

EVALUATION OF ANTIOXIDANT AND ANTIBACTERIAL ACTIVITIES OF FRESH AND FREEZE-DRIED SELECTED FRUIT JUICESNARITSARA TOOBPENG¹, PANNAPA POWTHONG², PATTRA SUNTORNTHITICHAROEN^{3*}

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ABSTRACT

Objective: The objective of this investigation was to assess the biological activity of fresh fruit juices and freeze dried fruit juices including antioxidant and antibacterial activity.

Methods: The fruits used in this study were *Schleichera oleosa* (Lour.) Oken, *Carissa carandas* L., and *Sandoricum koetjape* (Burm.f.) Merr. The fresh juice sample (FJS) was extracted and also prepared for freeze drying sample (FDS) by freeze dryer. The antioxidant capacity of FJS and FDS was assessed using the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) assays and determination for total phenolic contents (TPCs) by Folin-Ciocalteu's reagent. Antimicrobial tests were carried out by agar diffusion methods and evaluated by measuring the zone of inhibition against 10 isolates of pathogenic bacteria.

Results: The highest antioxidant activity of FJS and FDS was found in *S. oleosa* and related to the scavenging effect on DPPH radical of FJS (100% concentration) and FDS (200 mg/ml) was found in the value of 93.05±0.31% and 82.18±0.33%, respectively. TPC of FJS and FDS from *S. oleosa* at the same concentration as in the DPPH assay was 1,003.53±1.96 ug GAE/300 g of matter and 827.77±23.15 ug GAE/100 ml of matter, respectively. Both FJS and FDS revealed antibacterial activity. The zone of inhibition of FJS was ranging from 8.3±0.6 to 24.0±1.0 mm and FDS values of 7.3±0.5-21.0±0.9 mm. The most potent sample against bacteria was FJS and FDS from *S. oleosa* which FJSs against *Escherichia coli* ESB1 and FDS against *Pseudomonas aeruginosa* MDR2.

Conclusion: Fruit juices of *S. oleosa*, *C. carandas*, and *S. koetjape* associated with antioxidant activity in the form of both fresh fruit juices and FDS. The result of this study showed that the value of DPPH and TPCs were slightly decreased in FDS compare with fresh juice. Screening of FJS and FDS of *C. carandas* revealed broad spectrum antibacterial activity. However, FJS of *S. oleosa* and *S. koetjape* showed inhibition of growth of bacteria, but few of FDS from these fruits could inhibit limited bacterial isolates. For antibacterial activity against pathogenic bacteria, the samples of fresh and freeze dried exhibited a different degree of activity.

Keywords: Fresh juices, Freeze dried, Antibacterial, Antioxidant, Fruit.

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INTRODUCTION

In the human living system, oxidation is known to involve a series of free radical in the form of reactive oxygen and nitrogen species-mediated chain reactions which antioxidants protect the body from damage caused by harmful molecules. Antioxidant could reduce reactive oxygen species or delay the chain reaction of an oxidizable substance, and scavenge free radical. Phenolic compounds are phytochemicals which play a major role in the protection of oxidation processes. There is some comfort knowing that such antioxidants are purified from natural products that have been consumed for generations [1]. Thus, they have a wide range of biological activities, such as excellent antioxidant activities and protected against antimicrobial activities. The researcher reported that the amount of phenolic compounds in plant depends on cultivation techniques, cultivar, growing conditions, extraction methods, extraction temperature, and ripening process, as well as processing and storage conditions [2].

Apart from plants, some naturally consumed fruits also have important bioactivity properties. Many of the health advantages of fruits are attributed to the phytonutrients or bioactive compounds within plants that provide health benefits beyond that of the traditional nutrients. Among many advantageous properties, phytonutrients contribute direct and indirect bioactivity attributable to their free radical scavenging

ability [3]. The majority of other compounds may be vitamin C, vitamin E, β -carotene, or flavonoids, which there are strong antioxidant activities [4]. Fruit juices are primarily considered as a main source of phenolic compounds. The high in polyphenols contents caused in a high antioxidant activity [5]. Natural products have interesting biological activity. It possesses an interesting challenge in organic chemistry, and has remarkable structural diversity and biological characteristics, providing researchers with exciting possibilities to develop novel molecular entities for human therapeutics [6]. Many natural products have a variety of biological activities and have always been a significant source of new lead compounds in pharmaceutical industries [7]. Increasing antibiotic resistant microbe problems lead to urgent needs for new antimicrobial agents, especially new sources of the agents.

