



## Proximate and *in-vitro* bioactivity analysis of fruit tea infusions prepared with locally available fruits of Manipur, India

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### ABSTRACT

Two different fruit tea infusions (dragon tea and roselle-pineapple tea) were formulated using various locally available fruits and analyzed the proximate, phenolic, flavonoid content and antioxidant activities. Further, the fruit teas were evaluated for sensory quality for consumer acceptance. Both the tea infusions contained a high amount of total phenolic content corresponding to 368.9±0.07 mg GAE 100g<sup>-1</sup> (Gallic acid equivalent 100g<sup>-1</sup> sample) in dragon fruit and 664.9±0.31 mg GAE 100g<sup>-1</sup> in roselle-pineapple fruit tea, while flavonoid content of dragon fruit and roselle-pineapple tea was recorded as 111.0 ±0.14 mg QE 100g<sup>-1</sup> (Quercetin Equivalent per 100g sample) and 232.2±0.12 mg QE 100g<sup>-1</sup> respectively. Higher antioxidant activity was shown by roselle-pineapple (460.0±0.2mg Ascorbic acid Equivalent per 100 g sample) as compared to dragon fruit (129.7±0.07mg Ascorbic acid Equivalent per 100 g sample). Both the fruit tea infusions also contained high vitamin C content. Moreover, the present fruit tea infusions have shown high sensory quality. Time of infusion is directly correlated with sensory quality.

**Keywords :** Antioxidant activity, dragon fruit, flavonoid content, fruit tea, phenolic content, roselle-pineapple

Among the beverages, tea is one of the most consumed beverages all over the world (Zieniewska *et al.* 2020). There are different types of teas however all the types of tea are made from the leaves of the *Camellia sinensis*. The differences in the processing of the tea leaves are the primary reason for producing various types of tea with different tastes and appearances even though they all are made from the leaves of the same plant. They are rich in natural polyphenols and other bioactive compounds which are known to have health-beneficial effects. However, tea contains caffeine (3.5%) which upon regular and high consumption is known to have sides effects on human health. The major side effect of caffeine includes insomnia, low calcium absorption, restlessness, nausea and irregular heartbeat (Nawrot *et al.*, 2003). So, people are looking for other beverages which are rich in natural antioxidants but free of caffeine. One such alternative is herbal tea; generally, they are prepared from natural ingredients of different morphological plant parts such as leaves, stems, roots, fruits, buds and flowers (Chandrasekara and Shahidi, 2018). Herbal teas or beverages are rich in natural bioactive compounds *viz*; alkaloids, anthocyanins, carotenoids, coumarins, flavonoids, polyacetylenes, polyphenols, saponins, terpenoids, etc. The formulation of herbal tea may be of a single or mixture of compositions of several plants.

As herbal teas are rich in natural antioxidants, they can be considered as an important part of a healthy diet. Antioxidants are compounds that can scavenge the free radicals which are very reactive and harmful to healthy living. Our body also generated free radicals or reactive oxygen species (ROS) as a part of the body's metabolism and it also has the endogenous mechanism to deal with it. However the excess generation of ROS causes oxidative stress which is related to the etiology of many degenerative diseases (Singh *et al.*, 2021). So, the removal of such excess free radicals is very important for healthy living. Consumption of natural antioxidant-rich herbal tea preparations will help in scavenging harmful free radicals from our bodies. Besides antioxidant activity, herbal teas are reported to have several bioactivities such as antidiabetic, anticholesterol, antihypertensive, antibacterial activity *etc.* Herbal teas especially fruit tea infusions are successfully replacing sweetened drinks and juices because of their taste, aroma, flavour and attractive appearance. Hence, there is increasing market demand for new products having a nutritive and appealing appearance. Considering this, the present study was undertaken with the specific objective of formulation of mixed fruit tea with locally available fruits and analysis of its nutritive and antioxidant activities.

## MATERIALS AND METHODS

### *Chemicals and reagents*

The chemicals used for the analysis of nutritional content were procured from Sigma Aldrich (Merck, India), Hi-media (India) and SRL (India). The teabags used for the study were of food-grade quality.

