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Characterization of secondary metabolites in local tomato (*Solanum lycopersicum*) extract for development of antioxidant-based product

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Abstract. Tomatoes (*Solanum lycopersicum*) are an important fruits source of carotenoid and other bioactive compounds with antioxidant properties. However, Studies on antioxidant potential of secondary metabolite in local tomato varieties remain limited. The lack of scientific data on their characteristics and activity constrain the development of functional product based on natural ingredients in the livestock sector. This study aimed to identify chemical compounds present in local tomato extracts as potential natural antioxidants. Three varieties were analyzed: Rose Tomato (RT) (*Solanum lycopersicum* L. *rugosum*) and Vegetable Tomato (VT) (*Solanum lycopersicum* L. *var. lycopersicum*), Cherry Tomato (CT) (*Solanum lycopersicum* *var. cerasiforme*). Sample were macerated in ethanol, concentrated using a rotary evaporator and analyzed by Gas Chromatography-Mass Spectrometry (GCMS). Result showed difference in compositions, with CT dominated compounds by 2-furanmethanol 52,58%. RT by 2-furanmethanol 23,17% and pentadecanoic acid 22,09%. And VT by 2-furanmetahnol 29,66%, 5-ethyl-2- furaldehyde 12,02% and nonadecanoic acid 8,28%. Vitamin E was also detected in RT and VT. These findings confirm local tomato extracts as source of diverse bioactive contain compounds with antioxidant potential.

1. Introduction

Tomato (*Solanum lycopersicum* L) is a fruit rich in antioxidants and plays an important role in preventing oxidative stress [1]. Oxidative stress occurs when there is an imbalance between the production of free radicals, such as *Reactive Oxygen Species* (ROS), and the ability of the body's antioxidant system to neutralize them. In livestock, this condition leads to excessive accumulation of free radicals that damage lipids, proteins, and DNA, resulting in various health problems. Its impacts include reduced productivity, lower, quality of animal products (meat and milk), weakened immune system[2], and decreased spermatozoa quality[3]. To overcome this condition, effective antioxidants, derived from secondary metabolite compounds, are required. Secondary metabolites are bioactive



compounds produced by plants and fruits including flavonoids, phenolics, terpenoids, alkaloids, and carotenoids. These compounds have been shown to neutralized free radicals, inhibit lipid peroxidation, and enhance the activity of endogenous antioxidant enzymes such as Superoxide Dismutase (SOD) and catalase [4]. Their utilization in modern livestock production is important to developed as preventive nutrition since they are safe, natural, and environmentally friendly, thus reducing dependence on synthetic substances with potential long-term toxic risks.

Tomato (*Solanum lycopersicum L*) is one of the sources of natural secondary metabolite compounds containing lycopene, flavonoids, and phenolics compounds [5]. Lycopene, a carotenoid compound, is known to have strong antioxidant activity and potential benefits in improving reproductive cell quality. Several studies have been conducted, especially the use of lycopene in the freezing buffalo spermatozoa that the addition of 1-2 % lycopene in 100 ml skimmed milk and egg yolk can increase the viability and motility of post-thawing buffalo spermatozoa. Therefore, the addition of lycopene up to 2% in 100 ml skimmed milk-egg yolk is highly recommended [6]. Although various studies have revealed the antioxidant potential of secondary metabolite compounds in imported tomatoes, characterisation of the secondary metabolite content of local tomatoes is still limited. The lack of scientific data on the characteristics of local tomatoes and their antioxidant properties is an obstacle in the development of functional products based on natural ingredients. In addition, this study focuses on local tomatoes because genetic diversity and the environment in which they grow are likely to have different and distinctive compounds profile. In addition, local tomatoes have not been scientifically explored for their secondary metabolite compounds, so it is necessary to conduct characterisation research on local tomato extracts to support their use as a potential source of natural antioxidants in the livestock sector.

2. Materials and methods

2.1. Research Sample

Three local tomato variants were used in this study: Rose Tomato (RT) (*Solanum lycopersicum var rugosum*), Vegetable Tomato (VT) (*Solanum lycopersium L. var. lycopersicum*), Cherry Tomato (CT) (*Solanum lycopersicum var cerasiforme*). The plants were cultivated for six months in the experimental garden under organic conditions, without the application of chemical fertilisers. Fully ripened fruits from each variant were harvested and subsequently used as research samples.

