This research aimed to analyze the antioxidant potential and metabolomic compounds of the rhizomes of the Zingiberaceae tribe using GC-MS after the application of the elicitor methyl jasmonate and Plant Growth Promoting Rhizobacteria (PGPR). Experimental method research with stages: 1) Collection of rhizomes of the Zingiberaceae family, Genus *Curcuma*; 2) Preparation of experimental land and fertilization with Petroganik organic fertilizer, 3) Planting *Curcuma* rhizomes; 4) Application of elicitor methyl jasmonate 60 ppm at 25 and 50 days after planting (DAP), 5) Application of PGPR 10 mL/L water at 30 and 60 DAP. Antioxidant activity testing using a spectrophotometer with DPPH solution. Metabolomic profile analysis using GC-MS. The GC-MS results, 3 with the highest area (%) of *Curcuma xanthorrhiza* rhizomes, obtained 18 metabolite compounds, namely 1-Naphthaleneethanol, Ethyl orthoformate and Cyclopentanol. The GC-MS results of *Curcuma longa* rhizomes obtained 17 metabolite compounds: Turmerone, Curlone and Acetylene. The GC-MS results of *Curcuma caesia* rhizomes obtained 21 metabolite compounds: Isoprene, Imidazol-4-one and 3-ethenyl-3-ethylcyclopentanone. The GC-MS results of *Curcuma zedoria* rhizomes yielded 15 metabolite compounds, namely 3,4,5-trimethylphenol, Naphthalene, and Acetic acid. Based on the DPPH test, the IC<sub>50</sub> values mg/L ± SD (category) for the control group were *Curcuma xanthorrhiza* 51.02 ± 4.53 (strong), *Curcuma longa* 124.06 ± 3.71 (medium), *Curcuma zedoria* 112.46 ± 3.145 (medium), and *Curcuma caesia* 154.18 ± 3.629 (weak). The IC<sub>50</sub> values (mg/L) for the treatment group were *Curcuma xanthorrhiza* 31.50 ± 3.373 (very strong), *Curcuma longa* 97.20 ± 2.940 (strong), *Curcuma zedoria* 88.18 ± 2.924 (strong), and *Curcuma caesia* 143.69 ± 3.500 (medium). The results of the t-test, between the control and treatment groups of *Curcuma xanthorrhiza*, *Curcuma longa*, *Curcuma zedoria* (sig. 0.001), *Curcuma caesia* (sig. 0.004), sig value <0.05, then it is stated that the IC<sub>50</sub> value between the control and treatment groups is different. Application of methyl jasmonate and PGPR elicitors increased antioxidant potential.

**Keywords:** Zingiberaceae Family, Antioxidants, Metabolomics

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## Introduction

Zingiberaceae is the most prominent family in the order Zingiberales, comprising perennial herbs with around 1400 species that grow in tropical to subtropical areas [1]. These plants are characterized as aromatic herbaceous plants with rhizome roots. Zingiberaceae rhizomes are utilised in Indonesia for culinary purposes, medicinal applications, as cosmetic ingredients, and as ingredients in beverages [2]. The chemical constituents of Zingiberaceae rhizomes include flavonoids, phenols, terpenoids, essential oils, and potential biopharmaceuticals [3,4]. Zingiberaceae rhizomes have the potential to serve as immunostimulants and immunomodulators as well as antioxidants [5–7].

Antioxidants are chemical compounds that bind to free oxygen radicals, preventing them from damaging healthy cells [8]. Antioxidants are categorized into two types: natural antioxidants and synthetic antioxidants. Some examples of synthetic antioxidants commonly used in food are Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT) [9]. Long-term use of synthetic antioxidants can be toxic, so safe natural antioxidants are needed [10]. Natural antioxidants are found in fruits, vegetables, and grains and are rich in vitamins E, C, A, phenolic acids, flavonoids, and carotene. One source of natural antioxidants is the rhizome of the Zingiberaceae genus, specifically *Curcuma*, which contains various bioactive components that can combat free radicals.

Antioxidant testing can be done using the DPPH (2,2-Diphenyl-1-Picrylhydrazyl) method. The basis of the DPPH reaction is the capture of hydrogen from antioxidants by DPPH free radicals, which are converted into 2,2-diphenyl-1-picrylhydrazyl. Activity is expressed as IC<sub>50</sub>, which represents the minimum concentration required to inhibit 50% of free radicals. The use of DPPH in antioxidant tests has been carried out on *Solanum Lycopersicum* [11], *Rhizopora apiculata* [12], *Berberchia discolor* [13], *Citrus reticulata* [14], and *Foeniculum vulgare* [15].