Thailand is a major source of tropical plant varieties, which are associated with many medicinal properties. Fruits are the excellent sources of antioxidant and antimicrobial from natures. There are a large number of fruits in Thailand. *Schleichera oleosa* (Lour.) Oken. is classified in a family of Sapindaceae. The fruits tasted sour and could prepare for fruit juices. Many parts of *S. oleosa* have been studied about pharmacological importance, including antioxidant, antiulcer, anticancer, and antimicrobial activities [8]. *Carissa carandas* L. belongs to family Apocynaceae. The crude extracts of *C. carandas* leaves have

been evaluated for antioxidant [9]. In addition, fruits of *C. carandas* were examined for antioxidant and nutraceutical property [10]. In addition, fruits, leaf, and root of *C. carandas* has been reported from the crude extract and their different fractions and isolates which were evaluated for antioxidant [9]. *Sandoricum koetjape* (Burm.f.) Merr. is the plant belonging to the family Meliaceae. The phytochemical and pharmacological properties from fruits, seeds, leaves, and bark were studied [11]. Numerous triterpenoids have been found from stem bark. Although earlier reports show the antimicrobial and antioxidant potential in some part of them, but there are many of them that remain unexplored.

In the season of fruits, lots of product is easily found in the market. The limitation of the short season of fresh fruit and large amounts of product in the market could be solved by processing the fruits. Freeze dried method is the way that decrease water from the substances [12]. This method is a more powerful process for preserving the contents of the fruits for value adding of fruits when its flood in the market. The composition of the phenolic compounds in juice products depends on the juice treating application [13]. There are physical-chemical changes in fruit products due to lyophilize processing. Values of antioxidant activity also depend strongly on the preparation of samples. The stability of components and bioactive properties require exploring for proving the properties of processing fruits while the fruits are limited in very short periods of the year. The processed food in the form of freeze drying could be used for promoting for healthy food and more precious than fresh fruits which are easily denatured.

This study aims to evaluate the antioxidant and antimicrobial activities of fresh fruit juices and freeze dried fruit juices from *S. oleosa*, *C. carandas*, and *S. koetjape*.

METHODS

Bacterial strains

About 10 isolates of multidrug-resistant bacteria, which contains seven Gram-negative strains: *Pseudomonas aeruginosa* MDR1, *P. aeruginosa* MDR2, *Klebsiella pneumoniae* ESBL, *Escherichia coli* P174 ESBL, *E. coli* ESBL, *Acinetobacter baumannii* MDR1, and *A. baumannii* MDR2 and three Gram-positive strains: *Enterococcus faecalis*, *Staphylococcus aureus* methicillin-resistant *S. aureus* MRSA1, and *S. aureus* MRSA2 were used. Bacteria were stored at -80°C .

Chemicals

2, 2-diphenyl-1-picryl-hydrazyl (DPPH), Gallic acid, and Folin-Ciocalteu reagents were purchased from Sigma-Aldrich, Germany. Ascorbic acid was purchased from Unilab, England. All other chemicals and reagents used in the study were of analytical grade.

Collection of plant material

Three ripe fruits, *S. oleosa* (Lour.) Oken, *C. carandas* L., and *S. koetjape* (Burm.f.) Merr., were obtained from the Nakhon Ratchasima province, Thailand on July 2016. The fruits were washed with tap water and air dried before used.

Preparation of fresh fruit juice samples

About 300 g of each ripe fruit were washed, peeled and cut into smaller bits, then was extracted by extractor for 30 seconds. The fruit juice was filtered through a Whatman filter paper and kept as fresh juice samples (FJS) at -20°C until use.

Preparation of freeze dried samples (FDS)

About 100 ml of fresh fruit juice has been freeze dried by vacuum freeze dryer for 24 hrs. Then, the FDS were kept on -20°C until use.

