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Research Article

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Antioxidant ability of pepino fruits (*Solanum muricatum* Aiton) in apoptotic modulation of yeast cells (*Saccaromyces cerevisiae* FNCC 3012)

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ABSTRACT

Various plants have been used traditionally by communities for treatment cardiovascular disease. In this research, fruit extracts of Solanum Muricatum Aitonwas studied. The aim of this research was to determine the effect of antioxidative potential of these extracts on the ability to modulate apoptotic in yeast (Saccharomyces cerevisiae FNCC 3012) cells. Antioxidative potentials were analyzed using Thiobarbituric acid (TBA) assay by measuring the concentration of Malonaldehyde (MDA) upon linoleic acid oxidation using spectrophotometer UV-Vis at 532 nm and vitamin E was used as a control antioxidant. Apoptotic activity was determined by counting the colony of yeast cells and measuring the frequency of petite yeast cells after treatment with extract followed by incubation. The results of viability test showed that petit cells at glucose 4% more than 60%, whereas control of aquadest seen its amount only 35,05%. The raising level of concentration caused petit cells percentage is bigger, whereas at concentration 300 ppm it was be down. From this research, it can be concluded that Solanum Muricatum Aiton extract showed midle antioxidative potential as well as midle inhibition of apoptotic activity in yeast cells (Saccharomyces cerevisiae FNCC 3012).

Keywords: *Solanum Muricatum* Aiton; Antioxidant; *Saccharomyces cerevisiae* FNCC 3012.

INTRODUCTION

Reactive oxygen (ROS) and nitrogen (RNS) species, as well as other free radicals are potentially capable of producing damage in various biological macromolecules (DNA, proteins and lipids) playing an important role in the etiology of various diseases such as cancer, diabetes, cardiovascular diseases and premature aging [1-3]. Some enzymatic antioxidant mechanisms promote the protection of human cells against the harmful effects of free radicals, but these antioxidant mechanisms may not be enough to combat oxidative stress, which results from the imbalance between production and elimination of free radicals [4]. Accordingly, certain amounts of antioxidant supplements are necessary to ensure the balance of reactive species derived from oxygen and nitrogen produced by pathophysiological metabolism.

One of means that should be done in order to prevent cardiovascular disesase is to consume of antioxidant which neutralize free radical [5]. It can be functionized as modulator of apoptosis and fasten metal ion in forming of ROS (Reactive Oxygen Substrate) species which finally generate degenerative problem specially disesase, i.e cardiovascular [6]. One of plants with potential antioxidant is *Solanum Muricatum* Aiton.

The Pepino fruit (Solanummuricatum Aiton.) which is an exotic fruit is also known as melon pear and sweet cucumber. Altough is is native to South America, it is also grown in Australia, New Zealand and USA. It contains a high percentage of their fresh weight as water (92%), it is low in calories, very rich in minerals and contains vitamins like thiamine, niacin, riboflavin and and ascorbic acid (vitamin C), ideal for a number of metabolic and antioxidant reactions [7], also contain flavonoid 53.6 mg/RE with total phenol as 24.68 mg/GAE [8].

Over the last decade, interest in the search for antioxidants from natural sources has increased and various studies by the scientific community have reported the importance of fruits and vegetables in the prevention of chronic diseases resulting from oxidative stress [9]. The plants have a rich source of molecules with antioxidant potential, as phenolic com-pounds, carotenoids, vitamins, flavonoids and terpenoids [10-11]. Therefore, greater attention is turned to the antioxidants of natural origin, which may act by inhibiting lipid peroxidation and/or neutralizing reactive oxygen species and nitrogen, resulting in the modulation of oxidative stress [12].

The interest in working with medicinal plants is the therapeutic value they have and because there are few studies on the antioxidant capacity of *Solanum Muricatum* Aiton. Thus, this study aimed at providing scientific evidence of the antioxidant capacity of the methanol extract of fruits of *Solanum Muricatum* Aiton through various tests. Tests were conducted to study the antioxidant capacity in *Saccharomyces cerevisiae* proficient and deficient in antioxidant defenses, evaluation of antioxidant capacity *in vitro* method for inhibition of hydrogen peroxide and lipid peroxidation.

