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ARTIKEL

EFFECT OF STORAGE TIME VARIATION OF ANDALIMAN FRUIT ON FREE RADICAL SCAVENGING AND PROLIFERATION INHIBITION ON MCF-7 CELLS

[*Pengaruh variasi waktu penyimpanan buah Andaliman terhadap aktivitas penangkap radikal bebas dan penghambatan proliferasi sel MCF-7*]

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ABSTRACT

Andaliman (*Zanthoxylum acanthopodium* D.C) is a plant widely used by the Batak people in North Sumatera. Several studies demonstrated that Andaliman fruit exhibits antioxidant activities and cytotoxic effects on cancer cells. Quality of Andaliman fruit could affected by post-harvest process including storage time. This research aims to analyze the compound, antioxidant activity and proliferation inhibition on MCF-7 cells of Andaliman fruit extracts (AFE) stored at varying storage time. A series of fresh 250 g Andaliman fruit sealed in a closed container and stored in the oven at 30ºC. After storage at 0, 24, 48, 72, 96, 120 and 144 hours, Andaliman fruit macerated using methanol and obtained successive extract yields of 4.12; 3.70; 12.79; 8.17; 8.15; 3.7 and 2.21%. These extracts were analyzed for total phenolic, total flavonoids, free radical scavenging activity and proliferation inhibition on MCF-7 cells. The chemical compound analysis of AFE was performed by ultra performance liquid chromatography (UPLC). Results showed that the AFE stored at zero hours had higher total phenol content (47.32 mg gallic acid equivalent (GAE)/g of extract) and free radical scavenging activity (50.38%) at 500 µg/mL significantly compared to other extracts. The total flavonoid content of all extracts showed no difference level except for the extract at 120 hours of storage. The proliferation inhibition test on MCF-7 cells at 100 µg/mL showed that AFE stored at 72, 96 and 144 hours could inhibit MCF-7 cells above 50%. The storage time variation of Andaliman fruit may affect the total phenolic and flavonoid content, and also activity of free radical scavenging and proliferation inhibition on MCF-7. The UPLC analysis founded the major compound of AFE was predicted as α-sanshool. Analysis of chemicals substance in Andaliman fruit with varying of storage time need to be conducted to evaluated alteration of secondary metabolites contained in Andaliman fruit.

Keywords: Methanol extract of Andaliman fruit, storage time, radical scavenging, proliferation inhibition of MCF-7

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ABSTRAK

Andaliman (Zanthoxylum acanthopodium D.C) merupakan tanaman yang banyak dimanfaatkan oleh masyarakat Batak di Sumatera Utara. Beberapa penelitian menunjukkan bahwa buah Andaliman memiliki aktivitas antioksidan dan efek sitotoksik terhadap sel kanker. Kualitas buah Andaliman dapat dipengaruhi oleh proses pasca panen termasuk lama penyimpanan. Penelitian ini bertujuan untuk menganalisis senyawa, aktivitas penangkal radikal bebas dan penghambatan proliferasi MCF-7 ekstrak buah Andaliman (AFE) yang disimpan pada berbagai waktu penyimpanan. Sebanyak 250 g buah Andaliman segar dalam wadah tertutup disimpan dalam oven pada suhu 30ºC. Setelah penyimpanan, pada jam ke-0, 24, 48, 72, 96, 120 dan 144, buah Andaliman dimaserasi menggunakan metanol dan diperoleh rendemen ekstrak berturut-turut sebesar 4,12; 3,70; 12,79; 8,17; 8,15; 3,71 dan 2,21%. Ekstrak-kemudian dianalisis kadar total fenolik, total flavonoid, aktivitas penangkal radikal bebas dan penghambatan proliferasi pada sel MCF-7. Analisa kandungan senyawa dilakukan dengan ultra performance liquid chromatography (UPLC). Hasil penelitian menunjukkan bahwa AFE yang disimpan pada nol jam mempunyai kandungan total fenol lebih tinggi (47,32 mg ekivalen asam galat (GAE)/ g ekstrak) dan aktivitas penangkal radikal bebas (50,38%) pada 500 µg/mL secara signifikan dibandingkan ekstrak lainnya. Kadar flavonoid total seluruh AFE tidak menunjukkan perbedaan kecuali ekstrak pada penyimpanan 120 jam. Uji penghambatan proliferasi sel MCF-7 pada dosis 100 µg/mL menunjukkan bahwa AFE yang disimpan pada suhu 72, 96 dan 144 jam mempunyai kemampuan menghambat sel MCF-7 di atas 50%. Variasi waktu penyimpanan buah Andaliman dapat mempengaruhi kandungan total fenolik dan flavonoid, serta aktivitas penangkal radikal bebas dan penghambatan proliferasi MCF-7. Hasil Analisa UPLC menunjukkan adanya senyawa utama yang diprediksi sebagai α-sanshool. Analisis kandungan kimia pada buah Andaliman dengan variasi waktu penyimpanan perlu dilakukan untuk mengevaluasi perubahan komposisi kimia atau metabolit sekunder.