Antioxidant activity

DPPH assay

The potential antioxidant activities of the samples were determined using DPPH according to Powthong and co-workers (2013) [14]. Briefly,

100 μl of the fresh juice (100%, 50%, 25%, 12.5%, and 6.25% serially) and FDS (concentration of 12.5, 25, 50, 100, and 200 mg/ml) prepared in ethanol was mixed with 100 μl of 0.2 mM DPPH (DPPH, Sigma) prepared in methanol. The mixture was shaken vigorously and incubated under dark at room temperature for 30 minutes, and the absorbance was then measured at 517 nm against a blank. Simultaneously, a control was prepared without sample extracts. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\text{DPPH scavenging effect (\%)} = [(A_0 - A_1/A_0) \times 100]$$

Where A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample. All measurements were performed in triplicate.

Determination of total phenol content

Total phenolic compounds were determined using Folin-Ciocalteu's method [14]. Briefly, 20 μl of the fresh juice (100%, 50%, 25%, 12.5%, and 6.25% serially) and FDS (concentration of 12.5, 25, 50, 100, and 200 mg/ml) prepared in ethanol was mixed with 100 μl of Folin-Ciocalteu's reagent. Then, 80 μl of 2% aqueous sodium carbonate was added into the mixture. The mixture was incubated for 30 minutes, and the absorbance of the mixture was measured at 765 nm against the reagent blank. The content of total phenol was calculated on the basis of the calibration curve of gallic acid, and the results were expressed as μg of gallic acid equivalents (GAEs) per 300 g or 100 ml of matter. All measurements were performed in triplicate.

Screening of antibacterial activity by agar diffusion method

Antimicrobial activity was determined by agar diffusion methods. All microorganisms were first left 16-18 hrs at 37°C in nutrient agar base, then touch at least colonies with a sterile loop for inoculum into 0.85% NaCl. Cellular density of inoculum was obtained by optical densities measured at 625 nm by a spectrophotometer to obtain the required bacterial concentration as 10^8 CFU/ml or OD of 0.08-0.1 [15].

A suspension of any tested bacteria containing about 10^8 CFU/ml was spread on a Mueller-Hinton agar plate by three-way swab technique using sterile cotton swabs. For FJS, the Mueller-Hinton agar plate was pouched by cork borer (\varnothing 6 mm), and 30 μl of the fresh samples were added into agar well. Gentamicin (300 μg) was used as positive control.

FDS were prepared in DMSO at a concentration of 200 mg/ml. Paper discs were placed on Mueller-Hinton agar plates containing tested bacteria then added 10 μl of each FDS into paper disk. Which each paper disc contains 2 mg of FDS. 10 μl of DMSO were tested as a negative control. The plates were then incubated at 37°C for 24 hrs, and the diameter of the inhibition zone was measured. Triplicates of each sample have been done. The results were revealed by the average \pm standard deviation (SD).

RESULTS AND DISCUSSION

The difference in total antioxidant capacity (DPPH) and total phenolic content (TPC) among selected fresh fruit juice and its FDS showed in Figs. 1 and 2. DPPH scavenging activity was varied in all selected fruit juice, and its freeze-dried form. It was exhibited that the DPPH scavenging activity of FJSs was ranging from $4.50 \pm 3.50\%$ to $93.05 \pm 0.31\%$. Among the FJSs, the highest scavenging activity was found in FJSs of *S. oleosa* with a value of $93.05 \pm 0.31\%$ at a concentration of 100%. While the DPPH scavenging activity of FDS was ranging from $5.67 \pm 2.60\%$ to $82.18 \pm 0.33\%$. Among the FDS, the highest scavenging activity was found in *S. oleosa* with a value of $82.18 \pm 0.33\%$ at a concentration of 200 mg/ml. We found that when various concentrations of either fresh fruit juice or its FDS were subjected to determined total antioxidant capacity, DPPH levels revealed increasing in a dose-dependent manner. Moreover, DPPH radical scavenging activity values of FDS showed a nonstatistically significant decreased when compared to its fresh fruit juice.