EXPERIMENTAL SECTION

Materials and Methods

The raw pepino fruits was obtained from a farm in Rejang Lebong, Bengkulu, Indonesia. The fruits were carefully selected in order to obtain a uniform batch in relation to size and degree of maturity. Methnaol P.a, ethanol P.a, acetic acid, and hydrochloric acid (HCl) were purchased from Merck, 1.1.2-trimethoxypropane (TMP), trichloroacetic (TCA), α-tocopherol and thiostembituric acid (TBA) from Sigma-Aldrich, linoleic acid from Fluka were used as received. Destilled water, phosphate buffer pH 7, yeast extract, glucose P.a, pepton, Nutrient agar and Mg ribbon are available at Chemistry Laboratory, Faculty of Mathematics and Natural Science, University of Bengkulu, Indonesia.The strains of *S. cerevisiae* was kindly provided by The Indonesian Institute of Science, Jakarta.

Preparation of sample and extraction

The fruits were cleaned and cut into small pieces before being dried over the sunshine. After drying, all samples had water contents below 10 %. They were ground to a fine powder in a mechanical blender and kept at room temperature prior to extraction. It was macerated by using technical methanol 96 % for 3 days and than was filtered by using filter paper to dissociate filtrate with residue of pepino fruits. Filtrate condensed by uing rotary evaporator with 90 rpm at temperature 50^0 C. It was stored in refrigerator until it was used.

Qualitative test of flavonoid

Extract was dripped on drip plate, then given of methanol 96%, added 2 cutting-ribbon of Mg and 2 mL drip of HCL condensed 98%. The red colour was formed by addition of concentrate HCL showing the existence of flavonoids. As comparator used betelnut with the same procedure [13].

Determination of total phenolic content

Total phenol content was determined by the method adapted with some modifications using the Folin-Ciocalteu reagent. One ml of the extract was mixed with 1 ml of Folin-Ciocalteu's phenol reagent. After 3 min, 1 ml of saturated Na_2CO_3 (35%) was added to the mixture and it was made up to 10 ml by adding deionised distilled water. The mixture was kept for 90 min at room temperature in the dark. The absorbance was measured at 725 nm against the blank. The total phenolic content is expressed as mg of gallic acid equivalents (GAE) per gram of dry extract [14].

Analysis of hydroperoxide from oxidation of linoleic acid by method of diene conjugation

Briefly, analysis of hydroperoxide from oxidation of linoleic acid done by added 2 mL phosphate buffer 0.1 M pH 7, 2 mL of linoleic acid 50 mM in etanol 99.8%, and 1 mL destilled water into dark bottle, then incubated at temperature 40°C. Mixture of sample taken by 50 µL, and then added into 6 mL ethanol 75%. Absorbance of diene conjugation of sampel measured by used of UV-VIS Spectrophotometer at wavelength 234 nm. Analyze hydroperoxide measured every day till got maximum absorbance [15].

Concentration analysis of malonadialdehida (MDA) with method of TBA

Extract of pepino fuits made in variety concentration (100 ppm, 200 ppm and 300 ppm). Each sampel was taken 1 mL, it was added 2 mL phosphate buffer 0.1 M pH 7.0 and 2 mL linoleic acid 50 mM in etanol 99.8%. As positive control used 1 mL α-tocopherol, 2 mL phosphate buffer mL 0.1 M pH 7.0 and 2 mL linoleic acid 50 mM in etanol 99.8%, while for negative control 1 mL α-tocopherol replaced by 1 mL destilled water. All mixtures packed into dark bottle and incubated at temperature 40°C during optimum incubation. At the time of maximum incubation, measurement of TBARS (*Thiobarbituric Acid Reactive Substance*) through method of TBA by taking 1 mL from each samples was added 2 mL of TCA 20% and 2 mL of TBA 1% in acetate acid 50%. As blank is used destilled

water with same treatment. The mixture put down in bath at 100°C during 10 minutes. After chilled, it was centrifuge 3000 rpm during 15 minutes and then solution is measured at λ 532 nm.

Standard curve have been made by using 1.1.2-trimetoxypropane (TMP) with variety concentration, such as 1.5, 3, 6, 9, 12, 15, and 18 µM, every solution was taken 1 mL and added 2 mL TCA 20% and 2 mL of TBA 1% in acetate acid 50%. The mixture put down in bath at 100 °C during 10 minutes. After chilled, it was centrifuge 3000 rpm during 15 minutes and then solution is measured at λ 532 nm. The destilled water with same treatment was used as a blank.

