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Nutritional Properties of Flower Petals of Four *Rosa Hybrida* **Cultivars**

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Article Information

Received: Sep 19, 2024 **Accepted:** Nov 01, 2024 **Published:** Nov 08, 2024

SciBase Human Nutrition & Food Science - scibasejournals.org Khoo HE et al. © All rights are reserved

Citation: Liu Y, Vun VF, Yim HS, Khoo HE. Nutritional Properties of Flower Petals of Four *Rosa Hybrida* Cultivars. SciBase Hum Nutr Food Sci. 2024; 1(2): 1007.

Abstract

Roses are among the most essential ornamentals in the world, offering a high nutritional value. The objective of this study is to determine and compare the nutritional composition and antioxidative properties of the rose petals of four different cultivars of *Rosa hybrida* L. from Cameron Highland in Malaysia. Proximate analyses including those of protein, lipid, crude fiber, ash, and carbohydrate, were conducted. The Total Phenolic Content (TPC), Total Flavonoid Content (TFC), and Total Monomeric Anthocyanin Content (TMAC) were determined, as well as the antioxidant capacity of the samples, which was evaluated using the 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) and [2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)] diammonium salt (ABTS) scavenging capacity assays. The two major macronutrients in all rose petal samples were carbohydrates and proteins. Cultivar 302 exhibited the highest levels of TPC, TFC, and ABTS, while cultivar 201 demonstrated the highest level of TMAC. The DDPH scavenging activity for all cultivars exhibited a range of 78.58±2.09% to 82.21±0.98%. This study presents the nutritional composition and antioxidant activity of four distinct cultivars of rose petals sourced from Cameron Highland, Malaysia. All samples demonstrated antioxidant properties at a moderately high level.

Keywords: Antioxidant activity; Phenolic; Nutrient; Proximate composition; *Rosa hybrida*.

Introduction

Flowers have been consumed by humans since ancient times, offering nutritional value and medicinal properties. Edible flowers are employed for a variety of purposes in culinary practices. They are utilized to enhance the visual appeal, color, and nutritional value of the dishes, as well as to impart a distinctive flavor and aroma to the food being prepared [1]. In the contemporary era, edible flowers are utilized as garnishes for culinary preparations and are typically consumed in a fresh state, such as in a salad [2]. However, these flowers can be consumed in a dried state, incorporated into cocktails (in ice cubes), and preserved in distillates [2]. Moreover, they can be incorporated into a variety of other culinary preparations, including sauces, tarts, preserves, pickles, fritters, and cream cheeses [3]. Furthermore, the phytochemicals present in flowers exhibit considerable nutraceutical potential. A recent surge in research activity has been observed in the field of floral nutrition and medicine [4-6].

Roses are among the most widely cultivated and appreciated ornamental plants worldwide, offering a substantial nutritional value. They are valuable perennial flower shrubs of the genus Rosa, which belongs to the Rosaceae family and are widely cultivated as cut flowers, potted plants, and in home gardens [7]. The genus Rosa comprises over 100 species, which are widely distributed across Europe, Asia, the Middle East, and North America [8]. These species give rise to thousands of cultivars, which exhibit a wide range of colors [7,9]. Although roses are primarily cultivated for their ornamental value, they have been employed in culinary, functional food, medicinal, cosmetic, and aromatic applications for millennia [3]. Rose petals have

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been utilized in the preparation of jams, teas, cakes, and flavor extracts [10]. In ancient Rome, roses were employed in the preparation of a variety of purées and omelets [1]. It has been documented that Chinese rose flowers have been traditionally utilized for the treatment of menstrual disorders, trauma, and blood disorders, as well as for the maintenance of homeostasis and the management of pain and diarrhea [9]. Rose oil is composed of citronellol, nerol, and geraniol as its primary components [11]. It is one of the most expensive essential oils in the global market, with Damask roses representing the most renowned Rosa species for rose oil production worldwide [12].

Rosa hybrida is one of the most economically important horticultural crops, and its color is attributed to the presence of anthocyanins and carotenoids [13]. As a member of the genus Rosa, roses are reported to comprise a few types of secondary metabolites, including phenolic acids (e.g., gallic acid, chlorogenic acid), flavonoids (e.g., flavonols, anthocyanins), fragrant components (essential oils, e.g., monoterpenes, sesquiterpenes), and hydrolyzable and condensed tannins (e.g., rugosins, procyanidins) [9]. These metabolites are active antioxidants, and they are known to possess significant medicinal, nutraceutical, and cosmetic values [14].