Research on antioxidant potential in the *Curcuma* genus has been carried out on *Curcuma xanthorrhiza* [16,17], *Curcuma longa* [18,19], *Curcuma zedoria* [20,21] and *Curcuma caesia* [22–24]. The rhizomes of the *Curcuma* genus have moderate to very strong antioxidant activity.

Phytochemical characteristics and antioxidant properties can be induced with the elicitor methyl jasmonate and Plant Growth Promoting Rhizobacteria (PGPR). Research on the potential of methyl jasmonate to induce phytochemical compounds was carried out by Acikgoz et al. on *Achillea gypsicola* plants [25], Sanchez et al. on suspension culture of *Piper cumanense* [26], Nguyen et al. on root culture of *Centela asiatica* [27], and Rampe et al. (2023) on *Ipomoea batatas* [28].

Regarding phytochemicals, jasmonic acid is a natural compound synthesized by plants in response to pathogen attack and external damage [26,29,30]. Sun et al. found methyl jasmonate as an inducer of triterpenoid biosynthesis in *Inonotus baumii* [31]. Research by Saeed et al. found that applying the elicitors methyl jasmonate and phenylacetic acid increased the total phenolic and flavonoid content in the root suspension of *Ajuga bracteosa* [32].

PGPR can act as biofertilizers, biopesticides, and bioprotectants, stimulating plant growth. PGPR can increase plant growth through various mechanisms such as solubilization of phosphorus, nitrogen fixation, production of cyanide acid Compounds (HCN), and Indole Acetic Acid (IAA) compounds [33]. Many PGPRs produce the Hormones IAA, gibberellin and cytokinins, influencing plant growth and architecture [34,35]. The application of Methyl jasmonate and PGPR can increase secondary metabolites, such as flavonoids, which possess antioxidant potential.

## Materials and Methods

Research activities were carried out in experimental gardens located in Tomohon City, North Sulawesi Province and the advanced biology laboratory of the Biology Department at Sam Ratulangi University Indonesia. GC-MS analysis at the North Sulawesi Regional Police Forensic Laboratory, in 2024.

### Research Procedures

The research was conducted as an experimental study consisting of two groups, namely control and methyl jasmonate and PGPR treatment, with three replications. The experimental plot measured 4x4m<sup>2</sup> and was planted with 16 plants:

1. Collection of plants from the *Curcuma* genus, namely *Curcuma Zanthorrhiza*, *Curcuma longa*, *Curcuma zedoria* and *Curcuma caesia*, in the form of saplings from farmers in Tomohon City

2. Preparation of experimental land and Petroganic fertilization
3. Planting a collection of Curcuma Genus plants
4. Application of methyl jasmonate elicitor

Methyl jasmonate was applied at 60 ppm at 25 and 50 Days after Planting (DAP). The methyl jasmonate concentration is used by research results [36]. PGPR was applied at 30 and 60 DAP. Application of PGPR 10 mL/L water at 30 and 60 DAP. PGPR comes from the Center for Protection and Quality Testing of Food Crops and Horticulture in Minahasa Regency, North Sulawesi. Bacteria in PGPR: Flavobacterium sp., Acetobacter sp., Paracoccus sp., Serratia sp., Arthrobacter sp. Application of treatment by spraying on the stems and leaves.

## Fresh Rhizomes of the Genus Curcuma Were Crushed and Extracted

The rhizomes of each type of plant are crushed and then squeezed with a filter cloth; then, the extract is filtered with filter paper to obtain juice.

## Antioxidant Activity Testing

Each test sample was made into a stock solution of 500 ppm by weighing the sample 0.005 g and then dissolving it in 10 ml methanol to obtain a sample of 500 ppm. Next, the mother liquor was diluted to 20, 40, 60, 80 and 100 ppm. Antioxidant activity testing used a spectrophotometer, DPPH (1,1-diphenyl-2-picrylhydrazyl) solution, and ascorbic acid as a positive control. Extract 2 ml of the sample and add 2 ml of DPPH; the sample is incubated for 30 minutes in dark conditions. Antioxidant activity was determined by decolourising DPPH at a wavelength of 517 nm using a UV-Vis spectrophotometer. Through a linear equation that states the relationship between the concentration of the test extract (x) and the free radical scavenging activity (y). The antioxidant activity of the sample extract was determined based on the percentage of inhibitory power relative to the control using the equation of Handayani et al. [37]:

## The Level of Antioxidant Power Using the DPPH Method

IC<sub>50</sub> value (ppm) <50 (very strong), 50-100 (strong), 100-150 (medium), 150-200 (weak) and >200 (very weak) [38].

