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Analysis of Metabolomic Compounds and Antioxidant Potential of Solanaceae Family Plants using Gcms after Elicitor Induction Methyl Jasmonate and Salicylic Acid

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ABSTRACT

Background: Solanaceae plants have the potential to be a source of food, medicine, industrial materials and antioxidants. Antioxidants are compounds that can delay or prevent oxidation caused by free radicals significantly. Metabolomic analysis is a method used to study metabolite profiles. It is necessary to use elicitors to optimize the synthesis of metabolomic compounds and antioxidant potential: methyl jasmonate and salicylic acid as elicitors.

Methods: This research aims to analyze the Solanaceae plant's metabolomic compounds and antioxidant potential using GCMS after the elicitors methyl jasmonate and salicylic acid induction. Experimental method research with stages: 1) Observation, collection and identification of Solanaceae plants, 2) Preparation of experimental land, 3) Planting of plant collections, 4) Application of elicitor methyl jasmonate 60 ppm and salicylic acid 0.45 g/L, 5) Metabolomic and antioxidant analysis on fresh extracts of samples. Testing antioxidant activity using a spectrophotometer with DPPH solution. Metabolomic profile analysis using GCMS.

Result: The results of the non-targeted metabolomic analysis with GCMS and antioxidant activity with IC_{50} in the *Solanum genus*, namely *Solanum betaceum, Solanum melongena*, *Solanum torvum*, *Solanum Lycopersicum* and *Solanum tuberosum,* respectively identified 18, 16, 14, 13 and 20 compounds. The IC_{50} values were 51.32 mg/L (strong category), 56.64 mg/L (strong category), 65.86 mg/L (strong category), 113.60 mg/L (medium category), 102.71 mg/L (medium category), respectively. In the *Capsicum* genus, namely *Capsicum frutescens* and *Capsicum annuum*, 21 and 37 compounds were identified, respectively, with IC_{50} values of 124.76 mg/L (medium category) and 63.23 mg/L (strong category). In *Physalis angulata*, 19 compounds were identified, IC_{50} was 44.04 mg/L (very strong category), six compounds were identified in *Nicotiana tabacum* and IC_{50} was 67.55 mg/L (strong category). Solanaceae plants have moderate to very strong antioxidant potential.

Key words: Antioxidants, Metabolomics, Solanaceae family.

INTRODUCTION

The Solanaceae family is a group of shrubs or trees with single or compound leaves and trumpet-shaped flowers. Some important genera from the Solanaceae family are *Solanum*, *Capsicum*, *Nicotiana* and *Physalis*. Solanaceae plants have the potential to be a source of food, medicine, industrial materials and antioxidants (Artanti and Lisnasari, 2018; Krisnawati and Febrianti, 2019; Azzikri *et al.,* 2020). Antioxidants are compounds that can significantly delay or prevent oxidation caused by free radicals. Antioxidants result from metabolite activity produced by various compounds, which can be studied through metabolomic analysis (Kumari *et al.,* 2023).

Several studies on the antioxidant potential of Solanaceae plants have been carried out on *Capsicum annuum* (Ananta and Anjasmara, 2022), *Capsicum frutescens* (Misfadhila *et al.,* 2022), *Solanum tuberosum* (Hellmann *et al.,* 2021; Dewi *et al.,* 2024). *Solanum lycopersicum* (Pujiastuti *et al.,* 2019; Hasfikasari, 2024), *Physalis angulata* (Putra *et al.,* 2023; Sukmawati *et al.,* 2024), *Datura metel* (Mbida *et al.,* 2022). *Solanum torvum* (Helilusiatiningsih and Soenyoto, 2020), *Nicotiana tabacum* (Al-Lahham *et al.,* 2020; Zou *et al.,* 2021). Antioxidant activity was determined through decolorization of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The use of DPPH in the antioxidant potential test has been carried out on extracts (Leaves and Fruit) of *Cucurbita maxima* (Wafa,

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2024), *Syzygium cumini* (Devi *et al.,* 2023), Turnip Roots (*Brassica rapa*) Beniwal *et al.,* (2022), Nardostachys Jatamansi (Pant *et al.,* 2024).