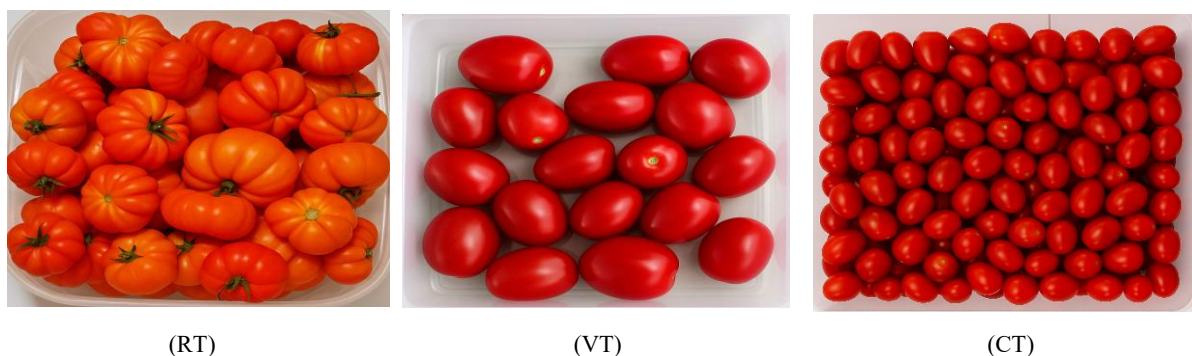


Figure 1. Research samples; *Solanum lycopersicum var. rugosum*. Rose Tomato (RT) *Solanum lycopersicum L.var. lycopersicum*, Vegetable Tomato (VT). *Solanum lycopersicum var cerasiforme* Cherry Tomato (CT).

2.2. Preparation Sample

Extraction method using conventional method. Before use, all tomatoes are washed with clean water and drained. Each 1 kg of tomato (RT, VT, CT) are sliced put into oven drying for 2 x 24 hours at 50°C. dried tomatoes are grounds using a blender until smooth. The resulting tomato powder, weighed using digital scales. Tomato powder was macerated for 2 x 24 hours without light, using 95% ethanol solvent

in a ratio of 1:4 (1 dry extract:4 ethanol 95%). Stored in a dark place. Filter the precipitate using whattman filter paper and a vacuum/buchnerr funnel to filter out the precipitate. The filtrate was then evaporated using a rotary evaporator (IKA Model: RV 10 B, spec: 100-240V-50/60 Hz 75 W IP 20) at 50-60°C water bath to evaporate ethanol and obtain dark red tomato extract. The tomato extract was stored in a dark bottle at 4°C to avoid compound degradation due to light and oxygen (8).

2.3. GCMS Analysis of secondary metabolite content

GC-MS analyses were performed on a Shimadzu QP 2010 Ultra GC-MS equipped with Rxi-1MS capillary column (30 m x 0,25 mm x 0,25 μ m). Helium was used as a carrier gas under constant linear velocity (39 cm s⁻¹; initial flow $\approx$ 1,2 mL min⁻¹). The injector (250 °C) operated in splitless mode using a deactivated single taper-liner. The oven program was 35°C (2 min), ramped at 10°C min⁻¹ to 300°C (5min). The MS operated in EI at 70 eV, source 200°C, interface 270°C, full scan m/z 35-500 (solvent delay 4 min). identification was based on NIST library matches and kovats retention indices determined with n-alkane standards: quantification used SIM with matrix-matched calibration and an internal standard. Each batch include a solvent blank, procedural blank, calibration standard, a continuing calibration verification ($\pm$ 15%), and a QC sample. After each sequence, the column was baked at 300°C to remove residuals.

2.4. Identification compounds

Identification of secondary metabolite compound on retention time and mass spectrum of GC-MS was done using the database of National Institute Standard and Technology (NIST).

3. Results and discussion

3.1. Secondary metabolite of Rose Tomato (RT).

Table. 1. Secondary metabolite content of Rose Tomato (RT) extract.

