

(RESEARCH ARTICLE)



Metabolite profile of tree marigold (*Tithonia diversifolia* (Hemsley) A. Grey) leaves

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Abstract

Tree marigold or *Tithonia diversifolia* (Hemsley) A. Grey also known as Insulin leaves has traditionally been used as an herb for the treatment of diabetes mellitus (DM). Tree marigolds contain various metabolite compounds, which have various bioactivities, so research was carried out to study metabolite profiles using a non-target metabolism approach using a GC-MS (Gas Chromatography-Mass Spectrometry) instrument. The results of GC-MS analysis of Tree marigold ethanol extract showed 30 peaks in the chromatogram, of which 7 were dominant metabolite compounds including 1-butanol, 3-methyl acetate (28.11%), Thunbergol (14.28%), Geranyl acetate (12.13%), D-Limonene (6.64%), 4H-Pyran-4-one, 2,3-Dihydro-3,5-dihydroxy-6-methyl (5.05%), acetic acid (4.10%), 7,8-epoxy- α -ionone (2.91%). The various categories of metabolites consist of the monoterpene class followed by fatty acids, terpenes, and sesquiterpenoids, as well as other compounds in the terpenoid esters class, alkanolic acids, fatty acids, proteins, amino acids, furanoids, ketones, and others. These metabolite compounds have antidiabetic, antioxidant, anti-inflammatory, antimicrobial, antibacterial, anticancer, and antitumor effects so they have potential as antidiabetic therapy.

Keywords: Bioactivity; GCMS; Metabolite profile; *Tithonia diversifolia* (Hemsley) A. Grey); Tree marigold (Insulin leaves)

1. Introduction

The tree marigold (*Tithonia diversifolia* (Hemsley) A. Grey)) is known to some Indonesian people as an insulin leaf and can be said to be a plant with local wisdom that has been used by the community as an alternative treatment for diabetes mellitus (DM) instead of chemical drugs. The part of the marigold tree that is used is the leaves which are then processed and consumed regularly. The part commonly used is the leaf which contains metabolite compounds that then act as antioxidants so that they can bind excessive free radicals in the body. According to previous research [1], it was explained that the results of testing the content of Tree marigold leaves have an antidiabetic effect or act as an antihyperglycemic. In addition, a phytochemical screening test [2], showed that tree marigold leaves contain secondary metabolites such as flavonoids, alkaloids, terpenoids, saponines, and phenolics. The flavonoid content in tree marigold leaves can function as a compound that has insulin-like effects. The effect of reducing glucose levels caused by flavonoids will work by regenerating and protecting damaged pancreatic cells and stimulating the release of insulin [3].

The compound content in Tree marigold leaves has the ability to inhibit ROS (radical oxygen species) chain reactions in accordance with [2], that phytochemical screening of Tree marigold leaf extracts shows that there are active compounds of flavonoids, alkaloids, saponins, terpenoids, as well as phenolics. This is also in line with research by [4], that the content of active compounds such as fructooligosaccharides, phenols, chlorogenic acid, and flavonoids from tree marigold leaves can reduce blood glucose levels. Therefore, Tree marigold leaves are considered capable of being used as an alternative medicine for treating DM because they play a role in reducing blood glucose levels.

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The use of metabolite compounds from tree marigold leaves to treat DM disease needs to be followed up with metabolomic analysis. Metabolomic analysis can be carried out using various methods, one of which is using a Gas Chromatography-Mass Spectrometry (GC-MS) instrument. GC-MS-based metabolic profiling has been frequently used in many metabolomic studies of plants, animals, and microorganisms [5]. GC-MS can be used to identify metabolite compounds in Tree marigold leaves. So research was carried out to determine the metabolite profile of the ethanol extract of Tree marigold leaves and the dominant compounds from its metabolites.

2. Materials and Methods

2.1. Sampling

Tree marigold (*T. diversifolia*) leaf samples were taken in the Banyuasin area at a geographical position of 1.30°-4.0° South Latitude and 104° 00'-105° 35' East Longitude. Leaves are taken from the 3rd-5th order of mature leaves from the shoots in the amount of 2 kg, then the leaves are sorted and dried. The dried leaves are ground into simplicia powder and then filtered through a sieve to obtain fine simplicia powder.