Metabolomic analysis is a method used to study metabolite profiles. It is necessary to use elicitors to optimize the synthesis of metabolomic compounds and antioxidant potential methyl jasmonate and salicylic acid as elicitors (Diaz *et al.,* 2016). Jasmonic acid can induce hormone synthesis and gene expression (Huang *et al.,* 2017; Ruan *et al.,* 2019). Jasmonic acid is a natural compound synthesized by plants as a resistance response to pathogen attack and external damage (Siddiqi and Husen, 2019; Gomi, 2020; Torres *et al.,* 2020). Salicylic acid is an endogenous hormone that plays a role in plant physiological processes, protein synthesis and abiotic stress (Khatun *et al.,* 2016).

Research on induction of the elicitor methyl jasmonate (MJ) (Rampe *et al.,* 2022), a combination of the elicitors salicylic acid and methyl jasmonic (Rampe *et al.,* 2023) has been carried out on sweet potatoes. The results showed increased quantitative morphoanatomical characters and flavonoid and tannin content. Tam-Ho *et al.* (2020) found that applying exogenous jasmonic acid and methyl jasmonate induced reactive oxygen species and resistance mechanisms with the accumulation of secondary metabolites. Testing of MJ *in vitro* cultures has induced antioxidant enzyme activity, expression of resistance-related genes and increased production of secondary metabolites. The potential for methyl jasmonate to induce secondary metabolite biosynthesis has been carried out by Sanchez *et al.* (2020) on the suspension culture of *Piper cumanense*, Nguyen *et al.* (2019) on the root culture of *Centella asiatica*. Solanaceae plants have antioxidant potential (Ralte *et al.,* 2021) because they contain metabolomic compounds, namely alkaloids, flavonoids, saponins, tannins and terpenoids. This research focuses on the induction of elicitors methyl jasmonate and salicylic acid on antioxidant potential and metabolomic compound content.

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. GCMS analysis at the North Sulawesi Regional Police Forensic Laboratory, in 2024.

Research procedures

a. Observation, collection and identification of *Solannaceae* plants in Tomohon City.
b. Preparation of experimental land. The experimental plots were 10 x 10 m in size.
c. Planting a collection of solanaceae plants. Each plot is planted with 16 plants.
d. The elicitor methyl jasmonate 60 ppm and salicylic acid 0.45 g/L, 25 days after planting (DAP) and 50 DAP were applied by spraying on the whole plant. Control without methyl jasmonate and salicylic acid treatment.
e. Metabolomic and antioxidant analysis on fresh extracts of samples. The materials used were fruits of *Solanum betaceum*, *Solanum melongena*, *Solanum torvum*, *Solanum lycopersicum*, *Capsicum frutescens*, *Capsicum annuum*. Furthermore, leaves on *Physalis angulata* and *Nicotiana tabacum* and tubers on *Solanum tuberosum*. The material (fruit/leaf/tuber) of each plant sample was pulverized, squeezed with a filter cloth; then, the extract was filtered to obtain the filtrate.

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. 1.5 mg of fresh sample extract was dissolved in 1 mL of DMSO to obtain a solution with a concentration of 50/ mL. The sample was incubated for 20 minutes. 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 through decolorization of DPPH at a wavelength of 514 nm using a UV-Vis spectrophotometer.

Through a linear equation that states the relationship between the concentration of the test extract (x) and 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.* (2018), namely.

$$\%\ \text{Inhibition} = \frac{\text{A DPPH - A Sample}}{\text{A DPPH}} \times 100$$

The level of antioxidant power using the DPPH method: IC_{50} value (ppm)<50 (very strong), 50-100 (strong), 100-150 (medium), 150-200 (weak) and >200 (very weak) (Molyneux, 2004).

Metabolomic compound analysis using GCMS version of Agilent 8890 GC System. The sample used was a fresh extract solution of Solanaceae plants. The sample extract was dissolved in methanol (1:1) and 2 μL was injected into the GCMS.

RESULTS AND DISCUSSION

The samples used were fresh extract solutions from Solanaceae plants. The entire area was then identified qualitatively through the method of comparing the spectrum data obtained with the spectrum data in the data bank. Next, look for the compound chain in PubChem and then copy the chain obtained to way2drug.com to find out the potential of the metabolite compound. The study is focused on identifying large percent areas that show the greatest composition of compounds contained in the sample.