### *Collection of raw materials*

The matured and healthy dragon fruits, orange, lemon, pineapple, roselle and ginger were procured from Imphal market, Manipur. The fruits were washed thoroughly with tap water 2 to 3 times. The peels of the fruits were removed and the fruit pulp was sliced with stainless steel knife then kept separately. Dehydration of the peel and fruit was done by using an electric dehydrator at 58°C at different times. Dragon fruits and peels were dehydrated at 58°C for 15-16 h, orange peels were dehydrated for 10hr, lemon pulp was dehydrated for 15hrs, pineapples slices were dehydrated for 17-18 h, roselle fruits were dehydrated for 12-13 h and ginger slices were dehydrated for 10 h respectively till it retained 3-5% moisture level. The dehydrated fruits pulps, peels and ginger were separately transferred to a food-grade polythene pouch after sealing with an electric sealer then stored at room temperature till used.

### *Preparation of fruit tea*

For the preparation of blended herbal tea, the different components were separately ground for 20-30 seconds using a kitchen blender. Fine powders were removed using a sieve (2 mm pore size) and the coarse sizes were selected for the purpose. Herbal tea was formulated by mixing different ingredients (Table 1). For packaging, 2.5 gm of the mixture was transferred to each food-grade tea bag or sachet and packed with an electric sealer. The individual teabag was transferred to a plastic pouch and sealed again with the electric sealer.

### *Proximate composition*

Ash (Method No. 930.05), crude protein (Method no. 955.04), and fat (Method No. 2003.05) contents were determined according to AOAC methods (2005). Nelson-Somogyi method was used for the estimation of reducing and non-reducing sugar (Sadasivam and Manickam, 2008). While anthrone method was followed for the estimation of total carbohydrates. Vitamin C present in the sample was determined by the spectrophotometric method (Sadasivam and Manickam, 2008).

### *Sample preparation for in-vitro bioactivity*

One gram of fruit tea samples was soaked into 100 mL (80°C) and kept for 10 min. The mixture was sieved

and the tea infusion was used for further analysis of antioxidant activity, flavonoid and total phenol.

### *Total phenolic and flavonoid content*

The content of polyphenols in every sample was assayed following the Folin–Ciocalteu method (Singleton and Rossi, 1965). The value was expressed as GAE 100g<sup>-1</sup> DW (Gallic acid equivalent per 100-gram dry weight). Flavonoid content was determined according to Barros *et al.* (2008) and the value was expressed as QE 100g<sup>-1</sup> DW (Quercetin equivalent per 100 gram dry weight).

### *Antioxidant assay*

Antioxidant activity was determined by the DPPH method (Thaipong *et al.*, 2006). The antioxidant activity was expressed as mg ascorbic acid equivalent per gram of dry weight sample (AAE g<sup>-1</sup> DW).

### *Sensory evaluation of fruit tea*

Sensory evaluation of fruit tea was conducted to determine the consumer preference rating and acceptability. The sensory qualities were estimated by the 30 semi-trained panelists. Panelists were familiar with product sensory evaluation; most of them had knowledge of fruit tea preparation. Analysis was done using Nine Point Hedonic Scale (Peryam and Pilgrim, 1957; Larmond, 1977). In order to assess the effect of infusion time on sensory parameters, the fruit tea samples (2.5gm) were infused with 100 ml freshly boiled water in a glass cup for 2-minute intervals (2,4,6,8 and 10 minutes). One sample was provided at a time and panelists were asked to give scores for the sensory characteristic such as colour, flavour, taste, appearance and overall acceptability of the blended fruit tea. Before starting another sample, water was given to neutralize the taste and other samples were provided to give their preference and overall acceptability. The experiment was replicated thrice.

### *Statistical analysis*

All the analytical assays were performed in triplicate and data are presented as means ± standard deviations (SD). Descriptive analyses, one-way ANOVA (p = 0.05), and Duncan's multiple range test (DMRT) at 5% level of significance was used for separation of the mean (Rovira *et al.*, 2011). Pearson's coefficient correlation analysis between the different mean sensory scores of tea types was performed using SPSS version 22. The heat map was created using the R package "gplot" as an upgraded version of the basic stats function (Warnes *et al.*, 2005). Simple regression was worked out using Microsoft XLSTAT.