Cultures of *Saccharomyces cerevisiae.*

To evaluate the antioxidant capacity of the pepino fruits extract against oxidative damage, strains of *S. cerevisiae* FNCC 3012 was used in antioxidant defense system. *S. cerevisiae* cultures were grown in YEPD (1% yeast extract, 2% peptone, 2% dextrose), it was incubated at temperature 28° C for 2 days. The sporulated diploid cells were transferred to liquid YEPD medium and 0.1 % glucose was added. The suspension was again incubated at 28°C for 4 days still got stationary-phase. The cells were pelleted at 4000 rpm, washed twice in 40 ml of water, and resuspended in small volume of water.

Antioxidant capacity in *S. cerevisiae* cellsand Viability Tests

The mixture containing 600 µL pellets and pepino fruits extract at concentrations of 100, 150, 200 and 300 ppm was incubated at 37° C for 24 h. As negative control is used destilled water and 4% glucose as positive control, it was done by same procedure. The procedure were carried out in triplicate. Cells were grown exponentially in YEPD medium to 2 x 10^7 cells per ml for stationary-phase cells, it were incubated after they reached 5 x 10^8 cells per ml. 50 µL cells of *S. cerevisiae* has been cultured and treatment were transferred to solid YEPD medium, it was incubated at 28°C for 3 days. The colony was counted and compared by control, the formula showed below.

$$\frac{\sum petit\_colony}{\sum petit\ colony + \sum normalcolony}\ x\,100\%$$

RESULTS AND DISCUSSION

Based on qualitative analysis of flavonoid, it showed that *Solanum Muricatum* Aiton extract contained of flavonoid (+++), which means that sample contain plenty of flavonoid compared to betelnut (+++). More content of flavonoid in samples, more effective activity of antioxidant yielded. Most interest has been devoted to the antioxidant activity of flavonoids, which is due to their ability to reduce free radical formation and to scavenge free radicals [16].

Flavonoid is chemical compound which included into group of antioxidant could be used to lessen continuous reaction related to free radical [17]. Compound of flavonoid was assumed to component of bioactive could be function as natural antioxidant capable to inhibition produce of peroxide lipid at system of linoleic [18]. The total phenol and percent yield of ripe methanol extract of pepino fruit were found to be 23,96 mg GAE dry weight, 13.3% respectively.

Analysis of hydroperoxide from oxidation of linoleic acid by method of diene conjugation

Determination of linoleic acid incubation time by the use of diena conjugation method is conducted before measurement of activity of sample oxidation. Analyze the hydroperoxide measured every day till got maximum absorbance.

Table 1. Absorbance of diene conjugation

Days	Absorbance			Average absorbance
	Replicate 1	Replicate 2	Replicate 3	
1	0.0906	0.0622	0.0562	0.0696
2	0.1275	0.0944	0.0890	0.1036
3	0.2264	0.1170	0.0090	0.1174
4	0.1329	0.1290	0.1236	0.1285
5	0.1622	0.1454	0.2018	0.1698
6	0.1271	0.1231	0.1315	0.1272
7	0.1374	0.1138	0.1351	0.1287