***Kata Kunci**: Ekstrak methanol buah Andaliman, waktu penyimpanan, penangkal radikal, penghambatan proliferasi sel MCF-7*

INTRODUCTION

Andaliman is the fruit of the Andaliman plant (*Zanthoxylum acanthopodium* DC) family Rutaceae which is widely consumed by the Batak tribe in North Sumatra. The distinctive taste and smell of this fruit attracts people to use Andaliman fruit as a cooking spice. Andaliman fruit also has benefits for maintaining health, including as an antioxidant and anticancer. Andaliman fruit ethanol extract can increase superoxide dismutase (SOD) activity of test animals at a dose of 300 mg/kg BW (Arsiaty *et al*., 2022) and is categorized as having strong antioxidant activity in a test using DPPH with an IC_{50} value of 17.97 µg/mL (Syaputri *et al.*, 2022) and ABTS with IC_{50} *value of 64.45 mg/mL (Dewana et al., 2022).* Previous research also showed that petroleum extract and ethyl acetate of Andaliman fruit have a cytotoxic effect on T47D cells with an IC_{50} of 149.4 and 48.94 µg/mL (Kristanty and Suriawati., 2014; Satria *et al*., 2019), inhibits the proliferation of HepG2 cells through the apoptosis mechanism with an IC_{50} of 122.65 µg/mL (Tala and Siregar., 2022) and MCF-7 cells with an IC_{50} of 221.31 µg/mL (Arsita *et al*., 2019). Moreover, ethanol and ethyl acetate fraction of Andaliman fruit have cytotoxic effect in 4TI cells, it's were IC_{50} of 54.48 µg/mL and 48.1 µg/mL (Rosidah *et al.*, 2019; Harahap *et al*., 2018).

Andaliman fruit was known to contain terpenoids, alkaloids, flavonoids, saponin, tannin, anthraquinone glycosides, and other aromatic compounds (Muzafri *et al*., 2018; Lelyana *et al*., 2021). A terpenoid group are dominant chemical compound in ethanol extract of Andaliman fruit. The biological activity of terpenoid compounds contained in Andaliman fruit such as geranyl acetate, limonin, myrcene, ocimene, linalool, citronellol, neral, and geraniol exerted anticancer activity (Adrian *et al*., 2023). Andaliman is one of traditional spice with a unique volatile citrus-like aroma and numbing trigeminal sensation. The aroma of Andaliman comes from the content of citronellol, geranyl acetate, and limonene (Wijaya *et al*., 2001). Andaliman also has a unique trigeminal sensation described as tingling and numbing in the mouth, which reported that Andaliman contained substituted alkylamides or also known as sanshool (Wijaya *et al*., 2001; Sugai *et al.*, 2005).

Andaliman fruit changes its color from green to red when ripens. Although both the green and red fruit can be used as spices, red fruits easily rot and turn black, which causes losing the characteristic flavour of Andaliman (Wijaya *et al.*, 2018). Hence, the proper postharvest handling of

Andaliman is important to be developed to preserve the quality (Marpaung *et al.,* 2019). Various efforts have been conducted to overcome this such as packaging with aluminum foil and propilen plastic were shown able to keep the pericarp during room temperature storage within 3 days (Marpaung *et al.,* 2019). The best quality of Andaliman powder is obtained in the type of glass and plastic bottles which can be seen from the water content, oleoresin content and organoleptic value (Sitohang *et al.,* 2020). Moreover, the best drying method for Andaliman using oven drying because of its high acceptability, intensities of favorable aroma attributes, volatiles, and sanshools content as well as its short duration and low water activity (Napitupulu *et al.,*2014; Suharta *et a.,l* 2022). Therefore, the storage time of Andaliman fruit might affect the activity of fruit regarding secondary metabolite decomposition. This research was conducted to determine the effect of storage time of Andaliman fruit on free radical scavenging and proliferation inhibition on MCF-7 cells.