## RESULTS AND DISCUSSION

Proximate analysis of the fruit tea was presented in Table 2. Dragon fruit tea and roselle tea had similar

protein content. While total sugar and total carbohydrate content were higher in dragon fruit as compared to roselle-pineapple fruit tea. Zieniewska *et al.* (2020) had analyzed seventeen fruit teas available in the market where they observed that the protein content ranged from 0.7- 1.2 g 100g<sup>-1</sup>, fat content ranged from 0.1-0.7 g 100g<sup>-1</sup> and carbohydrate content from 10.3-18.9 g 100g<sup>-1</sup>. The protein and carbohydrate content of the present study was higher than these seventeen reported commercially available fruit teas. The main components of the present fruit tea *viz.* dragon, roselle and pineapple fruit were not present in the composition of fruit teas used in their studies. Both the fruit tea infusion was found to contain a good amount of vitamin C. The vitamin C content in roselle-pineapple tea and dragon fruit tea was recorded as 83.86 ±3 mg 100g<sup>-1</sup> and 86.29 ±4.2 mg 100g<sup>-1</sup>. This value is comparable with fruits that contain a high amount of vitamin C such as oranges and lemons.

Polyphenols and flavonoids are the two important phytochemicals present in plants. These phytochemicals are responsible for many bioactivities exerted by the plant extracts. Roselle-pineapple tea had a higher TPC and flavonoid content than the dragon fruit tea. The TPC value of roselle-pineapple and dragon fruit tea was found to be 664.9 ±0.3 mg GAE 100<sup>-1</sup>g DW and 368.9±0.07 mg GAE 100g<sup>-1</sup> DW respectively. Zieniewska *et al.* (2020) had studied the nutritional and antioxidant properties of seven different fruit teas available in the market, where they found TPC values ranging from 0. 699± 0.19 mg to 51.31± 0.9 mg GAE 100g<sup>-1</sup> DW, while the flavonoid content of roselle-pineapple and dragon fruit tea was found to be 111.0 ±0.14 and 232.2 ±0.12 mg QE 100g<sup>-1</sup> DW respectively. This showed that roselle-pineapple tea had a much higher flavonoid content than the dragon fruit tea infusions.

The antioxidant activity analysis revealed that roselle-pineapple tea (460±0.2 mg AAE 100g<sup>-1</sup>) exhibited higher antioxidant activity than the dragon fruit tea (129±0.07 mg AAE 100g<sup>-1</sup>) in the DPPH assay. The higher antioxidant activity of roselle-pineapple tea may be contributed to higher phenolic and flavonoid content. It is well established that phenolic and flavonoid content is positively correlated to antioxidant activity.

The fruits used in this study are well-known for exhibiting health beneficial properties. For instance, dragon fruits not only have nutrition properties but are also reported for medicinal properties including antioxidant, antimicrobial, antidiabetic and anticancer (Joshi and Pravakar, 2020). Pineapple has been reported for anti-inflammatory, antioxidant activity, healing of bowel movement, and monitoring nervous system function (Ali *et al.*, 2020). While the roselle calyces have been used in traditional medicine to mitigate various diseases like diabetes, hypertension, and liver

disorders (Nguyen and Chuyen, 2020). Hence, consumption of these fruit tea infusions may give various health-beneficial effects.

### Sensory evaluation of fruit tea

Sensory evaluation is an indispensable part of the analysis of food products because it helps in designing and marketing products to meet consumers' sensory needs, which helped to reduce the risk of product failure (Sidel and Stone, 1993). The mean sensory scores of dragon fruit tea and roselle-pineapple fruit tea are presented in Table 3. It was observed that in both the tea infusions S<sub>5</sub> scored the highest while S<sub>1</sub> scored the lowest for all the sensory attributes. This showed that the sensory qualities of tea were improved with the increase in time of infusion. The interactions between the sensory parameters were evaluated by correlation, cluster and regression analysis.

### Correlation analysis between the sensory parameters

Results of the Pearson's correlation coefficient analysis (Table 4) in respect of dragon fruit tea revealed the existence of highly significant positive correlation between colour with flavour (p < 0.01 and r = 0.959), taste (r = 0.962), appearance (r = 0.999), and overall acceptability, (r = 0.995). Similarly, flavour showed a high significant positive correlation (p < 0.01) with taste (r = 0.998), appearance (r = 0.962), and overall acceptability, (r = 0.966). Taste was found to correlate significantly (p < 0.01), appearance (r = 0.965) and overall acceptability, (r = 0.967). Appearance and overall acceptability observed a significant positive correlation (p < 0.01 and r = 0.991).