2.2. Extraction of Tree Marigold Leaves

After obtaining fine simplicia powder, extract Tree marigold leaves using the maceration method with 70% ethanol in a ratio of 1:5 within 72 hours. Filtered to obtain leaf filtrate then the filtrate was evaporated using a rotary evaporator at a temperature of 80°C. Next, the extract yield was used for GC-MS analysis.

2.3. Gas Chromatography-Mass Spectrometry (GC-MS) Analysis

Analysis of the ethanol extract of tree marigold leaves using GC-MS was carried out to determine the metabolite groups. carried out at the Chemical Analysis Division, Central Laboratory, Padjadjaran University with a GC-MS Agilent 5977B GCMSD instrument. Ethanol extract of tree marigold leaves was 0.1 µl and 2 ml of meOH, and 2 ml of chloroform were added, then sonicated for 10 minutes then centrifuged for 5 minutes at 10,000 rpm. The supernatant was taken and injected into the GC-MS according to the working protocol of the GC-MS Agilent 5977B GCMSD.

3. Results and Discussion

The results of GC-MS analysis of the ethanol extract of Tree marigold leaves obtained a chromatogram which showed the presence of peaks at different specific times. Each peak shows the diversity of metabolite compounds from Tree marigold which were traced from the PubChem, KEGG, Spectrabase, PlantCye, and ChEBI websites. The chromatogram of the metabolite profile results in the ethanol extract of Tree marigold leaves can be seen in Figure 1.

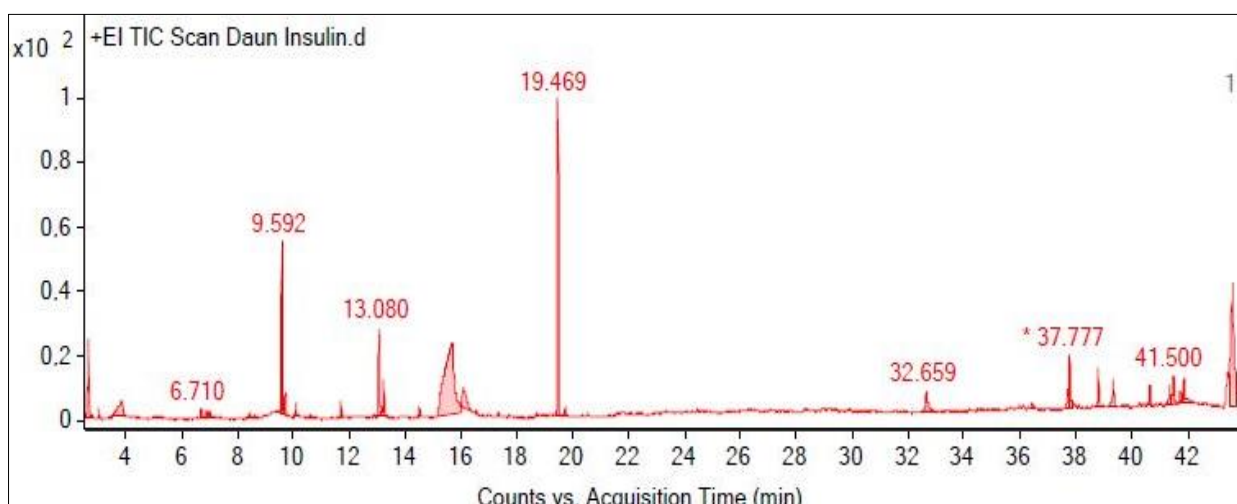


Figure 1 Chromatogram of ethanol extract of Tree marigold leaves using GC-MS

The chromatogram shows 30 peaks detected by GC-MS, 7 of which are the main peaks. This indicates that each peak will receive a different compound with a different retention time. Based on the area percentage, the dominant compounds from tree marigold leaves include *1-Butanol, 3-methyl-, acetate* (28.11%), *Thunbergol* (14.28%), *Geranyl acetate* (12.13%), *D-Limonene* (6.64%), *4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl* (5.05%), *Acetic acid* (4.10%), *7,8-Epoxy-.alpha.-ionone* (2.91%), *2-Piperidinone, N-[4-bromo-n-butyl]* (2.59%), *Nonanoic acid, 2,3-dimethylphenyl ester* (2.59%), and *6-Octenal, 3,7-dimethyl- (R)* (1.56%).