MATERIALS AND METHODS

Materials

Andaliman fruit was obtained from Eden Gardens, Toba Regency, North Sumatra, Indonesia. Methanol as solvent extraction obtained from CV. Amor, Indonesia. Gallic acid, quercetin, Folin–Ciocalteu's reagent, 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), aluminium chloride, sodium carbonate was purchased from Sigma Chemical Co., St. Louis, MO, USA. Dulbecco's modified Eagle's medium (DMEM), FBS, penicillin, streptomycin, PBS, 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) and other cell culture reagents were purchased from Gibco BRL Life Technologies Inc.

Preparation of extract

A series of fresh Andaliman fruit in a closed container of 250 g, stored in the oven at 30ºC. After storage at 0, 24, 48, 72, 96, 120 and 144 hours, Andaliman fruit macerated using 2 L methanol with stirring for 2 hours, then filtered. Maceration was repeated 3 times, then the methanol filtrate was evaporated at a temperature of 40ºC by a rotary evaporator. Andaliman fruit extract (AFE) was kept in a bottle at prior to analysis.

Determination of total phenolic content

The total content of phenolic in AFE was measured using the Folin–Ciocalteu method (Ghafar *et al.,* 2010). The extract was dissolved in ethanol Then, 25 µL AFE (500 µg/mL) was transferred into microplate, mixed with 75 µL of 10% (v/v) Folin-Ciocalteu reagent in deionized water. The mixture thoroughly at room temperature for 5 min. Then, 75 µL of sodium carbonate (6%) solution was added to the mixture. The solution was incubated for 90 min in the dark at room temperature. Absorbance was measured at 725 nm using a microplate reader (BioTek Instruments, German). The total phenolic content was determined using a standard curve of gallic acid at 6.25-200 µg/mL concentrations. Total phenolic content was calculated and expressed as mg of gallic acid equivalent per gram of the extract (mg GAE/g extract).

Determination of total flavonoid content

The flavonoid content of AFE was measured using aluminium chloride colorimetry method as described by Singha *et al.,* 2021. Briefly, 50 µL of AFE (500 µg/ml) was added into a microplate containing 50 µL of 95% ethanol (v/v). Then, 20 µL of 10% (b/v) aluminium chloride hexahydrate, 20 µL of 1 M sodium acetate, and 100 µL of water were added to the 96-well plate. The mixture was incubated for 30 min at room temperature and the absorbance was read at 425 nm using a microplate reader (BioTek Instrument, German). Quercetin was used as the standard to determine the total flavonoid content values of the samples. A standard curve was using concentrations of quercetin in the 10–160 µg/mL range and the total flavonoid content values are presented as mg quercetin equivalent per gram of extract (mg QE/g extract).

DPPH free radical-scavenging assay

Scavenging activity of AFE on DPPH radicals was determined using the modified method of Zhao *et al*., 2020. Briefly, 50 µl of AFE (500 µg/mL) was placed in 96-well plates. The reaction was initiated by adding 150 µL of DPPH solution (0.1 mM in methanol). The mixture was left to stand at room temperature for 30 min. After that, the absorbance values were read at 517 nm using microplate reader (BioTek Instruments, German). Ascorbic acid as positive control and assay was carried out in triplicates. The percentage inhibition of DPPH radical scavenging activity was evaluated by the following equation: DPPH radical scavenging activity (%) =[(A1-A2)/A1] ×100, where A1 refers to the absorbance of the blank solution and A2 refers to the absorbance of the sample solution in ethanol.

MTT assay detect cell proliferation

MCF-7 cell proliferation of AFE was evaluated using MTT (3-(4,5-dimethlylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay using a previously described protocol Twentyman and Luscombe, 1987. MCF.7 cells were seeded into 96-well plates at a density of 10^5 cells/mL then incubated the cells at 37°C in a humidified 5% CO_2 atmosphere for 24 h. Andaliman fruit extract, AFE (100 µg/mL) were added into each well followed by incubation for 24 h. Thus, 100 µL 0,5 mg/ml MTT solution was added, and the cells were then incubated for another 4 h, the formazan crystals were dissolved in 100 µL 10% Sodium Dodecyl Sulphate in 0.1% HCl overnight. The absorbance was measured at 450 nm by a microplate reader (BioTek Instruments, German).