In respect of roselle-pineapple fruit tea also similar correlation was observed, wherein, colour was significantly and positively correlated (p < 0.01) with flavour r = 0.963), taste (r = 0.979), appearance (r = 0.996), and overall acceptability, (r = 0.987). Similarly, flavour (r = 0.952), appearance (r = 0.973), and overall acceptance (r = 0.923) all revealed a highly significant positive connection (p < 0.01). Taste, appearance (r = 0.981), and overall acceptance (r = 0.941) were shown

**Table 1: Recipe of herbal tea per sachet**

Type of herbal tea	Ingredients	Quantity 100g <sup>-1</sup>
Dragon fruit tea	Dragon fruit pulp	52.0
	Dragon peel	20.0
	Lemon pulp	12.0
	Orange peel	8.0
	Ginger	8.0
Roselle-pineapple tea	Roselle	55.55
	Pineapple	37.03
	Ginger	29.63

Table 2: Proximate composition and *in-vitro* bioactivity of fruit tea

Sample	Parameters (g 100g <sup>-1</sup> sample)			Reducing sugar	Total sugar	Vitamin C mg 100g <sup>-1</sup>	Antioxidant activity (mg AAE 100g <sup>-1</sup> )	Phenolic content (mg GAE 100g <sup>-1</sup> )	Flavonoid content (mg QE 100g <sup>-1</sup> )
	Ash (%)	Protein	Fat						
Dragon fruit tea	7.4±0.02	5.6±0.1	1.65±0.002	0.16±0.002	3.22±0.02	86.29±0.6	129.7±0.07	368.9±0.07	111.0±0.14
Roselle- Pineapple tea	5.9±0.008	5.08±0.02	0.16±0.001	0.27±0.004	4.68±0.01	83.86±0.8	460.0±0.2	664.9±0.31	232.20±0.12

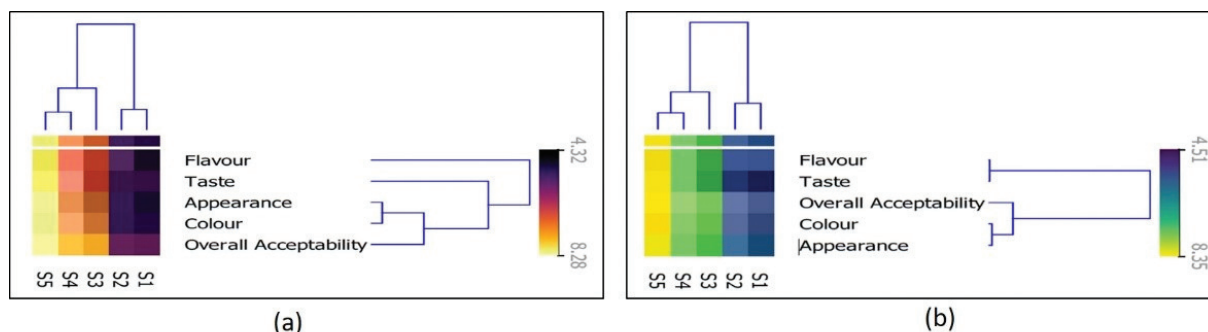
Table 3: Mean score for the performance of colour, flavour, texture, taste and overall acceptability of dragon fruit tea with different times of infusion