The results of data interpretation in the chromatogram, the total compounds from the ethanol extract of tree marigold leaves can be seen in Table 1.

Table 1 Metabolite profile of metabolites, chemical formula, retention time and area of abundance in extract ethanol Tree marigold (*Tithonia diversifolia*) leaves

Metabolites	Chemical Formula	Retention Time	Area (%)
<i>Acetic acid</i>	C ₂ H ₄ O ₂	2.67	4.10
<i>Propanoic acid, 2-methyl</i>	C ₄ H ₈ O ₂	3.85	3.08
<i>Urea</i>	CH ₄ N ₂ O	5.06	0.40
<i>Butanoic acid, 4-hydroxy</i>	C ₄ H ₈ O ₃	6.71	0.44
<i>Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)</i>	C ₁₀ H ₁₆	6.90	0.30
<i>2-Cyclopenten-1-one, 2-hydroxy</i>	C ₅ H ₆ O ₂	6.99	0.77
<i>Diisopropyl azodicarboxylate</i>	C ₈ H ₁₄ N ₂ O ₄	8.43	0.43
<i>D-Limonene</i>	C ₁₀ H ₁₆	9.59	6.64
<i>Eucalyptol</i>	C ₁₀ H ₁₈ O	9.71	0.82
<i>trans-.beta.-Ocimene</i>	C ₁₀ H ₁₆	10.10	0.63
<i>1.5-Dimethyl-1-vinyl-4-hexenyl butyrate</i>	C ₁₄ H ₂₄ O ₂	11.71	0.66
<i>4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl</i>	C ₆ H ₈ O ₄	13.08	5.05
<i>6-Octenal, 3,7-dimethyl- (R)</i>	C ₁₀ H ₁₈ O	13.23	1.56
<i>.alpha.-Terpineol</i>	C ₁₀ H ₁₈ O	14.52	0.44
<i>1-Butanol, 3-methyl-, acetate</i>	C ₇ H ₁₄ O ₂	15.70	28.11
<i>2,6-Octadien-i-ol, 3,7-dimethyl-, (Z)</i>	C ₁₀ H ₁₈ O	16.07	3.02
<i>Geranyl acetate</i>	C ₁₂ H ₂₀ O ₂	19.47	12.13
<i>2-Propenoic acid, 3-phenyl-, methyl ester</i>	C ₁₀ H ₁₀ O ₂	19.73	0.34
<i>Octanal, 7-methoxy-3,7-dimethyl-</i>	C ₁₁ H ₂₂ O ₂	32.66	2.19
<i>4-Octanol, 7-methyl</i>	C ₉ H ₂₀ O	36.43	0.31
<i>Tetradecane, 1-chloro-</i>	C ₁₄ H ₂₉ Cl	37.70	0.97
<i>7,8-Epoxy-.alpha.-ionone</i>	C ₁₃ H ₂₀ O ₂	37.78	2.91
<i>Nonanoic acid, 2,3-dimethylphenyl ester</i>	C ₁₇ H ₂₆ O ₂	38.80	1.85
<i>2-Methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane</i>	C ₁₅ H ₂₆ O	39.35	1.87
<i>2,5,5-Trimethyl-3-hexyn-2-ol</i>	C ₉ H ₁₆ O	40.65	1.09
<i>1,6-Octadiene, 3-ethoxy-3,7-dimethyl-</i>	C ₁₂ H ₂₂ O	41.36	0.93
<i>2-Piperidinone, N-[4-bromo-n-butyl]</i>	C ₉ H ₁₆ BrNO	41.50	2.04
<i>Epoxy-.alpha.-terpenyl acetate</i>	C ₁₂ H ₂₀ O ₃	41.75	0.64
<i>Tetradecane, 1-chloro-</i>	C ₁₄ H ₂₉ Cl	41.88	2.00
<i>Thunbergol</i>	C ₂₀ H ₃₄ O	43.60	14.28
Total 30 compounds			

Based on Table 1, it can be seen that the number of metabolite compounds detected was 30 compounds. This can happen because the type of organic solvent used is ethanol. It's different if what is analyzed is Tree marigold leaf aquabidestic extract. According to [6], the GC-MS results of aquabidest leaf tree marigold extract only produced 12 compounds.