R.Time	Area	Area %	Height	Height%	A/H	Name
5670	59855	4,85	22753	5,91	2,63	<i>Furfural</i>
7582	122054	9,89	50934	13,23	2,4	<i>2,5 -Furandione, dihydro-3-methylene</i>
8751	96822	7,84	43022	11,17	2,25	<i>1,6-Heptadiyne</i>
11020	92257	7,47	28756	7,47	3,21	<i>4H-Pyran-4-one, 2,3 dihydro 3,5,-dihydrox</i>
12445	286012	23,17	48213	12,52	5,93	<i>2-Furanmtanol</i>
18671	53603	4,34	21170	5,5	2,53	<i>2-Butanone, 4-(dimethylamino)-3-methyl</i>
21071	272681	22,09	62805	16,31	4,34	<i>Pentadecanoic acid, 2,6,10,14-tetramethyl</i>
22670	58145	4,71	32326	8,39	1,8	<i>9-octadecynoic acid</i>
30406	129146	10,46	52353	13,6	2,47	<i>Vitamin E</i>
31512	63871	5.17	22742	5,91	2,81	<i>Z-10-Octadecen-1-ol acetate</i>

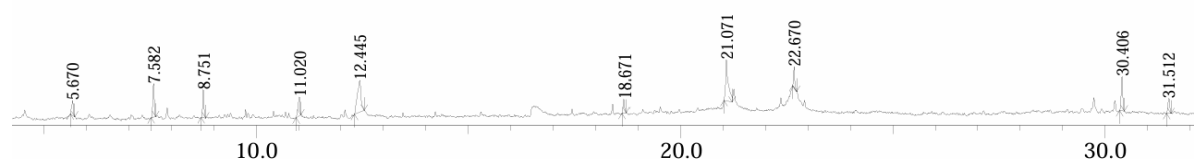


Figure 2. Chromatogram of Rose Tomato (RT) extract.

Based on the Table 1. the Result of the analysis of the secondary metabolite content of Rose Tomato (RT) extract found 10 secondary metabolite compounds. Of the 10 compounds, there are 3 compounds that dominate the results; *2-furanmethanol* with a content of 23,17%. These compounds are furan derivative formed during the tomato fruit extraction process. *Pentadecanoic acid, 2,6,10,14-tetramethyl* by 22,09%. These compounds in tomato extract as part of the lipid secondary metabolite profile during the extraction process. This compound is a branched fatty acid compound that shows potential antimicrobial activity. Furthermore, the dominating compound in tomato extract (RT) is vitamin E at 10, 46%.

Vitamin E in tomatoes is generally found in the form of γ -tocopherol. The *tocopherol content* may increase or decrease depending on the type of processing applied, such as heating, drying or storage conditions. Generally, vitamin E in tomatoes is mostly contained in the seeds. If seeds are removed then, reducing γ -tocopherol.[7]. *Furfural* a compound derived from pentose (xylose) formed from carbohydrate degradation. *9-octadecynoic acid* is an unsaturated fatty acid found in the seed tissue, skin and fruit extract of tomatoes. *Z-10-Octadecen-1-ol acetate* is a class of naturally occurring ester compounds of unsaturated fatty alcohols that can be produced by plants as volatile or semi-volatile components for scent. *2,5 -Furandione, dihydro-3-methylene* at 9,89 % is a furan derivative and an anhydride compound. It is not considered a natural metabolite of tomatoes, but rather a degradation product formed during thermal processing. *1,6-Heptadiyne* with 7,84% in tomatoes extract, belongs to non-polar volatile compounds, unsaturated aliphatic carbon hydrocarbon compounds (terminal alkuna). *4H-Pyran-4-one, 2,3 dihydro 3,5, dihydrox* is a maillard product (heat reaction between sugars and amino acids) not a result of natural biosynthesis from tomatoes. *2-Butanone, 4-(dimethylamino)-3-methyl* is not a natural metabolite of the tomatoes extract, it is suspected that this compound a rises from contaminants between the device and the solution.