Sample Code	Colour		Flavour		Taste		Appearance		Overall Acceptability	
	Dragon fruit	Roselle- pineapple tea	Dragon fruit	Roselle- pineapple tea	Dragon fruit	Roselle- pineapple tea	Dragon fruit	Roselle- pineapple tea	Dragon fruit	Roselle- pineapple tea
S <sub>1</sub>	5.21 <sup>c</sup>	4.82 <sup>c</sup>	5.42 <sup>c</sup>	4.32 <sup>c</sup>	4.51 <sup>c</sup>	5.13 <sup>d</sup>	5.12 <sup>d</sup>	4.61 <sup>d</sup>	5.82 <sup>d</sup>	5.57 <sup>c</sup>
S <sub>2</sub>	6.32 <sup>b</sup>	5.26 <sup>c</sup>	5.62 <sup>c</sup>	5.67 <sup>bc</sup>	4.85 <sup>c</sup>	5.25 <sup>c</sup>	6.26 <sup>c</sup>	5.17 <sup>c</sup>	6.57 <sup>c</sup>	5.78 <sup>c</sup>
S <sub>3</sub>	7.28 <sup>ab</sup>	6.84 <sup>bc</sup>	6.82 <sup>b</sup>	6.25 <sup>bc</sup>	6.47 <sup>b</sup>	6.27 <sup>bc</sup>	7.12 <sup>b</sup>	6.62 <sup>b</sup>	7.67 <sup>bc</sup>	7.58 <sup>ab</sup>
S <sub>4</sub>	7.83 <sup>ab</sup>	7.75 <sup>b</sup>	7.64 <sup>b</sup>	7.34 <sup>b</sup>	7.65 <sup>b</sup>	7.58 <sup>b</sup>	7.75 <sup>b</sup>	7.43 <sup>ab</sup>	7.96 <sup>ab</sup>	7.85 <sup>ab</sup>
S <sub>5</sub>	8.25 <sup>a</sup>	8.20 <sup>a</sup>	8.14 <sup>a</sup>	8.00 <sup>a</sup>	8.32 <sup>a</sup>	8.15 <sup>a</sup>	8.25 <sup>a</sup>	8.24 <sup>a</sup>	8.35 <sup>a</sup>	8.28 <sup>a</sup>

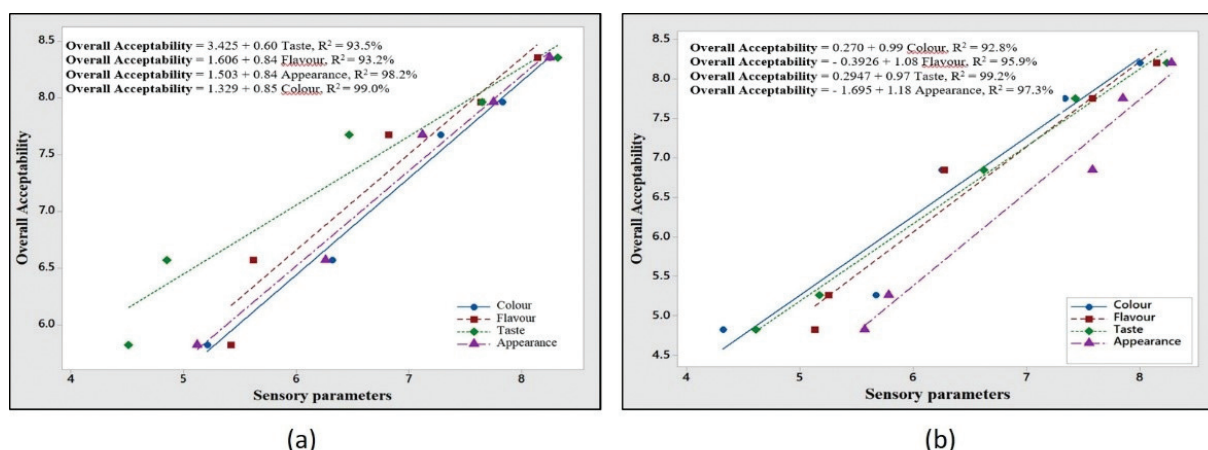
**Table 4: Pearson correlation coefficient (r) matrix between colour, flavour, texture, taste and overall acceptability of dragon fruit and roselle-pineapple fruit tea with different times of infusion**