Each detected compound originates from a different biosynthetic pathway, and metabolite class. Metabolite at biosynthetic pathways in Figure 2a., and classes group of active compounds in Tree marigold leaves can be seen in Figure 2b.

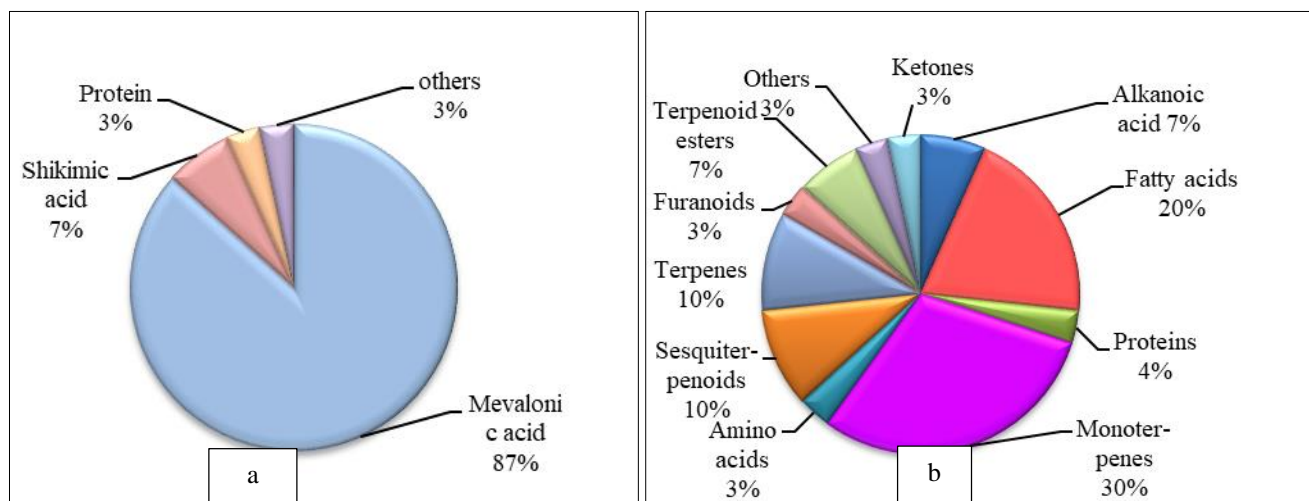


Figure 2a Biosynthetic pathways of metabolites Tree marigold, **2b.** metabolite classes in ethanol extract of Tree marigold leaves.

Comparison of the metabolite biosynthesis pathway of Tree marigold leaves based on Figure 2a, is dominated by the mevalonic acid pathway, amount 87%, while the comparison of various metabolite classes in Figure 2b, is dominated by the monoterpene class at 30%, followed by the fatty acid at 20%, terpenes and sesquiterpenoids 10% each, and other compounds in the terpenoid ester class, alkanolic acids, fatty acid, proteins, amino acids, furanoids, ketones, and others.

Components of monoterpene class compounds from the ethanol extract of Tree marigold leaves with active compounds such as *Bicyclo[3.1.0]hex-2-ene*, *2-methyl-5-(1-methyl ethyl)*, *D-Limonene*, *trans-β-Ocimene*, *6-Ocetal*, *3,7-dimethyl-(R)*, *αTerpineol*, *2,6-Octadien-i-ol*, *3,7-dimethyl-, (Z)*, *Geranyl acetate*, *1,6-Octadiene*, *3-ethoxy-3,7-dimethyl-*, and *Epoxy-α-terpenyl acetate*. Monoterpene compounds are obtained from the mevalonic acid biosynthesis pathway. The compounds from the monoterpene class on average have antioxidant bioactivity [7; 8; 9; 10], antibacterial, anticancer [11], anti-inflammatory [9; 11; 12], antitumor [9; 10], and antimicrobial [8; 9; 10; 12; 13; 14].