The result of analysis of the tomato extract (TM) contained 6 compounds which are secondary compounds derived from the biosynthetic tomato plant (TM), including: *2-furanmetanol, Pentadecanoic acid, 2,6,10,14-tetramethyl*, vitamin E, *Furfural, 9-octadecynoic acid, dan Z-10-Octadecen-1-ol acetate*. Sedangkan *2,5 -Furandione, dihydro-3-methylene, 1,6-Heptadiyne, 4H-Pyran-4-one, 2,3 dihydro 3,5,- dihydroxy dan 2-Butanone, 4-(dimethylamino)-3-methyl* are not natural metabolites but degraded compounds.

3.2. Secondary metabolite of Vegetable Tomato (VT).

Table 2. Metabolite content of Vegetable Tomato (VT) extract.

R.Time	Area	Area%	height	Height%	A/H	Name
5.703	378060	5.34	89794	5.33	4.21	<i>Furfural</i>
7.610	245487	3.47	58844	3.49	4.17	<i>2,5-Furandione, dihydro-3-methylene-</i>
7.668	60481	0.85	27388	1.62	2.21	<i>Butane, 1,1-diethoxy-3-methyl-</i>
7.935	303298	4.29	111796	6.63	6.63	<i>3(2H)-Pyridazinone, 6-methyl-</i>
8.761	438174	6.19	177325	10.52	2.47	<i>1,6-Heptadiyne</i>
9.966	97371	1.46	20716	1.23	4.70	<i>Acetic acid, 2-(2-furoylhydrazono)-</i>
10.434	102942	4.79	42579	2.53	2.42	<i>Levogluconone</i>
11.130	338652	4.79	77889	4.62	4.35	<i>4H-Pyran-4-one, 2,3-dihydro-3,5-dihydrox</i>
11.398	135950	1.92	57928	3.44	2.35	<i>2H-Pyran-2-one</i>
12.130	181120	2.56	72833	4.32	2.49	<i>Methyl-2-heptynoate</i>
12.743	2098317	29.66	135099	8.02	15.53	<i>2-Furanmethanol</i>
13.483	65334	0.92	41655	2.47	1.57	<i>Benzene, (2,2-diethoxyethyl)-</i>
17.460	67412	0.95	38460	2.28	1.75	<i>Benzophenone</i>
18.417	47762	0.68	25684	1.52	1.86	<i>Dehydroacetic Acid</i>
21.074	241107	3.41	110613	6.56	2.18	<i>Pentadecanoic acid, 2,6,10,14-tetramethyl</i>
21.179	585898	8.28	108411	6.43	5.40	<i>Nonadecanoic acid</i>

21.339	849950	12.02	157720	9.36	5.39	5-Ethyl-2-furaldehyde
22.683	227762	3.22	106971	6.35	2.13	9,12-Octadecadienoic acid, methyl ester,
22.751	263007	3.72	70772	4.20	3.72	9,12,15-Octadecatrienoic acid, (Z,Z,Z)-
27.145	82614	1.17	45293	2.69	1.82	5-Acetoxymethyl-2-furaldehyde
28.449	67810	0.96	35614	2.11	1.90	1-Cyclohexene-1-carboxaldehyde, 4-(1-m
29.749	53302	0.75	19523	1.16	2.73	Stigmastan-6,22-dien, 3,5-dedihydro-
30.411	142122	2.01	52605	3.12	2.70	Vitamin E

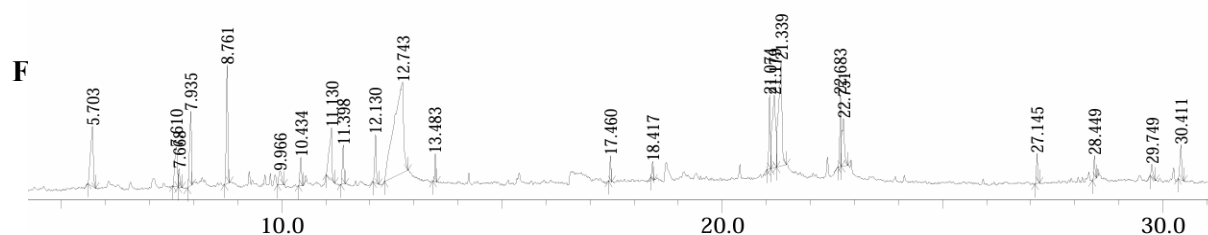


Figure 3. Chromatogram of Vegetable Tomato (VT) extract.