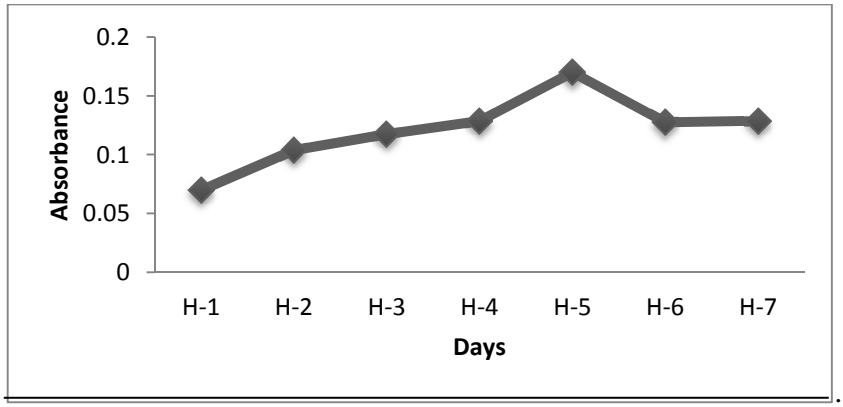

Figure 1. Maximum absorbance of diene conjugation

Analysis of diene conjugation gave result of measurement which reach from first day and down after 5 days. Figure 1 showed the maximum absorbance is 0.1698 on days to 5 which caused by the optimum product of hydroperoxide [19-20]. Linoleic acid was oxidized by oxidation agent at early stage to form hydroperoxide. Hydroperoxide will be decomposition form malondialdehida (MDA) as the final product reaction of lipid peroxidation. The purpose of measurement of MDA after 7 days was to give the chance of hydroperoxide have perfect decomposition. Determination of linoleic acid incubation time by the use of diene conjugation method, influenced by some factor could be assign value maximum absorbance which was different each other, such as the quality of linoleic acid, temperature of incubation which is not constant, and also existence of oxygen [21].

Analysis potency of antioxidant in methanol extract of pepino fruits

Compound of MDA (malondialdehida) was formed from hydroperoxide decomposition analyzed with method of TBA (Thiobarbituruc acid) to determine it's antioxidant activity which measured as *tiobarbituric acid reactive substance* (TSTEMS) and react with TBA form red coloured complex of MDA-TBA with maximum absorption at λ 532 nm. As its standard curve is used 1.1.2-trimethoxypropane (TMP) at various concentration such as: 1.5, 3, 6, 9, 12, 15, and 18 µM. TMP used as standard because could produced MDA if hydrolysis with acid [22]. Equation of linier regretion was calculated that is $y = 0.1718x + 0.0181$, $R^2 = 0.9993$ was used to determine the concentration of MDA and inhibition pepino fruit extract (Figure 2).

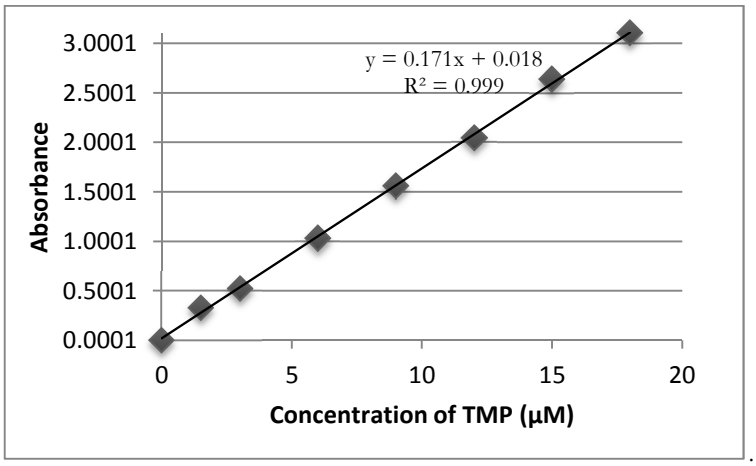

Figure 2. Curve ofstandard 1.1.2-trimethoxypropane (TMP)

Based on Figure 3, it showed that addition of methanol extract of stem purpel yam could inhibit oxidation of linoleic acid, marked with concentration of MDA measured smaller than negative control. Negative control has the highest concentration of MDA compared to other treatments, that was 5.6935 µM. It was caused by inexistence compound which can be function as antioxidant, so that linoleic acid continue to oxidation form much of MDA. α-tocopherol 200 ppm had concentration of MDA equal to 0.8463 µM. It indicates that α-tocopherol as antioxidant could inhibit produce of MDA. α- tocopherol as proton donor can change radical of peroxyl (product of lipid peroxidation) becoming radical of tocopherol which was less reactive, so that was unable to destroy chain of linoleic acid [23]. Autooxidation of linoleic acid inhibit in existence of antioxidant compound [24]. Addition of methanol stem purpel yam extract to linoleic mixture was done in early stage (days 0) to result antioxidant maximum effect. In existence

of antioxidant, concentration of malonaldehide (MDA) which produce from autooxidation smaller than without addition antioxidant. Absorbance value is proportional to concentration of MDA and inversely with activity of antioxidant [25].