The IC<sub>50</sub> value is a number that shows the concentration of the sample being tested which is able to inhibit oxidation by 50%. The percentage of antioxidant activity was graphed between the concentration and the average % of antioxidant activity to obtain the regression value  $y = bx + a$ . After that, the IC<sub>50</sub> (x) value is calculated using the equation:

$$IC_{50} = \frac{50 - a}{b}$$

Metabolomic compound analysis using the GC-MS version of Agilent 8890 GC System. The column used was DB-5MS UI (length 30 m, diameter 0.25 mm, film 0.25  $\mu$ m), injector temperature 250°C. The sample used was a fresh extract solution. The sample extract was dissolved in methanol (1:1), and 2  $\mu$ L was injected into the GC-MS.

## Statistical Analysis

To determine the difference in IC<sub>50</sub> values between the control and treatment groups for each type of curcuma, a t-test was performed at the 5% significance level.

## Research Results

The results of filtering fresh extracts of Curcuma xanthorrhiza, Curcuma longa, Curcuma zedoria and Curcuma caesia were then injected into the GC-MS instrument. All these areas were then identified qualitatively by comparing the spectrum data obtained with the spectrum data in the data bank. Next, look for the compound chain in PubChem and copy the chain obtained to way2drug.com to determine the potential of the metabolite compound. The GC-MS chromatography results show diversity in base-peak chromatograms. The chromatography results for the four plants are shown in Figures 1-4 and Tables 1-4.

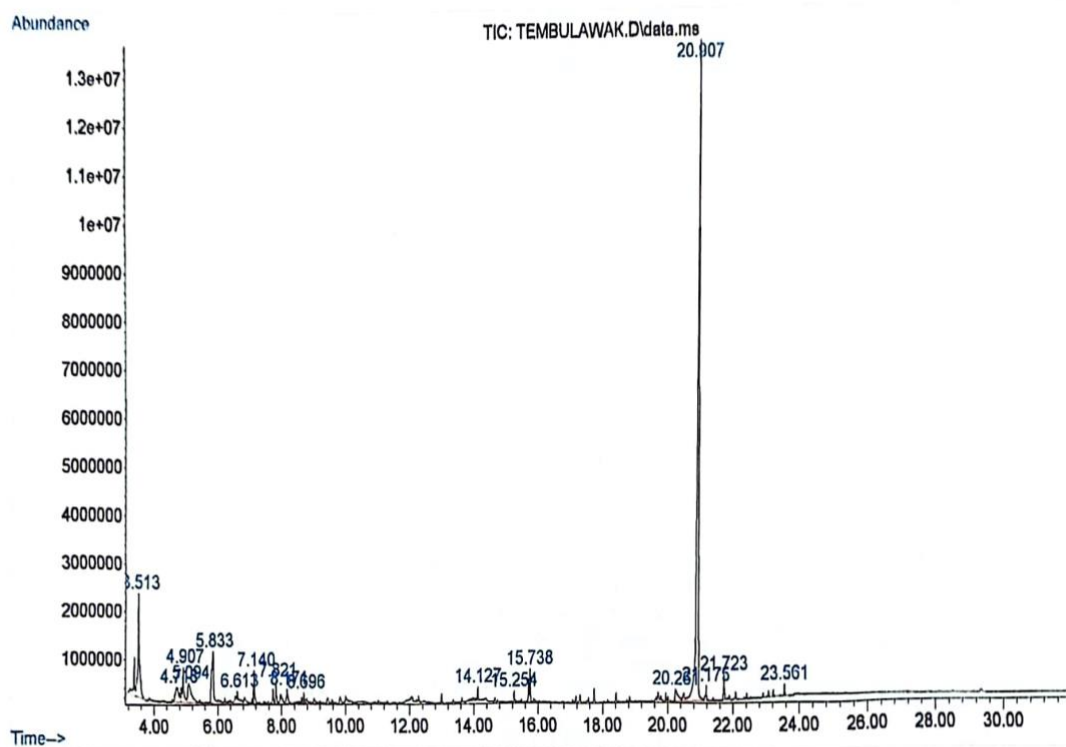


Fig. 1: GC-MS of methanol extract of Curcuma

Table 1: Bioactive compounds of *Curcuma xanthorrhiza* methanol extract

Number	rt	Area (%)	Compound Name
1.	3.513	10.65	Ethyl orthoformate
2.	4.718	2.67	Isoxazolidine
3.	4.907	2.95	2-Hydroxy-2-cyclopenten-1- one
4.	5.094	3.96	Beta-Myrcene
5.	5.833	6.15	Cyclopentanol
6.	6.613	2.79	3-Pentanone
7.	7.14	2.09	Cyclobutanol
8.	7.821	2.26	4H-Pyran-4-one
9.	8.171	1.24	1,2-Ethanediamine
10.	8.696	2.16	2,4-Dimethyl-3-pentanone
11.	14.127	1.09	2-Butanone
12.	15.254	0.71	Ethyl p-methoxycinnamate
13.	15.738	2.7	Ambrial
14.	20.261	2.14	Naphthalene
15.	20.907	53.3	1-Naphthaleneethanol
16.	21.175	0.99	15-Oxosteviol methyl ester
17.	21.723	1.17	1-Naphthyl phosphoramidate
18.	23.561	0.99	2,5-cyclohexadiene-1,7