Analysis of chemical composition of AFE

The AFE was analysis using UPLC (Waters Manchester, UK) with an Acquity UPLC BEH column (1.8 µm, 2.1 x 100 mm) as stationary phase. The UPLC spectrum of AFE was obtained with a mobile phase comprising ultrapure water grade (solvent A) and acetonitrile (solvent B), each with 0.1% formic acid. A gradient method was employed for separation, transitioning from 5% to 100% solvent B between 1 and 20 minutes, maintaining 100% solvent B from 20 to 22.3 minutes, and reverting to 5% solvent B from 22.4 to 25 minutes, with a flow rate of 0.4 mL/min and 1 µL volume injection. For MS analysis, quadrupole mass spectrometer with electrospray ionization was used as a detector. The analysis was conducted in positive ion mode, employing full-scan mass spectra acquisition within the range of 50-1200 m/z.

Statically analysis

All data are presented as mean ± standard deviation. Data were analyzed by ANOVA followed by Bonferroni test. Results were considered significant at $p < 0.05$. Data were analyzed using IBM SPSS 27.01 software.

RESULTS

Percentage yield of AFE

The AFE was obtained by maceration using methanol as a solvent. The extract was a sticky semisolid, dark brown colour with lemon-like aroma. The percentage yield of AFE stored at 0, 24, 48, 72, 96, 120 and 144 hours were 4.12; 3.70; 12.79; 8.17; 8.15; 3.71 and 2.21% respectively.

Total Phenolic content of AFE

Table 1. shows the total phenolic content of AFE measured using Folin-Ciocalteu method. Total phenolic values were obtained from the calibration curve $y = 0.0071x - 0.0076$ with $R^2 = 1$, where x is the absorbance and y is the concentration of gallic acid solution (mg/mL). The phenolic content of AFE range from 17.29 to 47.32 mg GAE/g of extract.

Total flavonoid content of AFE

Total flavonoid of AFE is reported in Table 1. The highest total flavonoid was obtained in storage time at 144 hours (48.30 ± 4.85 mg QE/g extract), which similarly not different with AFE storage time at zero hour (41.20 ± 4.57 mg QE/g extract).

Radical scavenging activity of AFE

The free radical scavenging activity was determined using DPPH method. The effect of storage time of AFE on DPPH scavenging ability is shown in Table 1. This activity was shown to decrease by increasing the storage time of AFE. The antioxidant property of DPPH was mainly based on the ability of DPPH and a stable-free radical, to decolorize in the presence of antioxidants.

Cell proliferation inhibition activity of AFE

The proliferation inhibition assay was performed by MTT method. The AFE was added at concentration of 100 µg/ml for 24 hours into MCF.7 cell. The result showed that AFE stored at 72, 96 and 144 hours inhibited MCF-7 cell proliferation above 50% as shown in Table 1.

Table 1. Total phenolic content, flavonoid content, radical scavenging and proliferation inhibition of AFE (*Total phenolic content, flavonoid content, radical scavenging and proliferation inhibition of AFE*).

Storage time (*Waktu Penyimpana*) **(hour)**	**Total Phenolic Content (***Kandungan Fenol Total***) (mg GAE/g extract)**	**Total Flavonoid Content (***Kandungan Flavonoid Total***) (mg QE/g extract)**	**Radical scavenging (***Penangkapan Radikal***) (%)**	**Proliferation inhibition** (*Penghambatan Proliferase*) **(%)**
0	47.32 ± 0.00	41.20 ± 4.57	50.38 ± 1.40	37.61 ± 4.84
24	24.51 ± 0.42[a]	38.79 ± 6.18	30.01 ± 0.69[a]	9.60 ± 0.70[a]
48	26.88 ± 0.66[a]	36.08 ± 3.61	25.70 ± 2.05[a]	47.23 ± 9.17
72	27.32 ± 0.0[a]	32.98 ± 2.96	26.63 ± 1.48[a]	63.72 ± 2.10[a]
96	19.26 ± 0.5[a]	37.77 ± 4.39	19.89 ± 1.48[a]	87.34 ± 1.33[a]
120	17.29 ± 0.40[a]	27.59 ± 1.54[a]	29.05 ± 1.79[a]	37.05 ± 1.36
144	24.06 ± 0.36[a]	48.30 ± 4.85	18.93 ± 0.59[a]	54.64 ± 9.19[a]

GAE= gallic acid equivalent, QE= quercetin equivalent, means within a column, followed by letters (a) is significantly different to zero hours by Bonferroni test, $p < 0.05$. (GAE= gallic acid equivalent, QE= quercetin equivalent, means within a column, followed by letters (a) is significantly different to zero hours by Bonferroni test, $p < 0.05$.(*GAE= setara asam galat, QE= setara quercetin, artinya dalam kolom diikuti huruf (a) berbeda nyata dengan nol jam dengan uji Bonferroni, $p < 0,05$*.)