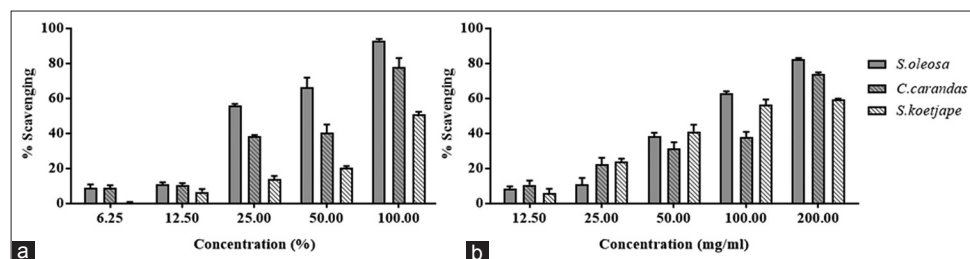


Fig. 1: The free radical scavenging activity of fruit juice samples and freeze dried samples. Antioxidant activity is expressed as the mean±standard deviation. (a) The free radical scavenging activity of fresh juice samples. (b) The free radical scavenging activity of freeze-dried samples

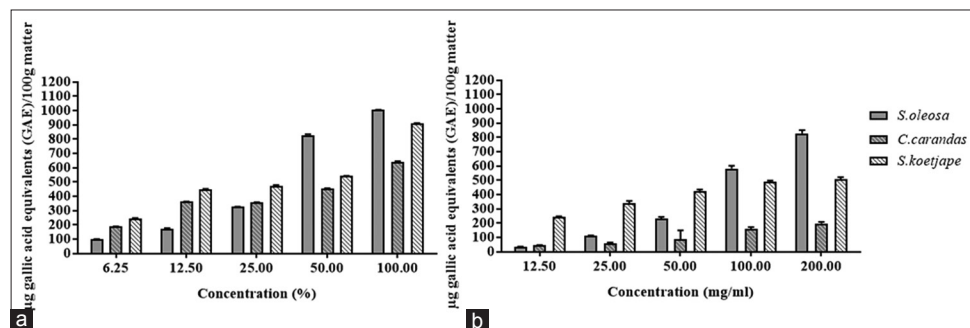


Fig. 2: Total phenolic contents of fruit juice samples and freeze dried samples. Antioxidant activity is expressed as the mean±standard deviation. (a) Total phenolic contents of fresh juice samples. (b) Total phenolic contents of freeze-dried samples

In addition, the TPC of FJSs ranging from 100.30 ± 0.52 mg GAE/300 g of matter to $1,003.53 \pm 1.96$ mg GAE/300 g of matter. Among the FJSs, the highest TPC was found in FJSs of *S. oleosa* with a value of $1,003.53 \pm 1.96$ mg GAE/300 g of matter at a concentration of 100%. The TPC of FDS was ranging from 32.00 ± 4.38 mg GAE/100 ml of matter to 827.77 ± 23.15 mg GAE/100 ml of matter. Furthermore, a highest TPC was found in FDS of *S. oleosa* with a value of 827.77 ± 23.15 mg GAE/100 ml of matter. It was observed that the concentration of TPC, both fresh fruit juice and its FDS increased in a dose-dependent manner. TPC values of FDS showed a nonstatistically significant decreased when compared to its fresh fruit juice same as we observed in DPPH assay.

The data presented in the study demonstrated that *S. oleosa* has phenolic content and show excellent activity against DPPH radicals. Previous phytochemical studies of *S. oleosa* fruit showed that it contains terpenoids, flavonoids, tannins, and steroids [16]. From this result suggests that *S. oleosa* is a good choice for medical used or cosmetic agent against free-radical-associated oxidative damage [17]. Therefore, it can be further subjected to isolate the therapeutic antioxidant compounds activity and evaluate their pharmacologically.

In a general way, the antioxidant values increased with the increase of concentration, possibly due to the higher extraction of antioxidant phenolic compounds. This fact can be associated with higher concentration of fresh juice and FDS. Moreover, we observed nonsignificant differences in both total antioxidant capacity (DPPH) and TPC between fresh fruit juice and its FDS. It was revealed that the value of DPPH and TPC was slightly decreased according to extraction procedure. In addition, there is some previous research which describes the extraction process depended on antioxidant activity of fresh juice from other plants. Banana peels were revealed their antioxidant capacities of various extracts using DPPH assays [18]. Phytochemical properties of fruit extract of *Elaeocarpus ganitrus* were also determined for phenolic and flavonoid compounds [19]. Yan and Kerr (2013) reported that higher temperature used during vacuum belt drying of pomace negatively impacted the TPC [20]. The results of this study are also similar to those reported by Sogi *et al.* (2013) for mango peel and kernel drying, they used four different drying methods; freeze drying -20°C , hot air drying at 60°C , vacuum drying