The GC-MS analysis of *Curcuma xanthorrhiza* rhizomes detected 18 metabolite compounds (Fig.1), three compounds that dominated with the highest area (%) namely 1-naphthaleneethanol, retention time (rt) 20,907, area 53.30%; ethyl orthoformate, rt 3.513 area 10.65%; cyclopentanol, rt 5.833 area 6.15%.

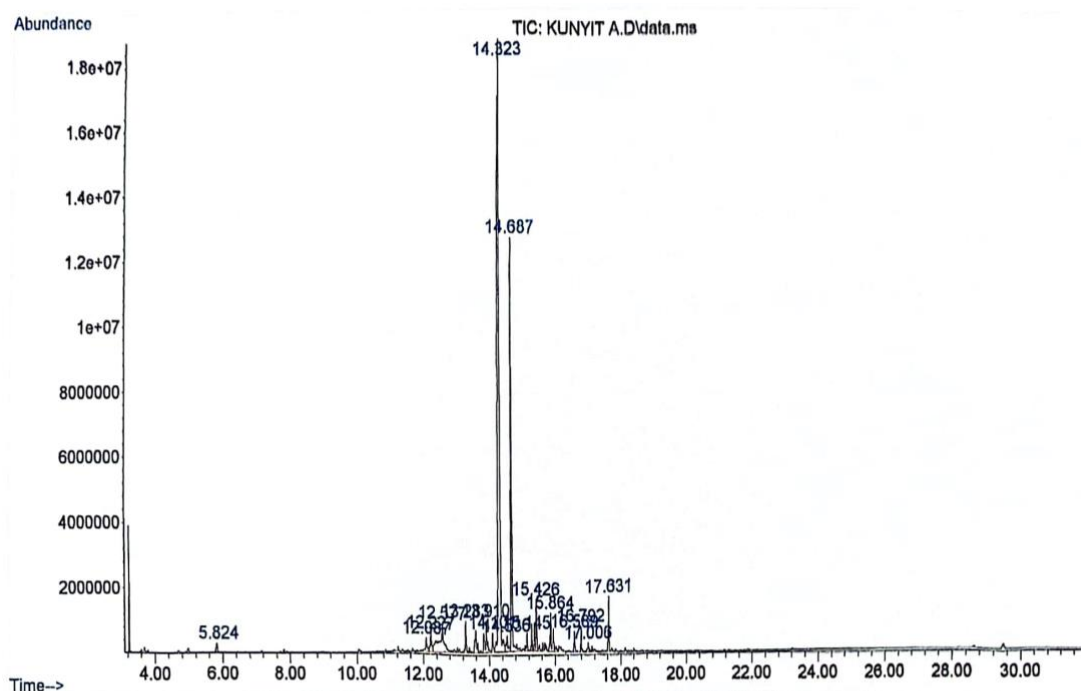


Fig. 2: GC-MS of methanol extract of *Curcuma*

Table 2: Bioactive compounds of *Curcuma longa* methanol extract

Number	rt	Area (%)	Compound Name
1.	5.824	0.54	2-hydroxy-gamma- butyrolactone
2.	12.087	1.08	Benzene
3.	12.227	1.32	1,3-Cyclohexadiene
4.	12.577	6.29	Cyclohexene
5.	13.283	4.32	Benzene
6.	13.91	2.78	Indolizine
7.	14.101	0.96	Bisabolene epoxide
8.	14.323	49.97	Turmerone
9.	14.536	1.35	5-Deuterioimidazo
10.	14.687	17.41	Curlone
11.	15.145	0.86	Bisabolene
12.	15.426	4.75	Benzene
13.	15.864	3.11	Methyl 2-cyclopropylacetate
14.	16.589	0.92	Silane
15.	16.792	0.93	2-Pyrazoline
16.	17.006	1.16	Carvyl angelate
17.	17.631	2.25	Acetylene

The GC-MS analysis of *Curcuma longa* rhizomes detected 17 metabolite compounds (Fig. 2), and three compounds dominated with the highest area (%), namely turmerone, rt 14,323 area 49.97%; curlone rt. 14,687 area 17.41%, Cyclohexene, rt. 12.577 area 6.29 %.