Among the identified compounds, 10 were classified as natural tomatoes metabolites (including 2-Furanmethanol, 2H-Pyran-2-one, Dehydroacetic Acid, Pentadecanoic acid, 2,6,10,14-tetramethyl, Nonadecanoic acid, 9,12-Octadecadienoic acid, methyl ester, Stigmastan-6,22-dien, 3,5-dedihydro-, 4H-Pyran-4-one, 2,3-dihydro-3,5-dihydrox and Vitamin E). The remaining 13 compounds (Furfural, 2,5-Furandione derivatives, levoglucosenone, furaldehyde). Are not considered natural metabolites, but are instead reported to arise from heating, thermal degradation, or processing artifacts during extraction and sterilization [8][9]

3.3. Secondary metabolite content of Cherry Tomato (CT).

Tabel 3. Secondary metabolite content of Cherry Tomato (CT).

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R.Time	Area	Area%	Height	Height%	A/H	Name
5.588	1959186	8.20	156430	9.63	12.52	Furfural
7.722	649858	2.72	104261	6.42	6.23	2,5-Furandione, dihydro-3-methylene-
7.966	1212234	5.07	178280	10.97	6.80	3(2H)-Pyridazinone, 6-methyl-
8.167	181743	0.76	43674	2.69	4.16	2,4-Dihydroxy-2,5-dimethyl-3(2H)-furan
9.252	141708	0.59	36306	2.23	3.90	Benzeneacetaldehyde
10.041	424923	1.78	101774	6.27	4.18	Acetic acid, 2-(2-furoylhydrazono)-
10.492	345514	1.45	93863	5.78	3.68	2-Oxabicyclo[2.2.0]hex-5-en-3-one
10.588	180478	0.76	42354	2.61	4.26	Maltol
11.337	1865842	7.81	167009	10.28	11.17	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydrox
11.943	135192	0.57	23808	1.47	5.68	Furazan-3-carboximide acid hydrazide, 4
13.381	12568461	52.58	366628	22.57	34.28	2-Furanmethanol
15.685	323510	1.35	46214	2.84	7.00	Propane, 2-isocyanato-2-methyl-
17.750	453240	1.90	19894	1.22	22.78	Dehydroacetic Acid
21.394	2063192	8.63	166842	10.27	12.37	5-Ethyl-2-furaldehyde
22.733	1399005	5.85	77130	4.75	18.14	9,12-Octadecadienal

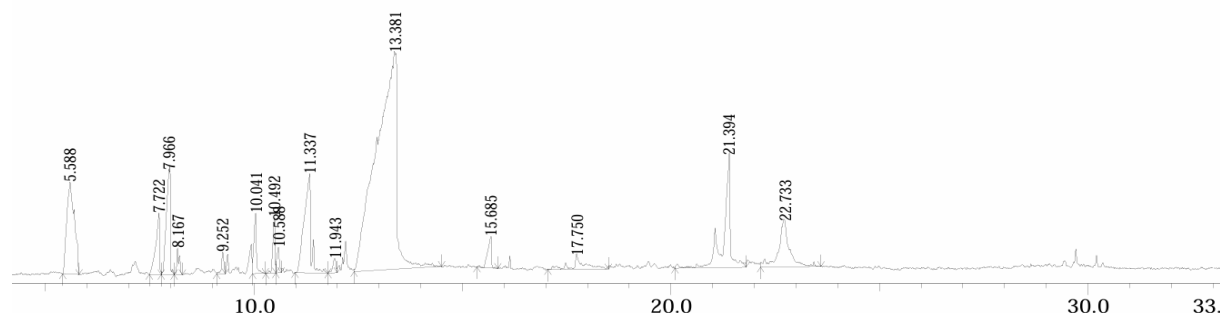


Figure 4. Chromatogram of Cherry Tomato (CT) extract.