at 60°C , and infra-red. The highest value of total phenolic was noticed in FDS and hot air drying at 60°C was lower than FDS [21]. According to Chellaram *et al.*, 2014 [22] purposed that the preparation of sample (freeze dehydration or lyophilization) is the cause of decreasing in antioxidant activity. The main reason may be the loss of phenolic compounds, which are the heat-sensitive bioactive compounds that can indicate the quantity of antioxidant activity. Therefore, the results of our study are similar to those in the previous reports and indicate that extraction process may serve as a slightly decreasing in antioxidant activity.

Identification of the antioxidants in *S. oleosa*, which are responsible for hydrogen and electron donation and metal chelation will supplement the findings of the study. The result of the trial is a good way to develop utilizing *S. oleosa* extracts as a commercial antioxidant will be greatly advanced through optimization of the extraction procedure. In another hand, it can also be further developed as a product to increase the value of the *S. oleosa* on the season, which has a large amount of output.

The result of *in vitro* antimicrobial activity of fresh and FDS was shown in Table 1 with different samples against the microorganisms. DMSO did not inhibit the growth of testing microorganisms (unpublished data), while gentamicin did. The fresh juice samples, *S. oleosa* pH 4.24, *C. carandas* pH 3.41, and *S. koetjape* pH 4.31, showed a zone of inhibition against almost all of the tested pathogenic bacteria with a zone of inhibition ranging from 8.3 ± 0.6 to 24.0 ± 1.0 mm. Among FJS, the most effective samples were FJS of *S. oleosa* against *E. coli* ESBL. For the FDS (2 mg), a zone of inhibition was ranging from 7.3 ± 0.5 to 21.0 ± 0.9 mm. The highest zone was FDS from *S. oleosa*. Only *C. carandas* inhibited all kinds of the tested bacteria (7.7 ± 0.5 to 17.0 ± 1.2 mm) while *S. oleosa* and *S. koetjape* were observed against *P. aeruginosa* MDR2. But *S. oleosa* could inhibit one more bacteria, *S. aureus* MRSA1.

In this study, antibacterial activity of fresh juices of all three fruits showed a broad spectrum against pathogenic bacteria. In general, pH of three selected fresh fruit juice samples was lower than 5. Evaluation of pH in foods is important because bacteria require a neutral pH environment to grow. The low pH of these fruit juices may affect the growth of bacteria. Previous study, leaf and seed extract of *S. oleosa* showed antibacterial

Table 1: Antibacterial activity of FJS and FDS on different bacterial strains

Microorganism	Zone of inhibition (mm)						
	<i>S. oleosa</i>		<i>C. carandas</i>		<i>S. koetjape</i>		Gentamicin
	FJS	FDS	FJS	FDS	FJS	FDS	FJS
<i>P. aeruginosa</i> MDR1	20.0±1.5	-	22.0±3.6	17.0±1.2	13.0±1.0	-	11.0±1.0
<i>P. aeruginosa</i> MDR2	22.0±1.0	21.0±0.9	23.0±1.0	15.0±1.2	15.0±1.5	10.0±4.9	10.0±0.6
<i>K. pneumoniae</i> ESBL	15.0±2.6	-	13.0±1.0	7.7±0.5	-	-	12.0±0.6
<i>E. coli</i> P174 ESBL	21.0±1.5	-	19.0±1.0	15.0±0.5	14.0±1.0	-	9.3±0.6
<i>E. coli</i> ESBL	24.0±1.0	-	22.0±2.0	9.7±1.9	15.0±0.6	-	12.0±0.6
<i>A. baumannii</i> MDR1	20.0±2.1	-	21.0±1.5	9.7±0.9	15.0±1.5	-	8.7±0.6
<i>A. baumannii</i> MDR2	20.0±2.0	-	21.0±1.2	11.0±1.2	15.0±1.0	-	11.0±0.6
<i>E. faecalis</i>	18.0±1.0	-	17.0±1.5	12.0±0.5	8.3±0.6	-	15.0±0.6
<i>S. aureus</i> MRSA1	20.0±2.5	7.3±0.5	21.0±1.5	10.0±0.5	16.0±1.5	-	10.0±1.0
<i>S. aureus</i> MRSA2	21.0±1.5	-	22.0±2.1	13.0±0.8	16.0±1.5	-	10.0±1.0