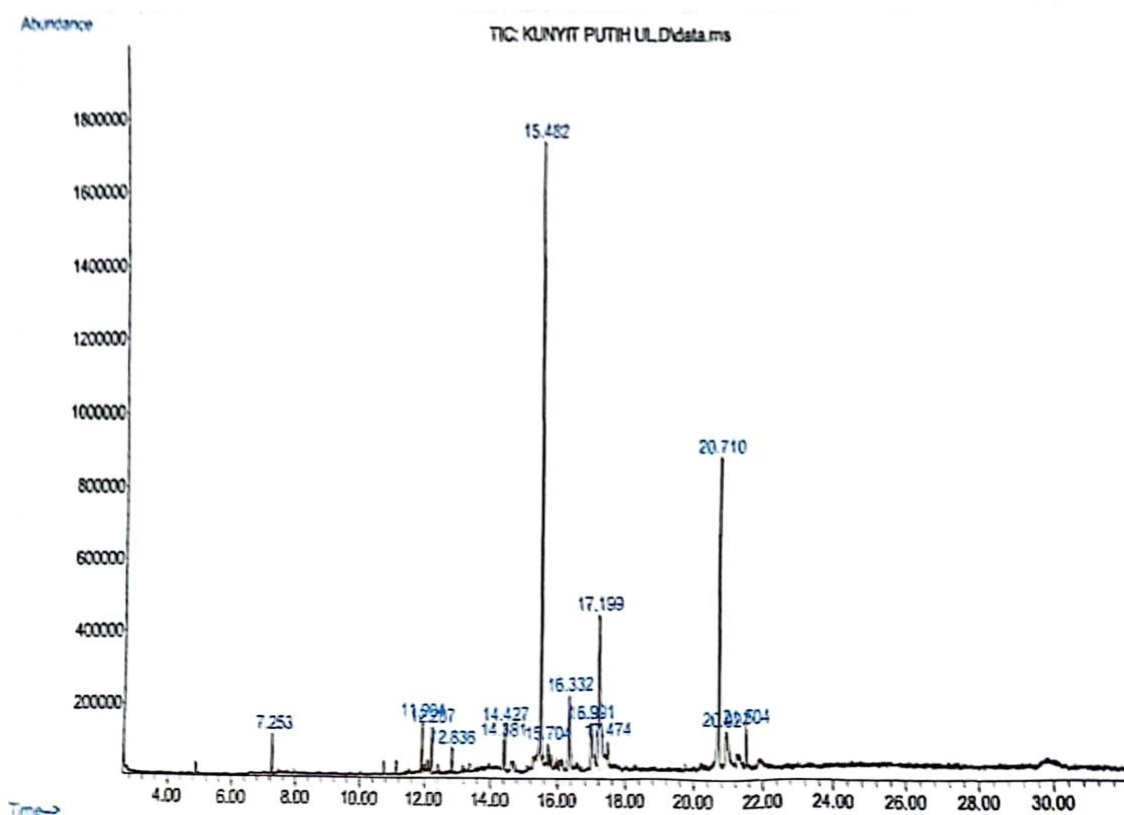


Fig. 3: GC-MS of methanol extract of *Curcuma*

Table 3: Bioactive compounds of *Curcuma zedoria* methanol extract

Number	rt	Area (%)	Compound Name
1.	7.253	2.04	2-Bornanone
2.	11.904	2.25	1H-Cyclopropa naphthalene
3.	12.207	2.38	1,3 Pentadiene
4.	12.836	1	11 Naphthalene
5.	14.381	1	36 Phenol
6.	14.427	1.76	1,5 Cyclooctadiene
7.	15.482	30.17	3,4,5-Trimethylphenol
8.	15.704	1.22	Cyclopentane
9.	16.332	7.04	Benzeneacetaldehyde
10.	16.991	5.38	Glutaric acid
11.	17.199	19.28	Acetic acid
12.	17.474	1.68	Methyl ester
13.	20.71	17.61	Naphthlene
14.	20.927	4.75	Acetic acid
15.	21.504	1.97	Benzene

The GC-MS analysis of *Curcuma zedoria* rhizomes detected 15 metabolite compounds (Fig. 3), three compounds that dominated with the highest area (%), namely 3,4,5-trimethylphenol, rt 15,482 area 30.17%; Acetic acid, rt. 17,199 area 19.28%, Naphthlene, rt. 20,710 area 17.61%;