The GCMS results of terong belanda/tamarillo (*Solanum betaceum*) (Fig 1), obtained 18 metabolite compounds, three compounds that dominate with the highest area (%) namely thiocyanic acid, retention time (rt)

8.952, area 8.64 %; 4H-pyran-4-one, rt 7,720 area 5.25% ; 2H-imidazole-2-one, rt 4.558 area 6.39%. Based on DPPH test, IC_{50} value = 51.32 mg/L strong antioxidant category, IC_{50} value of control 82.16 mg/L. The GCMS results of terong ungu/purple eggplant (*Solanum melongena*) fruit (Fig 1) showed that 16 metabolite compounds were obtained, three compounds that dominated with the highest area (%) namely 4H-pyran-4-one, rt 7.745, area 10.62%; 2-Cyclopenten-1-one rt. 4,721 area 13.36%, propane rt. 5,744 area 11.28%. Based on the DPPH test, IC_{50} value = 56.64 mg/L in the category of strong antioxidant properties, IC50 value of control 84.34 mg/L.

The GCMS results of takokak (*Solanum torvum*) leaves (Fig 1) showed that 14 metabolite compounds were obtained, three compounds that dominated namely phytol rt.18.461 area 37.13%; alpha-L-Galactopyranoside, rt. 13,783 area 14.03%; 2-Propen-1-ol, rt 5.563 area 6.36%, Based on DPPH test, IC_{50} value = 65.86 mg/L strong antioxidant category, IC_{50} value of control 94.64 mg/L. The GC-MS results of tomat / tomato (*Solanum lycopersicum*) (Fig 1) showed that 13 metabolite compounds were obtained, three compounds that dominated with the highest area (%) namely methyl glyoxal, rt 3,517 area 37.44%; (furan-2-yl) methanol, rt. 4,860 area 11.41%; 1,3-Cyclohexane-11, rt. 5,706 area 9.69%. Based on the DPPH test, IC50 value = 113.60 mg/L in the medium antioxidant category , IC50 value of control 136.12 mg/L. The GCMS results tubes of kentang / potato (*Solanum tuberosum*) (Fig 1) showed that 20 metabolite compounds were obtained, three compounds that dominated with the highest area (%) namely Hexadecanoic acid, rt.16,706 area 22.66%; 2-pyrrolidinone rt. 6,861 area 15.44%; methyl stearate, rt.18,552 area 1.74%. Based on the DPPH test, IC_{50} value = 102.71 mg/L in the medium antioxidant category, IC_{50} value of control 144.07 mg/L.

The GCMS results of cabai rawit/cayenne pepper (*Capsicum frutescens*) fruit (Fig 2), obtained 21 metabolite compounds, the three compounds that dominated were Capsaicin, rt. 22,379 area 63.15%; Dihydrocapsaicin, rt.22,521 area 19.92%; acetamide, rt.18,895 area 3.25%.