Values are means inhibition zone (mm)±SD of three replicates. FJS: Fresh juice sample, FDS: Freeze drying sample, *P. aeruginosa*: *Pseudomonas aeruginosa*, *K. pneumoniae*: *Klebsiella pneumoniae*, *E. coli*: *Escherichia coli*, *A. baumannii*: *Acinetobacter baumannii*, *E. faecalis*: *Enterococcus faecalis*, *S. aureus*: *Staphylococcus aureus*, MRSA: Methicillin-resistant *Staphylococcus aureus*, *S. oleosa*: *Schleichera oleosa*, *C. carandas*: *Carissa carandas*, and *S. koetjape*: *Sandoricum koetjape*

activity of against six organisms. The highest inhibition was found in *S. aureus* with an inhibition zone of 15 mm [23]. The presence of various phytochemical constituents in the extracts of *S. oleosa* influenced antioxidant activity. Leaf and seed extracts of *S. koetjape* was performed for the phytochemical constituents and antimicrobial activity on clinical isolates from patients [24]. The phytochemical showed the presence of tannins, phenols, flavonoids, alkaloids, saponins, steroids, and cardiac glycosides which involved in antioxidant and antimicrobial properties. The antibacterial susceptibility assay of *C. carandas* leaf extracts was done against the pathogenic microbes [25]. Fruits of *C. carandas* also had some inhibitory activity against selected bacterial cultures [26].

According to the components of FDS of *S. oleosa* and *S. koetjape*, especially the lyophilize process, it showed the moderate inhibitory effect against bacteria. This may be due to the chemical changes. The previous study demonstrated that the presence of polyphenols may have contributed to this result, as many molecules of this chemical group can appear antimicrobial activity [27]. In the investigation of the FDS of *C. carandas* demonstrated the antimicrobial activity against the 10 tested microorganisms, showed that *C. carandas* was the potential antibacterial agent. The process of freeze dryer on *C. carandas* may cause less effect on the active antibacterial compartments which could lead to study more in the future.

The previous study of many fruit extracts for antibacterial properties was revealed, for example, fruit extract of *Avicennia officinalis* [28], fruit of *Averrhoa carambola* Linn. and *Ziziphus mauritiana* Lam., [29] and the dried fruit of *Embelia basaal* [30] showed potent broad spectrum antibacterial properties. Fruits are important constituents in human and animal nutrition; however, storage of fruits has limitations, because they are perishable and deterioration by microbial and environmental factors that has a strong influence in shelf life. As a result, the stability of juice depends on the preparation, for example, lyophilize or freeze drying process becomes an important way to improve the value and storage of properties of fruit juices during the period of plenty and low costs of the seasonal fruits.

CONCLUSION

The results of this study reveal that the fresh juice extracts of *S. oleosa* have broad ranges of antibacterial activities and could be good potential sources for screening programs of bioactive natural products. The antioxidant activities demonstrated clearly that these extracts contain a number of antioxidant compounds which can effectively scavenge DPPH under *in vitro* condition. Its suggested that multiple mechanisms are involved. This study indicated that the extracts from *S. oleosa*, are the best sources of natural antimicrobial which might be useful in treating the diseases associated with pathogenic bacteria. Moreover, the freeze dry extraction process may be a cause of slightly

decreasing of these antioxidant activities, but significantly decreasing in antimicrobial activity, which results of loss of phenolic compounds due to its heat sensitive property. This report may be useful for evaluation of pharmaceutical or treatment of infections depending on the extraction process. Further analysis should be considered analysis of vitamins, minerals and dietary fiber of the selected fruit with increase options for extraction shelf life which serve as value-added products to local fruit. Furthermore, the processing fruit in the form of freeze drying could be the alternative method for providing the antioxidant properties for developed natural products for well preserve and save space. This report may useful for evaluation these advantage properties for pharmaceutical purpose of the fruits in the future.

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