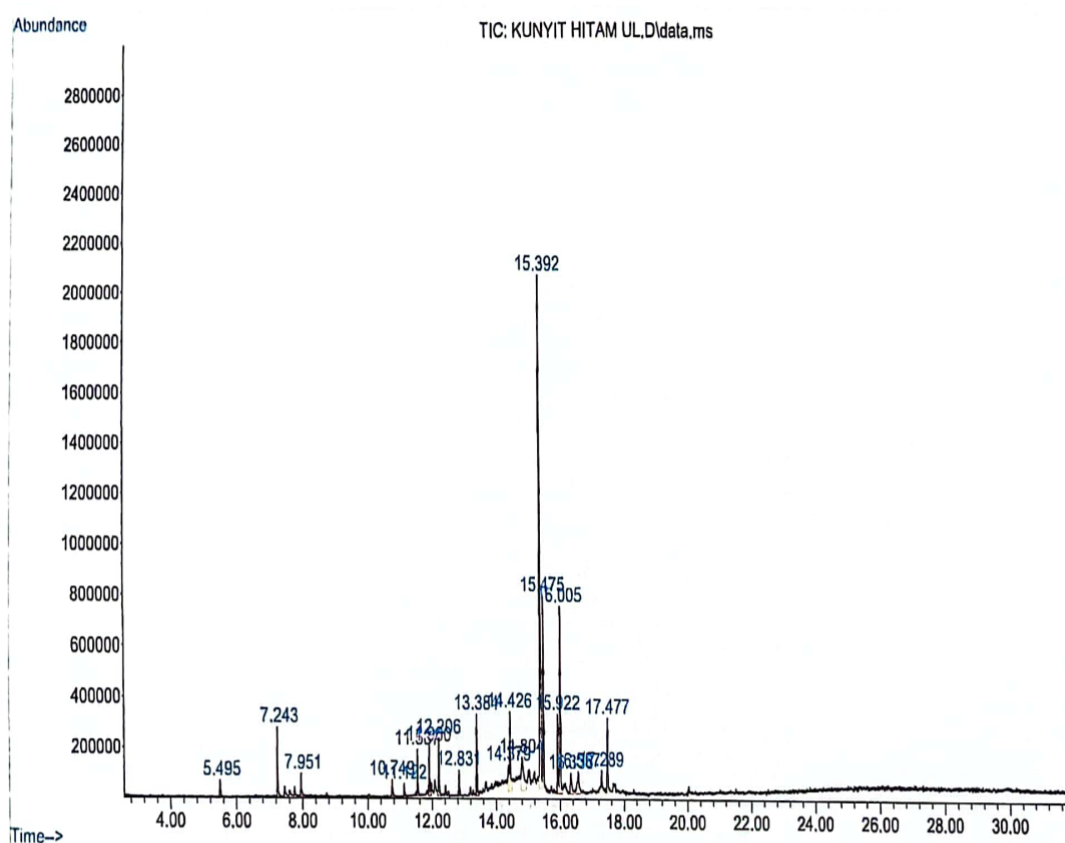


Fig. 4: GC-MS of methanol extract of *Curcuma caesia* rhizomes

Table 4: Bioactive compounds of *Curcuma caesia* methanol extract

Number	rt	Area (%)	Compound Name
1.	5.495	1.08	Eucalyptol
2.	7.243	3.56	2-Bornanone
3.	7.951	1.23	Alpha-terpineol
1.	10.749	1.06	1.3Pentadiene
2.	11.122	0.77	2-Carene
3.	11.537	2.4	Cis-beta-farnesene
4.	11.9	2.78	Benzene
5.	12.206	3.23	Isoprene
6.	12.831	1.34	Gamma-elemene
7.	13.384	3.95	Epicurzerenone
8.	14.37 9	1.94	Ethanone
9.	14.426	4.56	3.7-cyclodecadien-1-one
10.	14.804	5.72	Norbelladine
11.	15.392	30.34	Isoprene
12.	15.475	9.77	Imidazol-4-one

13.	15.922	4.01	Cyclododeca-5
14.	16.005	11.7	3-Ethenyl-3-ethylcyclopentanone
15.	16.33	1.92	Diethyl aminoalonnate
16.	16.557	2.44	Benzoic acid
17.	17.289	2.05	Coumarin-6-ol
18.	17.477	4.15	Benzaldehyde

The GC-MS analysis of *Curcuma caesia* rhizomes detected 21 metabolite compounds (Fig. 4), three compounds that dominated with the highest area (%) namely Isoprene, rt. 15,392 area 30.34 %; 3-ethenyl-3-ethylcyclopentanone, rt.16.005 area 11.70 %, Imidazole-4-one rt. 15,475 area 9.77 %;