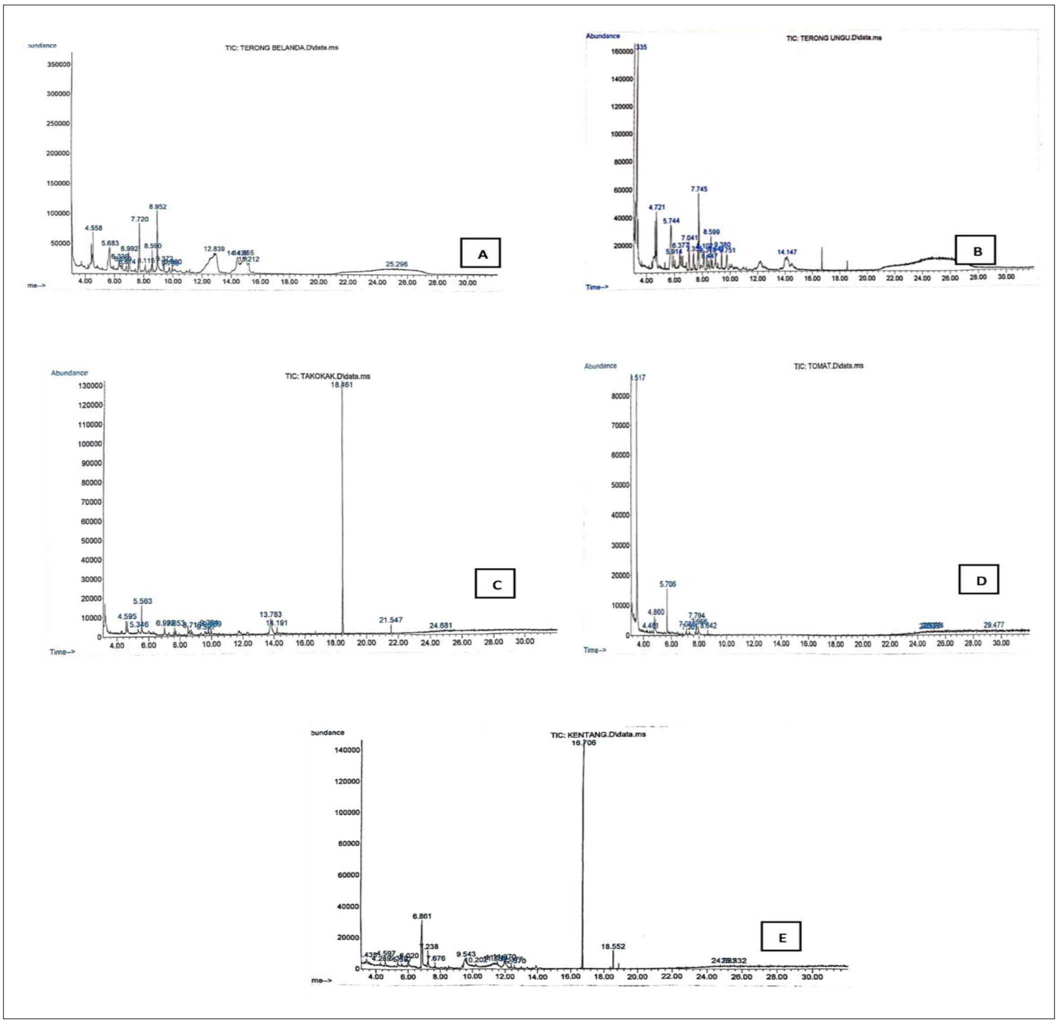

Fig 1: GCMS results of *S. betaceum* (A), *S. melongena*) (B), *S. torvum* (C), *S. lycopersicum* (D) and *S. tuberosum* (E).

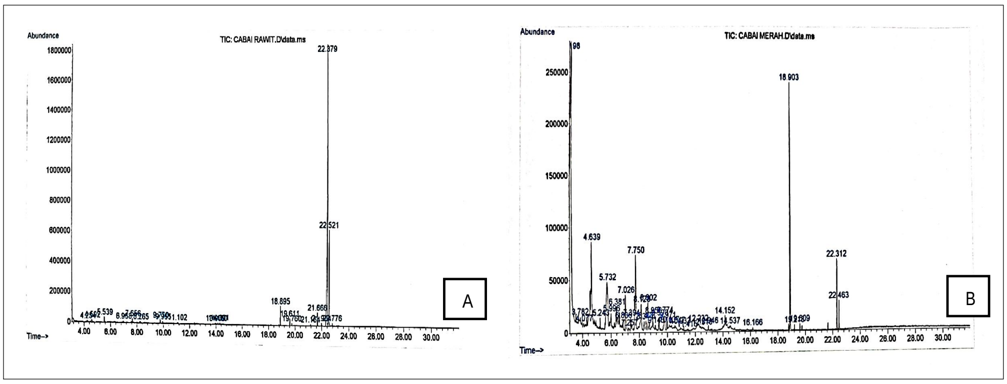

Fig 2: GCMS results of *C. frutescens* (A) and *C. annuum* (B).

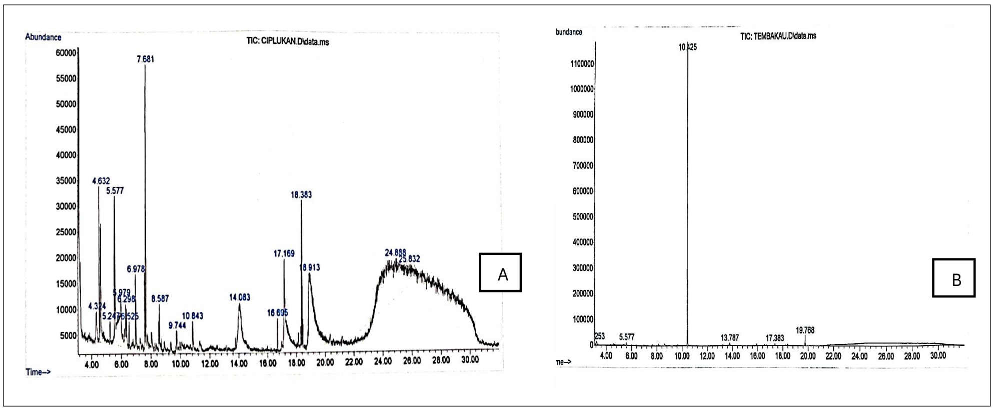

Fig 3: GCMS results of *P. angulata*) (A) and *N. tabacum* (B).