Chemical compound analysis of AFE

The UPLC chromatogram of AFE is presented in Figure 1 and Tabel 2. Based on the data, it shows that AFE has a major peak in the retention time range of 12.8 minutes and AU of 52.45-80.52%.

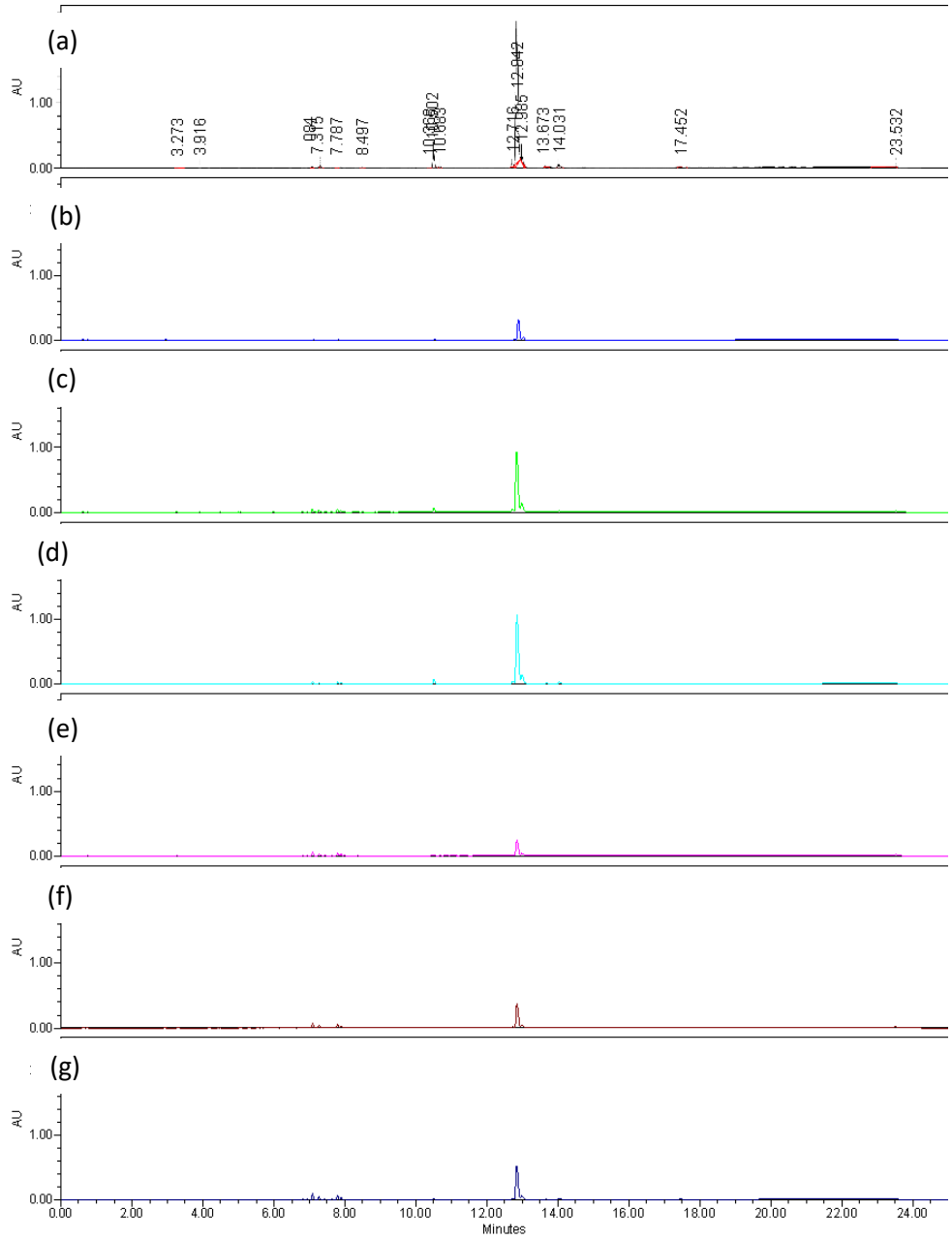

Figure 1. UPLC chromatogram of AFE at PDA spectrum 254 nm. Storage at (a) 0-hours, (b) 24-hours, (c) 48-hours, (d) 72-hours, (e) 96-hours, (f) 120-hours, and (g)144-hours (*Kromatogram UPLC AFE pada spektrum PDA 254 nm. Penyimpanan pada (a) 0 jam, (b) 24 jam, (c) 48 jam, (d) 72 jam, (e) 96 jam, (f) 120 jam, dan (g)144- jam*).