## Antioxidant Potential

The IC<sub>50</sub> value is a number that shows the concentration of the sample being tested which is able to inhibit oxidation by 50%. The lower the IC<sub>50</sub> value, the higher the free radical scavenging activity of DPPH, which shows that at this concentration it is able to neutralize up to 50% of free radicals. The IC<sub>50</sub> values mg/L  $\pm$  SD (category) for the control group were *Curcuma xanthorrhiza* 51.02  $\pm$  4.53 (strong), *Curcuma longa* 124.06  $\pm$  3.71 (medium), *Curcuma zedoria* 112.46  $\pm$  3.145 (medium), and *Curcuma caesia* 154.18  $\pm$  3.629 (weak). The IC<sub>50</sub> values (mg/L) for the treatment group were *Curcuma xanthorrhiza* 31.50  $\pm$  3.373 (very strong), *Curcuma longa* 97.20  $\pm$  2.940 (strong), *Curcuma zedoria* 88.18  $\pm$  2.924 (strong), and *Curcuma caesia* 143.69  $\pm$  3.500 (medium).

The results of the t-test, between the control and treatment groups of *Curcuma xanthorrhiza* (sig. 0.001), *Curcuma longa* (sig. 0.001), *Curcuma zedoria* (sig. 0.001), *Curcuma caesia* (sig. 0.004), sig value <0.05, then it is stated that the IC<sub>50</sub> value between the control and treatment groups is different.

## Discussion

Metabolomics is the systematic study of global metabolite profiles in biological samples. Metabolomics analysis is an approach used to determine metabolite profiles. Untargeted GC-MS is considered an appropriate method in analyzing metabolites in liquid samples. The diversity of chromatograms obtained in the Genus *Curcuma* shows differences in specific phenotypic and genotypic characteristics, and can also be influenced by external factors. The GC-MS results of *Curcuma xanthorrhiza*, *Curcuma longa*, *Curcuma zedoria* and *Curcuma caesia* obtained different amounts and types of metabolites. Curcumin, benzene and naphthalene group compounds are found in several *curcuma* genera.

The diversity of chromatograms obtained in the Genus *Curcuma* shows differences in specific phenotypic and genotypic characteristics, and can also be influenced by external factors. The application of elicitors can increase growth including the synthesis of potential antioxidant compounds. The results of research by Mendoza et al. showed that the application of 300  $\mu$ M salicylic acid and 3  $\mu$ M methyl jasmonate increased the content of enolic compounds and flavonoids, and induced the phenylpropanoid metabolite pathway [39]. Jasmonic acid and salicylic acid can stimulate the production of flavonoids and polyphenols in cell suspension, callus and cell culture [40].

Antioxidant activity is known from the IC<sub>50</sub> value. The IC<sub>50</sub> value is inversely proportional to the antioxidant content. A material that has a small IC<sub>50</sub> value indicates that the material has a large antioxidant content because IC<sub>50</sub> represents the sample concentration required to reduce 50% of the absorbance of a reducible DPPH solution. Genus *Curcuma* plants have different antioxidant potentials from medium to very high categories.

The results of the antioxidant potential test for the *Curcuma* rhizome treatment group showed a decrease in IC<sub>50</sub> values for all rhizomes tested. This indicates an increase in antioxidant potential. The IC<sub>50</sub> values (control and treatment groups) were *Curcuma xanthorrhiza* (51.02 and 31.50 (mg/L), *Curcuma longa* (124.06 and 97.20 (mg/L), *Curcuma zedoria* (112.46 and 88.18 (mg/L), and *Curcuma caesia* (154.18 and 143.69 (mg/L). The decrease in IC<sub>50</sub> values was 19.52, 26.86, 24.28 and 10.49 (mg/L), respectively. The most significant reduction in IC<sub>50</sub> values was in *Curcuma longa*. The antioxidant potential is closely related to the content of secondary metabolites in *curcuma* rhizomes, which are induced after the application of methyl jasmonate and PGPR. The IC<sub>50</sub> value in *Curcuma* can provide different results due to other test methods, sample sources, types of extracts and analysis procedures.

The use of methyl jasmonate and PGPR elicitors can trigger physiological and morphological responses, phytoalexin accumulation, and increase the synthesis of secondary metabolites. The results of Mendoza et al. study showed that the application of 3  $\mu\text{M}$  methyl jasmonate increased the content of phenolic and flavonoid compounds and induced the phenylpropanoid metabolite pathway. PGPR functions as: 1) a biostimulant by synthesizing and regulating the concentration of various phytohormones, such as Indole Acetic Acid (IAA), gibberellins, cytokinins, and ethylene in the root environment. 2) as biofertilizers by a symbiotically fixing  $\text{N}_2$  from the air and dissolving P nutrients bound in the soil; and 3) as bioprotectants by producing various antipathogenic compounds or metabolites [33,39].