The fatty acid class compounds found in Tree marigold leaves are the active compounds *Propanoic acid*, *2-methyl, Butanoic acid*, *4-hydroxy*, *2-Cyclopenten-1-one*, *2-hydroxy*, *1-Butanol*, *3-methyl-, acetate*, *Nonanoic acid*, *2,3-dimethylphenyl ester*, and *Tetradecane*, *1-chloro-*, which are compounds from the mevalonic acid biosynthesis pathway. The types of compounds from the fatty acid class generally have antidepressant bioactivity [15], antibiotics [16], antimicrobials [17; 18], antioxidants [17], anti-inflammatory and anticancer [19].

Terpene class, there are active compounds such as *1.5-Dimethyl-1-vinyl-4-hexenyl butyrate*, *2-Methyl-3-(3-methyl-but-2-enyl)-2-(4-methyl-pent-3-enyl)-oxetane*, and *Thunbergol*, which is a compound of the mevalonic acid biosynthesis pathway. Types of compounds from the terpene class have antioxidant bioactivity [20], antibacterial [21], anti-inflammatory [20], anticancer [21], and antimicrobial [20; 22]. Furthermore, compounds from the Sesquiterpenoid class with the active compounds found were *Eucalyptol*, *4-Octanol*, *7-methyl*, and *7,8-Epoxy-α-ionone*. Sesquiterpenoid compounds originate through the mevalonic acid biosynthesis pathway, which has antispasmodic bioactivity [23], antioxidant [23; 24], anti-inflammatory [24; 25], antitumor [24], anticancer [25], and antimicrobial [23; 25].

The next component of marigold leaf metabolite compounds comes from the terpenoid ester group which consists of active compounds such as *2-Propenoic acid*, *3-phenyl-, methyl ester*, and *Octanal*, *7-methoxy-3,7-dimethyl-*, derived from the mevalonic acid biosynthesis pathway. The terpenoid ester group has antioxidant bioactivity [26; 27], anti-

inflammatory, anticancer [26], and antimicrobial [26; 27]. Then there is the alkanolic acid group with active compounds such as *acetic acid* and *2-piperidinone, N-[4-bromo-n-butyl]*. Compounds in the alkanolic acid group have antibacterial [28], and antibiotic bioactivity [29]. originate from the mevalonic acid biosynthesis pathway.

The known furanoid group is the active compound *4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl* obtained from the shikimic acid biosynthesis pathway and has antidiabetic bioactivity [30]. The Ketone group with active compounds such as *2,5,5-Trimethyl-3-hexyn-2-ol* from the mevalonic acid biosynthesis pathway has bioactivity as antimicrobial, anti-inflammatory, antioxidant, and anticancer [31]. Another group was found to be an amino acid with a trans- β -Ocimene compound with anti-inflammatory bioactivity [14]. Hydrocarbon with the compound *Tetradecane, 1-chloro-* the result of other biosynthesis, and bioactivity as an antimicrobial [32]. As well as a protein with the active compound Urea which has bioactivity as antidiabetic, anticancer, and antibacterial [33].

4. Conclusion

The results of the analysis of the ethanol extract of Tree marigold leaves produced a chromatogram showing 30 peaks, 7 of which were dominant compounds consisting of *1-Butanol, 3-methyl-, acetate* (28.11%), *Thunbergol* (14.28%), *Geranyl acetate* (12.13%), *D-Limonene* (6.64%), *4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl* (5.05%), *Acetic acid* (4.10%), *7,8-Epoxy- α -ionone* (2.91%), *2-Piperidinone, N-[4-bromo-n-butyl]* (2.59%), *Nonanoic acid, 2,3-dimethylphenyl ester* (2.59%), and *6-Ocetalal, 3,7-dimethyl-(R)* (1.56%). The abundance of Tree marigold leaf compounds based on the biosynthetic pathway predominantly comes from the mevalonic acid pathway at 87% and is dominated by the monoterpene class at 30%, followed by the fatty acid class at 20%, terpenes and sesquiterpenoids at 10% each. The bioactivity of metabolite compounds from tree marigolds has antidiabetic, antibacterial, antibiotic, anticancer, antidepressant, antioxidant, antimicrobial, anti-inflammatory, antitumor, antiviral, antiposmodic, and antifungal properties. Further research is recommended on metabolomics testing with fractionation either *in vitro* or *in vivo* because the antidiabetic, antioxidant, and anti-inflammatory activity found in tree marigold leaf extract is quite high.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

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