(e)

Tabel 2. Retention time, abundant unit (AU), molecular weight (m/z) and prediction compound of AFE using PDA detector at 254 nm (*Retention time, abundant unit (AU), molecular weight (m/z) and prediction compound of AFE using PDA detector at 254 nm*).

Time storage (*Waktu Penyimpanan*) **(hours)**	**Retention time** (*Waktu Retensi*) **(min)**	**AU (%)**	**Molecular weight** (*Berat Molekul*) **(m/z)**	**Prediction compound** (*Senyawa Prediksi*)
0	12.842	80.52	248.45	Sanshool (Alkyl Amida)
24	12.884	64.48		
48	12.834	71.59		
72	12.843	75.28		
96	12.841	52.45		
120	12.842	57.83		
144	12.837	61.68		

DISCUSSION

Andaliman fruit extract (AFE) was obtained by the maceration method using methanol solvent. Extract yield represents the number of compounds that can be extracted from raw materials and the more compounds that can be extracted, the higher yield would be obtained. The extract was a sticky semisolid, dark brown with a lemon-like aroma. The percentage yield of AFE stored at 0, 24, 48, 72, 96, 120, and 144 hours were 4.12; 3.70; 12.79; 8.17; 8.15; 3.71, and 2.21% respectively. In the maceration, at storage time 48 hours (12.79%) was the maximum followed by 72 and 96 hours. Other studies have been carried out to show different yields of Andaliman fruit extract. Muzafri et.al 2018 showed that extracting Andaliman fruit with methanol for 72 hours followed by several shakes had a 4.15% yield (Muzafri *et al*., 2018). Maceration of Andaliman fruit powder using ethanol 96% also generated an extract with a yield of 11.9% (Arsiaty *et al*., 2022) and 13.19% (Suyanto *et al*., 2004).

Quality of fruit and plant could be affected by post-harvest process including storage time. Del-Toro-Sánchez *et al*., 2015, reported phenolic content of leaf, stem, and rhizome methanolic extracts of Anemopsis californica were stable during at least 60 days at 4ºC and at −20ºC, even though not stable at 50ºC and 35ºC. However, they decreased drastically of total phenolic after storage at 60 days on all treatments (50, 25, 4, and -20ºC). Galani *et al*., 2017, found that the dill leaves, onion leaves, green pepper, cauliflower, eggplant, tomato, carrot, potato, sugar beet, sapota, pomegranate, banana, grape had a lowest the phenolic content after storage during 15 hours at 4ºC. Sitohang *et al*., 2020, found the storage time of Andaliman fruit has a very significant effect on water content, total acid, oleoresin content and organoleptic value, but not significant to ash content. The oleoresin content and organoleptic values of Andaliman powder decrease after storage 2, 4, and 6-weeks.

Analysis of total phenol content in AFE based on the conversion of phenolic compounds expressed in gallic acid equivalents (GAE), as a response to phenolic compounds found in Andaliman fruit. Table 1, showed that the total phenol content of Andaliman fruit significantly decreased after zero hours and 30 ºC of storage, with 42-63% reduction compared to zero hours extracts ($P<0.05$). Polyphenol stability is a very important aspect to ensure the phenol component maintains activity during processing stages such as postharvest handling. The polyphenols are widely seen as very unstable and highly susceptible to degradation, which can involve high temperatures, light, oxygen, solvents, the presence of enzymes, proteins, metallic ions, or association with other food constituents (Volf *et al*., 2014). Total phenolic content also can be influenced by many factors including genotype, harvest time, growing location, solvent and method of extraction (Rumbawoa *et al.*, 2009; Madiwale *et al.*, 2011; Purbosari *et al.*, 2020). Decreasing of the total phenolic content of Andaliman fruit might be due to the process of enzymatic at the 30ºC. The results of total flavonoid content of AFE at stored for 120 hours is lower significantly compared to AFE at 0, 24, 48, 72, 96 and 144 hours of storage. Storage time of AFE for 144 hours revealed that flavonoid content was relatively stable.