Based on the analysis of Cherry Tomato extract (Table 3), a total of 15 metabolites were identified, consisting of seven natural metabolites derived from tomato and eight non-natural compounds generated from lipid oxidation and thermal degradation pathways. Among these, *2-furanmethanol* was the predominant compound 52,58% of the total metabolites detected. This compound is known to be formed through the thermal decomposition of carbohydrates and has also been reported to exhibit potential antioxidant activity [8]. In addition, 5-ethyl-2-furaldehyde (8,63%) and furfural (8,20%) were also detected as major furan-derived compounds. Furfural and its derivatives are volatile compounds typically generated from hemicellulose degradation during thermal food processing, particularly heating, sterilization, and concentration of tomato based products[8][9].

3.4. Comparison of analysis result of 3 tomato variants.

Table 4. Comparison of analysis result of 3 tomato variants.

Dominant component	RT (%)	VT (%)	CT (%)
<i>2-Furanmethanol</i>	23.17	29.66	52.58
<i>Furfural</i> / turunannya	4.85	5.34	~8.20
Vitamin E	10.46	2.01	-
Senyawa lipid (asam lemak, ester)	22.09	12.00+	-
<i>5-Ethyl-2-furaldehyde</i>	-	12.02	8.63
Key component	RT (%)	VT (%)	CT (%)
<i>2-Furanmethanol</i>	23.17	29.66	52.58
Vitamin E	10.46	2.01	-
Senyawa Lipid (Asam lemak & Ester)	high (22%)	medium (12%)	low
Senyawa Furan	medium	Dominant	Dominant

The CT sample showed a very high content of *2-furanmethanol*, signalling the dominance of carbohydrate degradation compounds. The RT dan VT sample contained vitamin E and lipid compounds, indicating successful extraction of lipophilic bioactive compounds, which are important in food and pharmaceutical applications. *2-Furanmethanol* is a furan-derived compound that plays a role in the natural flavouring of tomatoes [10]. Furfural, as a volatile antioxidant compound, not only functions as a natural flavouring agent but also demonstrates significant inhibitory activity against key enzymes such as tyrosinase. The reactivity of functional groups on the furan ring underlies this enzymatic inhibition. Moreover, empirical evidence shows that furfural can suppress the proliferation

of pathogenic bacteria *salmonella spp.* and *Bacillus subtilis*, with measurable minimum inhibitory concentration (MIC) confirming its antimicrobial potential [11]. A broader review of natural furan derivatives further emphasizes the diverse biological activities of this class, including antimicrobial and anti-inflammatory effects, likely mediated by their aromatic and hydroxyl moieties [12]. In the livestock sector which has been researched by researchers [13] the use of vitamin E as an ingredient in semen diluents that stabilises cellular unsaturated lipids against oxidative damage and maintains structural and functional integrity at the subcellular levels. [14] [15]. Vitamin E is a major component of the antioxidant system in sperm cells. [16]. Adding vitamin E to tris-yolk extender at 60 and 120 μm provides higher integrity of plasma membrane, mitochondria, male sheep [17] while the use of vitamin E tomato extract in semen diluent of Aceh cattle needs to be further researched and studied, especially in ranti/rose tomato (RT) which contains vitamin E of 10,46% which is the highest vitamin E content compared to vegetable tomato (VT).

3.5. Fatty acid and ester content

Roses Tomato (RT) contains *Pentadecanoic acid*, 2,6,10,14-tetramethyl by 22.09 %, Vegetable Tomatoes (VT) contains; *Nonadecanoic acid* 8.28%, 9,12-Octadecadienoic acid, methyl ester 3.22% and 9,12,15-Octadecatrienoic acid (linolenat) 3.72%. These compounds are saturated and unsaturated fatty acids with anti-inflammatory, antimicrobial effects and are important in the body's lipid metabolism [18]. Based on these contents, VT and RT are superior in lipid content compared to CT.

4. Conclusions

This study showed that extract of local tomato varieties; Roses Tomato (RT) and Vegetable Tomato (VT) contain secondary metabolite compounds that have potential as natural antioxidants such as vitamin E. anti-inflammatory and antimicrobial such as fatty acids and lipid compounds that are beneficial in lipid metabolism and maintaining cell integrity. Therefore, the use of VT and RT is recommended in Aceh cattle semen diluents as natural antioxidant to protect spermatozoa cells during storage, freezing and thawing.

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