	Colour		Flavour		Taste		Appearance		Overall Acceptability	
	Dragon fruit	Roselle-pineapple tea	Dragon fruit	Roselle-pineapple tea	Dragon fruit	Roselle-pineapple tea	Dragon fruit	Roselle-pineapple tea	Dragon fruit	Roselle-pineapple tea
Colour	<i>1.000</i>	<i>1.000</i>	<i>0.959</i>	<i>0.963</i>	<i>0.962</i>	<i>0.979</i>	<i>0.999</i>	<i>0.996</i>	<i>0.995</i>	<i>0.987</i>
Flavour	<i>0.959</i>	<i>0.963</i>	<i>1.000</i>	<i>1.000</i>	<i>1.000</i>	<i>0.952</i>	<i>0.962</i>	<i>0.973</i>	<i>0.966</i>	<i>0.923</i>
Taste	<i>0.962</i>	<i>0.979</i>	<i>0.998</i>	<i>0.952</i>	<i>1.000</i>	<i>1.000</i>	<i>0.965</i>	<i>0.981</i>	<i>0.967</i>	<i>0.941</i>
Appearance	<i>0.999</i>	<i>0.996</i>	<i>0.962</i>	<i>0.973</i>	<i>0.965</i>	<i>0.981</i>	<i>1.000</i>	<i>1.000</i>	<i>0.991</i>	<i>0.979</i>
Overall Acceptability	<i>0.995</i>	<i>0.987</i>	<i>0.966</i>	<i>0.923</i>	<i>0.967</i>	<i>0.941</i>	<i>0.991</i>	<i>0.979</i>	<i>1.000</i>	<i>1.000</i>

Boldfaced italics numerical values indicated that correlation coefficient (r) values are significantly positive at  $p < 0.01$  (2-tailed). The correlation coefficient (r) values correspond directly to the colour codes from green to yellow and red, respectively.



**Fig. 1 : Heat map depicting the clustering sensory parameters with different times of infusion, (a) Dragon fruit tea infusion (b) Roselle-pineapple fruit tea infusion.**



**Fig. 2 : Regression response curve depicting the relationship between differential sensory parameters with overall acceptability with different times of infusion (a) Dragon fruit (b) Roselle-pineapple fruit tea infusion**

to be strongly correlated ( $p < 0.01$ ). There was a substantial positive association between appearance and overall acceptability ( $p < 0.01$  and  $r = 0.979$ ). The overall results of correlation analysis (Table 4) indicate

that all the sensory parameters are correlated to each other and this implies that the increment in one of the sensory parameters improved the other parameters and *vice-versa*, and they are dependent on each other. A

similar finding was reported by Shanta *et al.* (2014) who also found a correlation between colour, flavour, texture and overall acceptability as if any one of the parameters increases the other parameters increase and the overall acceptability of the product also increases.

#### **Cluster analysis of sensory parameters**

Cluster analysis was used to see the affinity and extent of association between the visual parameters to validate the correlation study results. The dendrogram was used to show and depict the cluster analysis results and group the parameters based on Ward's hierarchical clustering (Strauss and Maltitz, 2017). In respect of dragon fruit tea cluster analysis, there was the formation of two dominant clusters *viz.*, cluster I comprising of flavour and taste and cluster II consisting of overall acceptability, colour and appearance. The most likely reason for the formation of similar clusters in respect of visual parameters is due to the existence of a highly positive and significant correlation between them as evident in correlation (Fig. 1 a). Likewise, in the case of roselle-pineapple fruit tea only one distinct cluster *i.e.*, Cluster I (Fig. 1 b) can be observed which consists of overall acceptability, colour and appearance. However, flavour and taste formed two simplifications outliers without forming any definite cluster.

#### **Regression analysis for screening of the significant sensory parameters**

To screen the magnitude of attribution of differential sensory parameters to overall acceptability simple linear regression with each sensory parameter and multiple stepwise regression was computed. To work out the regression the sensory parameters were assigned as independent attributes while overall acceptability was a dependent attribute. Upon iteration, the linear regression response curve for all the sensory parameters was laid in a single graph. Regression response curve in respect of dragon fruit tea (Fig. 2a) exhibited a highly significant  $R^2$  value of colour (99.0%), thereby indicating the strong dependence of colour on overall acceptability, which was closely followed by appearance, taste and flavour in decreasing order. A similar trend was recorded in respect of roselle-pineapple fruit tea (Fig. 2b), where taste showed a significant  $R^2$  value of 99.2% and can be ranked as appearance > flavour > colour.

#### **CONCLUSION**

The present study revealed that the locally prepared fruit tea infusions have shown promising antioxidant activities and vitamin C content. The antioxidant activity of the fruit tea is because of the presence of high phenolic and flavonoid content. From the sensory evaluation study, it was observed that both the fruit tea, increased in infusion time giving more attractive colour, pleasant

flavor and overall acceptability. These fruit tea infusions will be a good alternative for other sweetened drinks. Hence, these fruit tea infusions will not only serve as beverages but also act as dietary supplements of natural antioxidants and vitamin C.

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