The free-radical scavenging activity was carried out using the DPPH assay, based on the ability of a test compound to reduce the color intensity of DPPH radicals. The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay was chosen because this method is simple, easy, fast and sensitive and only requires a small sample to evaluate the antioxidant activity of natural compounds. The free-radical scavenging values at zero hours was significantly higher compared to another storage time (24, 48, 72, 96, 120 and 144-hours), which degradation range of radical scavenging from 40 to 62%. In this study, radical scavenging activity of AFE could be related with their phenolic content. This result shows that the radical scavenging activity decreased related to the total phenolic contents, which may be the phenolic compounds were unstable and degraded during storage after zero hours.

Effect of AFE on proliferation inhibition was carried out on MCF-7 cells by MTT method. Table 1, the effects of different storage time of AFE on the proliferation inhibition against MCF-7 cells, showed that all extracts of AFE reduced the viability of MCF.7 cells compared to untreated cells. Among all those extracts, AFE at 72, 96 and 144 hours of storage exerted proliferation inhibition on MCF-7 above 50%, which means that AFE at 72, 96 and 144 hours of storage had potential activity as an anticancer on MCF.7 cells. Several previous studies reported that Andaliman has a potential activity as an anticancer. Rosidah 2019, reported that ethanolic extract of Andaliman fruit had moderate cytotoxic activity on 4T1 breast cancer cells and the value of IC_{50} was 54.48 ± 0.22 µg/mL. Another study also reported that cytotoxic activity (IC_{50}) of ethyl acetate fraction of Andaliman fruit on 4T1 proliferation was 48.1 ± 1.06 µg/mL (Harahap *et al.*, 2018). On the other hand, ethanolic extracts of Andaliman seeds also reduced the growth of MCF.7 breast cancer cells with IC_{50} 221 µg/ml (Arsita *et al*., 2019).

The cytotoxic effect of Andaliman extract is associated with the content of active compounds present in Andaliman fruit. The Zanthoxylum genus contains important alkaloids, including benzophenanthridines, furoquinolines, oporphines, which have medicinal uses. Many studies reported that Alkaloids are group compounds responsible for the cytotoxic activity of Zanthoxylum (Pachón *et al*., 2007; Ngoumfo *et al*, 2010; Sardjo *et al*, 2014, Wang *et al*, 2015, Syari *et al*., 2019). Benzophenanthridine alkaloid from another species (*Z. madagascariense* Baker), rutaceline has been reported for its cytotoxic activity on Caco-2 cells by inducing apoptosis, cell cycle arrest at the G0/G1 phase and DNA fragmentation, and by inhibiting DNA synthesis (Pachón *et al*., 2007). Acridone alkaloids from *Zanthoxylum leprieurii* Guill exhibited a cytotoxic effect with LD_{50} 13.1 µg/mL in brine-shrimp (*Artemia salina* Leach) and demonstrated activity against A549 and DLD-1 cells (Ngoumfo *et al*., 2010). Benzophenanthridine alkaloid, 1-methoxy-12- methyl-12,13-dihydro-(1,3) dioxolo (4′,5′:4,5) benzo (1,2-c) phenanthridine-2,13-diol, contained in Z. buesgenii had moderate to strong cytotoxicity activity on several cell lines, including CCRFCEM, MDA-MB231, HCT and HepG2 (Sandjo *et al*., 2014). Benzophenanthridine derivates from *Zanthoxylum nitidum* exhibited A549, HeLa, SMMC-7721 and EJ cancer cells with IC_{50} 27.50; 37.50; 16.95; and 60.42 µM respectively (Wang *et al*., 2014). Alkaloid fraction from *Zanthoxylum acanthopodium* DC. fruits also have provided effectively as anticancer towards T47D, 4T1, MCF-7, HeLa, and Raji cells (Syari *et al.*, 2019)

The UPLC result of Andaliman fruit after 0, 24, 48, 72, 96, 120 and 144-hour storage is summarized in Table 2. The chromatogram from each extracts showed that the major peak was at the retention time of 12.8 minutes, showing the highest AU at 0-hour storage with m/z 248.45 and predicted as α-sanshool compounds (Suharta *et al*., 2022; Sugai *et al*., 2005).