Table 2. Absorbance of standard 1.1.2-trimethoxypropane (TMP)

TMP (µM)	Absorbance
0	0.0001
1,5	0.3267
3	0.5221
6	1.0307
9	1.5608
12	2.0411
15	2.6361
18	3.106

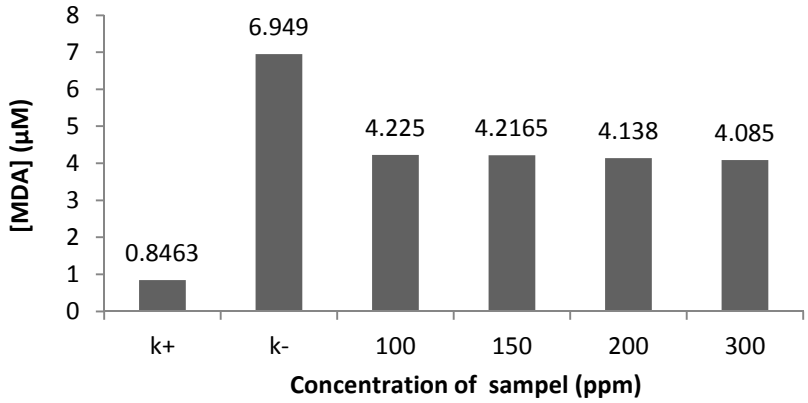

Figure 3. Concentration of MDA in variation of sample

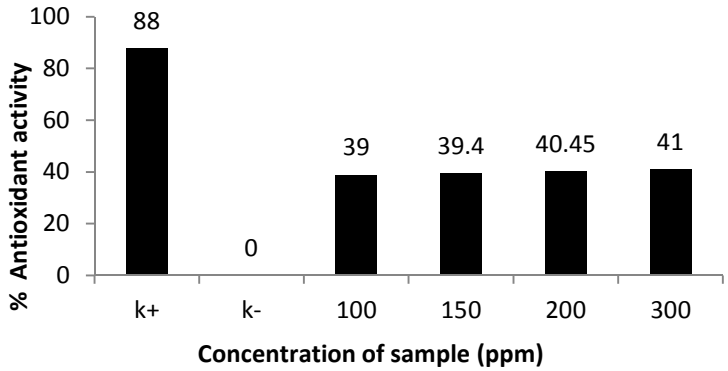

Figure 4. Percentage of inhibition of oxidation pepino fruits extract

Inhibition activity forming of MDA of each treatment calculated from rate of MDA obtained (done by tree repetition) showed at Figure 4. Percentage of inhibition show the level of potency each extract as antioxidant. Negative control used as reference to determine percentage of resistivity because no treatment on it, so that process oxidation is normal without existence of extract. α-tocopherol 200 ppm as positive control having inhibition ability for 88%. At positive control was used 200 ppm concentration, 200 ppm is the limit of existence antioxidant in our body [26]. Pepino fruits extract with concentration 100, 150, 200 and 300 ppm each has inhibition ability 39%, 39.4%, 40.45% and 41%. According to the research, in general, extract of pepino fruits have activity of antioxidant but still below α-tocopherol as positive control.

Ability of pepino fruits extractin apoptotic modulation of yeast cells (*Saccaromyces cerevisiae* FNCC 3012)

It has been reported that colony of yeast cells (*Saccaromyces cerevisiae*)was apoptotic can be differentiated from normal colony. One of characteristic apoptotic colony that is turning into colony of petit caused by losing of ability of respiration at mitokondria as effect of apoptotic process, so that cells growth rate of yeast was apoptotic slowly from cells of normal [27]. As a result, colony form and cell dimensions of yeast turning into colony of petit become smaller.

Cells of *S. cerevisiae* was apoptotic as effect of damage at mitocondria can't used ethanol as source of carbon. By minimum concentration of glucose, cells of *S. Cerevisiae* which have apoptotic can grew but small of the size (Figure 5A). While cell do not apoptotic can use etanol as source of carbon because its mitocondria doesn't damage, so that cell of *S. Cerevisiae* even grew better (Figure 5B).