Based on the DPPH test, IC50 value = 124.76 mg/L in the medium antioxidant category, IC_{50} value of control 165.94 mg/L. The GCMS results of cabai merah/red chili (*Capsicum annuum*) fruit (Fig 2), obtained 37 metabolite compounds, three compounds that dominated namely O-Isopropylhydroxylamine rt 3,198 area 27.84%; 6-Oxa-bicyclo (3.1.0) hexan-3-one rt 4.639 area 13.08%; Acetamide, rt 18.903, area 6.27%. Based on the DPPH test, IC50 value = 63.23 mg/L in the category of strong antioxidant properties, IC_{50} value of control 83.14 mg/L.

The GCMS results of ciplukan/morel berry (*Physalis angulata*) (Fig 3) obtained 19 metabolite compounds, three compounds that dominated namely 4H-Pyran-4-one rt 7,681 area 7.40%; Cyclopropane rt 4,632 area 5.14%; Topotecan rt 5,577 area 4.40%. Based on the DPPH test, the IC_{50} value 44.04 mg/L in the category of very strong antioxidant, IC_{50} value of control 79.16 mg/L. Furthermore, six metabolite compounds were obtained from tembakau /tobacco leaves (*Nicotiana tabacum*), three compounds dominated Pyridine, rt. 10,425 area 88.97 %; Caryophyllene oxide, rt. 19.768 area 3.63% and Scopoletine, rt.17.383 area 1.33%. Based on the DPPH test, IC_{50} value = 67.55 mg/L category of strong antioxidant, IC_{50} value of control 98.17 mg/L.

Metabolomics is the systematic study of global metabolite profiles in biological samples. Metabolomics analysis is an approach used to determine metabolite profiles. Untargeted GCMS is considered an appropriate method for analyzing metabolites in liquid samples. Based on Fig 1, the five species of the *Solanum* genus show chromatograms with different numbers of identified compounds, namely *Solanum tuberosum*, 20 compounds; Solanum *betaceum*, 18 compound and *Solanum melongena*, 16 compounds. Compounds found in the five types of plants include propane, acetaldehyde, 4H-Pyran-4-one-TU, 2-propen-1-ol, propen and 1,2 ethanediamine. Based on (Fig 2), it can be seen that there are differences in the chromatograms of the *Capsicum* genus species. In *Capsicum annuum*, 37 compounds were obtained and 21 compounds were found. Compounds identified in the two types of plants include capsaicin, acetamide, ethylene oxide, butanoic acid and acetic acid. The diversity of chromatograms obtained in the Solanaceae tribe shows differences in specific phenotypic and genotypic characteristics and can also be influenced by external factors. 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 μM salicylic acid and 3 μM methyl jasmonate increased the content of fenolic compounds and flavonoids and induced the phenylpropanoid metabolite pathway.

Antioxidant activity is known from the IC_{50} value. The IC_{50} value is inversely proportional to the antioxidant content. A material that has a small IC_{50} value indicates that the material has a large antioxidant content because IC_{50} represents the sample concentration required to reduce 50% of the absorbance of a reducible DPPH solution (Rusmana *et al.,* 2017). Solanaceae family plants have different antioxidant potentials from medium to very high categories. Antioxidant properties are closely related to metabolite compounds. Some classic non-enzymatic antioxidants are vitamins such as vitamin C or E, but flavonoids and other phenolic compounds are also potent antioxidants.

CONCLUSION

The results of non-targeted metabolomic analysis with GCMS and antioxidant activity with IC50 of the *Solanum* genus in *Solanum betaceum*, *Solanum melongena*, *Solanum torvum*, *Solanum lycopersicum*, *Solanum tuberosum* identified 18, 16, 14, 13 and 20 compounds respectively. The IC_{50} values were 51.32 mg/L (strong category), 56.64 mg/L (strong category), 65.86 mg/L (strong category), 113.60 mg/L (medium category), 102.71 mg/L (medium category), respectively. In the *Capsicum* genus, namely *Capsicum frutescens* and *Capsicum annuum*, 21 and 37 compounds were identified respectively, with IC50 values of 124.76 mg/L (medium category) and 63.23 mg/L (strong category). In *Physalis angulata* 19 compounds were identified, IC_{50} was 44.04 mg/L (very strong category), 6 compounds were identified in *Nicotiana tabacum*, IC_{50} was 67.55 mg/L (strong category). Solanaceae plants have moderate to very strong antioxidant potential.

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Conflict of interest

The authors declare no conflict of interest.

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