Based on UPLC measurements, α-sanshool was a major compound of Andaliman according to its concentration contained in Andaliman. α-sanshool reached the highest content in AFE at 0, 48 and 72 of storage which means that there was no correlation between α-sanshool content and cytotoxic activity of AFE on MCF.7 cells. Analysis of other compounds needs to be conducted to know the compounds responsible for cytotoxic activity on MCF.7 cells, especially benzophenanthridine and acridone. The hydroxy-α-sanshool or (2E,6Z,8E,10E)-N-(2′methylpropyl)-dodecatetraenamide is one of the primary compounds in Szechuan pepper (*Z. bungeanum*) which is often found in the fruit pericarp. The α-sanshool or hydroxy-α-sanshool and contained in Zanthoxylum are responsible for the numbing and tingling sensation. The α-sanshool was reported

to have a photoprotective effect of UVB irradiation by increasing cell viability, inhibiting MMP expression, and inducing autophagy in human dermal fibroblast (Hao *et al.*, 2019). Hydroxy-α-sanshool has also been reported to inhibit *Cutibacterium acnes* (*C. acnes*) growth, expression of reactive oxygen species (ROS), inflammatory mediators, and the activation of NF-κB and STAT3 pathways induced by *C. acnes* in HaCat and THP-1 cells (Zhou *et al*., 2023).

The post-harvest handling of Andaliman fruit remains a challenge for researchers fresh Andaliman fruit is prone to damage due to the instability of compounds. As shown in Figure 2, there is similar profile between the inhibition of free radicals and the inhibition of MCF.7 cells proliferation, and further studies still need to be conducted to find its correlation.

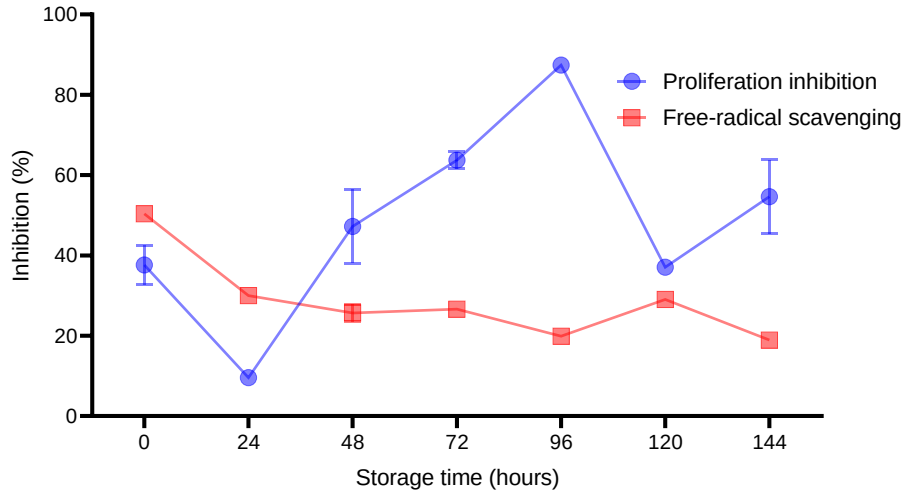

Figure 2. Effect of storage time of Andaliman fruit on free-radical scavenging and proliferation inhibition of MCF-7 cells (*Pengaruh Lama Penyimpanan Buah Andaliman Terhadap Penangkal Radikal Bebas dan Penghambatan Proliferasi Sel MCF-7*).

CONCLUSION

The storage time variation of Andaliman fruit may affect the activity of free radical scavenging and proliferation inhibition on MCF-7. From our research we can conclude that free radical scavenging activity decreased with the duration of storage time. The proliferation inhibition on MCF-7 of AFE stored at 72, 96 and 144 hours could inhibit MCF-7 cells above 50%. The major compound of AFE was predicted as α-sanshool.

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AUTHORSHIP CONTRIBUTION

All authors have contributed to this paper. IR, SAK, MD, MMM, RS and AEW: Conceptualization. IR, SAK, GSS, MD, MMM, RFK, and AEW: Data curation. IR and AEW: Formal analysis. AEW: Funding acquisition. IR, GSS, MMM, RFK and AEW: Investigation. IR, SAK, MD, MM, RSM, CC and AEW: Methodology. RS and AEW: Project administration. IR, SAK, MD, NN, MMM, RFK and AEW: Resources. MD: Software. IR, SAK, and AEW: Supervision. GSS: Validation. IR, SAK, MD, MMM, RFK and AEW: Visualization. IR, SAK and AEW: Writing-original draf and writing-review and editing.

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