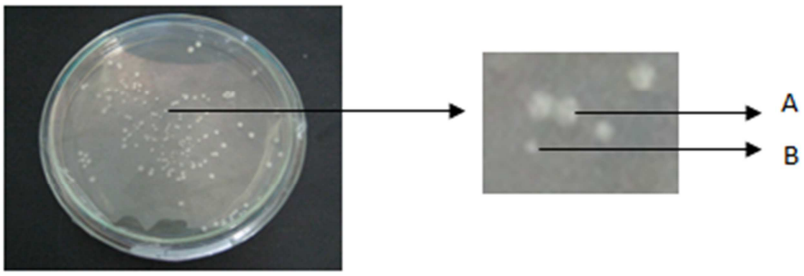

Figure 5. Colonicells of *S. Cerevisiae,* (a) normal cell and (b) petit cell

Apoptotic activity was determined by counting the colony of yeast cells and measuring the frequency of petite yeast cells after treatment with extract. Petit test at this research using glucose as positive control because glucose 2% can induce apoptotic at yeast cells [28]. Test of frequency of petite yeast cells which induction by glucose showed positive result, glucose influence yeast cells become petit. It was according to the theory mentioning that cell which apoptotic showed characteristic of morfologis for example creasing of cell or petit [29].

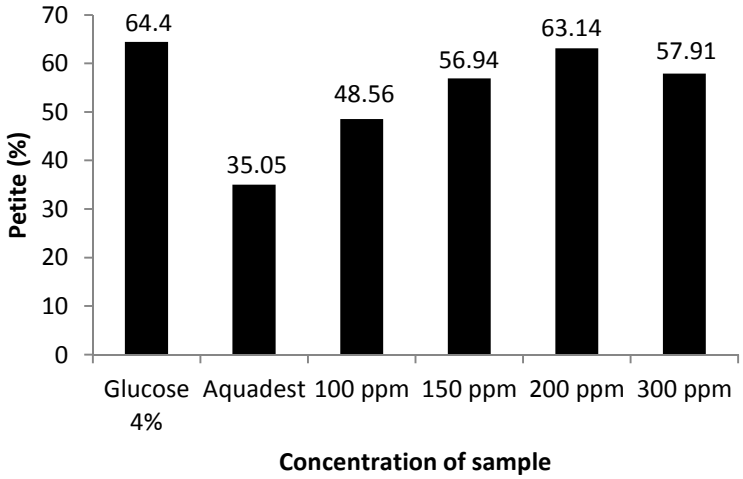

Figure 6. Percentage petite cells of *S. Cerevisiae*

The result of research seen that amount of petit cells at glucose 4% more than 60%, whereas control of aquadest seen its amount only 35,05% (Figure 6). Glucose can caused death of yeast cell in a few hours without addition of other nutrition to support its growth, marked with reactive oxygen species (ROS) production quickly, degradation RNA and DNA, damage of membrane, fragmentation and decrease of cell nucleus [28]. Figure 6 indicate that raising level of concentration caused petit cells percentage is bigger, whereas at concentration 300 ppm it was be down. Phenolic antioxidants i.e flavonoid are effective at low concentration, at high level phenolic compound become prooxidants because of their reactivity and participation in initiation process [30].

Flavonoid compound influence apoptoticby modulation protein expression of antiapoptotic (Bcl-2, Bcl-xl) or proapoptosis (Bax, Bid, Basin) [31]. Some other natural materials compound which contain polifenol compound like

kaemferol, inhibit induction of apoptosis in VSMCS by 7ß-hidroksikolesterol [32]. Newest research showed that flavonoid inhibit apoptosis in miocardial and protect normal cell (Et al Nandave. 2005).

CONCLUSION

Qualitative test of flavonoid showed that pepino fruit extract contained of higher flavonoid compared to betelnut. Pepino fruit extract which contained flavonoid at concentration 100, 150, 200 and 300 ppm each had inhibition ability 39%, 39.4%, 40.45% and 41%. In general, pepino fruit extract showed activity of antioxidant but it was still below α-tocopherol as positive control. In this research, activity of antioxidant was determined by calculating inhibition ability of the extract to inhibit oxidation of linoleic acid. The raising level of extract concentration caused petit cells percentage is bigger, whereas at concentration 300 ppm it was be down. From this research it can be concluded that *Solanum Muricatum* Aiton extract showed inhibition of apoptotic activity in yeast cells (*Saccharomyces cerevisiae* FNCC 3012).

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