Antioxidant properties are closely related to metabolite compounds [13,41]. Some classic non-enzymatic antioxidants are vitamins such as vitamin C or E, but flavonoids and other phenolic compounds are also potent antioxidants. According to Puspitasari, alkaloids act as antioxidants because they contain nitrogen atoms in their structure, which have lone electron pairs that neutralize free radical activity in the body [42]. Furthermore, tannins with OH groups whose hydrogen atoms can be donated to free radicals, produce non-radical compounds (DPPH-H). According to research by Kusuma, triterpenoids have antioxidant activity because they are included in the class of phenolic compounds that contain an OH group that is directly bound to the aromatic hydrocarbon ring [43]. The antioxidant mechanism of phenolic compounds is based on oxidation-reduction reactions. Flavonoids can act as antioxidants by donating hydrogen ions so that they can neutralize the toxic effects of free radicals by capturing free radicals. Tannin can function as an antioxidant because it can stop the formation of free radicals by chelating iron metal. Vitamin C, as a comparison, is a pure synthetic compound that has a hydroxyl group, so it can donate hydrogen atoms better, without any other interfering compounds to react with DPPH free radicals, so the IC<sub>50</sub> value of vitamin C is solid.

Applying elicitors can increase growth, including synthesizing potential antioxidant compounds. The results of research by Mendoza et al. (2018) showed that the application of 300  $\mu\text{M}$  salicylic acid and three  $\mu\text{M}$  methyl jasmonate increased the content of phenolic compounds and flavonoids and induced the phenylpropanoid metabolite pathway [39]. Jasmonic acid and salicylic acid can stimulate the production of flavonoids and polyphenols in cell suspension, callus and cell culture [40].

The use of methyl jasmonate and PGPR elicitors causes receptor-elicitor interactions. Changes in biochemical activity, such as: 1) Binding of elicitors in plasma membrane receptors, 2) Changes in ion flow across cell membranes, such as  $\text{K}^+$  and  $\text{Ca}^{2+}$  pumps, 3) Increased phospholipid activity in several plant tissues, 4) Activation of proteins that play a role in the initial response, and 6) Activation of NADPH oxidase.

## Conclusion

The GC-MS results, 3 with the highest area (%) of *Curcuma xanthorrhiza* rhizomes, obtained 18 metabolite compounds, namely 1-Naphthaleneethanol, Ethyl orthoformate and Cyclopentanol. The GC-MS results of *Curcuma longa* rhizomes obtained 17 metabolite compounds: Turmerone, Curlone and Acetylene. The GC-MS results of *Curcuma caesia* rhizomes obtained 21 metabolite compounds: Isoprene, Imidazol-4-one and 3-ethenyl-3-ethylcyclopentanone. The GC-MS results of *Curcuma zedoria* rhizomes obtained 15 metabolite compounds, namely 3,4,5-trimethyl phenol, Naphthalene and Acetic acid. Based on the DPPH test, The IC<sub>50</sub> values mg/L  $\pm$  SD (category) for the control group were *Curcuma xanthorrhiza* 51.02  $\pm$  4.53 (strong), *Curcuma longa* 124.06  $\pm$  3.71 (medium), *Curcuma zedoria* 112.46  $\pm$  3.145 (medium), and *Curcuma caesia* 154.18  $\pm$  3.629 (weak). The IC<sub>50</sub> values (mg/L) for the treatment group were *Curcuma xanthorrhiza* 31.50  $\pm$  3.373 (very strong), *Curcuma longa* 97.20  $\pm$  2.940 (strong), *Curcuma zedoria* 88.18  $\pm$  2.924 (strong), and *Curcuma caesia* 143.69  $\pm$  3.500 (medium). *Curcuma* rhizomes have medium to very strong antioxidant potential.

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## Author's Contributions

Henny Lieke Rampe: Coordinated and participated in all activities, including proposal preparation, plant sample collection, field experiments, laboratory sample analysis, data analysis, manuscript preparation and revision.

Hanny Hesky Pontororing: Involved in proposal preparation, experimental design, procurement of research equipment and materials, and field activities.

Harry Jefry Lengkong: Proposal preparation, field activities, data analysis, article preparation, and manuscript revision.

Meytij Jeanne Rampe: Laboratory activities, sample preparation, antioxidant testing, and metabolomic analysis using GC-MS.

Vistarani Arini Tiwow: Laboratory activities, data analysis and interpretation, manuscript preparation and revision.

## Ethics

There are no ethical issues that may arise after the publication of this manuscript because this research does not require ethical clearance.

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