To the best of our knowledge, there is a paucity of data available regarding the nutritional content and medicinal properties of Rosa species in Malaysia. Further scientific evidence is required to support the research and development of *R. hybrida* products in Malaysia and throughout the world, as well as nutraceutical and functional foods. Accordingly, the objective of this study was to ascertain and contrast the nutritional composition and antioxidative properties of rose petals from four distinct cultivars of R. hybrida originating from Cameron Highland, Malaysia. Moreover, the total polyphenolic content was also determined.

Materials and methods

Sample preparation

The fresh flowers of R. hybrida L. cultivars 201, 302, 402, and M301 were procured from Boon Sing Trading Sdn. Bhd., Cameron Highlands, Pahang, Malaysia. The fresh petals were obtained by removing the flower buds, washing them under the running tap water, and spin-drying them. Subsequently, the flower samples were subjected to freeze-drying under a vacuum pressure of 0.125 mbar for a period of 48 h using a freeze dryer (Alpha 1-4 plus, Martin Christ Gefrietrocknungsanlagen, Germany). This was conducted following the immediate freezing of the samples by liquid nitrogen. The freeze-dried petals were subjected to further processing, whereby they were transformed into fine particles. This was achieved through the utilization of an IKA®-Werke crusher, which was equipped with a sieve of MF-Sieb 1 mm (IKA-Werke GmbH & Co. KG, Germany). Subsequently, the lyophilized powders were vacuum-packed with the aid of a vacuum packager (DZQ 400/500, Clarity, China) and stored at -80°C until further analysis.

Proximate analysis

The lipid content of the rose samples was determined by the Soxhlet extraction method, in accordance with the AOAC standard [15], using hexane as the solvent. The protein content was determined in accordance with the Kjeldahl method [15], with a conversion factor of 6.25. The determination of crude fiber was conducted in accordance with the Weende method, as previously described by Jaafar et al. [16]. The ash content

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was determined by the dry ashing method, which involved incinerating the sample in a muffle furnace (Fisher Scientific, UK) set at 550°C until whitish ash was obtained. Subsequently, the carbohydrate content was subsequently calculated as the difference between 100 and the total sum of the protein, fat, ash, and crude fiber content. The results of the proximate analysis were expressed as a percentage of Dry Weight (DW).

Sample extraction

A lyophilized sample (1.0 g) was extracted with 10 ml of hexane in a 1:10 ratio (w/v). The mixture was agitated using an orbital shaker (Daihan LabTech Co. Ltd., Korea) set to 200 rpm at room temperature for 20 min. The extraction was repeated four to six times under the same conditions until a clear extracted solution was obtained. The combined hexane extracts were evaporated to dryness using a rotary evaporator (Rotavapor R-200, Buchi, Switzerland). Subsequently, the extracts were then reconstituted with 95% methanol to achieve a stock solution of 0.5 mg/ml and stored at -80°C until prior to antioxidant analysis. It should be noted that a clear layer of lipid can be observed at the base of the round-bottom flask.

Determination of total phenolic content

The Total Phenolic Content (TPC) was determined using the Folin-Ciocalteu reagent method, as previously described by Lim and Murtijaya [17]. A solution of the diluted rose petal lipid extract (300 μl) was prepared by mixing it with 1.5 ml of the diluted Folin-Ciocalteu reagent (10×) and 1.2 ml of sodium carbonate (7.5 g/100 ml). The solutions were thoroughly mixed and stored in the dark for 30 min prior to being read at 765 nm using a UV-Vis spectrophotometer (UviLine 9400, Secoman, France). A standard calibration curve of gallic acid (0-0.15 mg/ ml) was constructed in order to calculate the results. The results were expressed as mg of Gallic Acid Equivalent (GAE) per g DW.

Determination of total flavonoid content

The Total Flavonoid Content (TFC) of the lipid extracts derived from rose petals was determined by the aluminum chloride colorimetric assay as previously described by Liu et al. [18]. A diluted sample (2.0 ml) was combined with 0.2 ml of 5% sodium nitrite. Subsequently, following an interval of time, 0.2 ml of 10% aluminum chloride was introduced to the mixture. Subsequently, 2 ml of 1 M sodium hydroxide was added after a 6-min incubation period. The reaction mixture was diluted with 50% ethanol and topped up to a final volume of 5.0 ml. The absorbance was then measured at 510 nm against a blank. TFC was determined based on a standard calibration curve of rutin (0-500 mg/ml), and the results were expressed as mg of Rutin Equivalent (RE) per g DW.

Determination of total monomeric anthocyanin content

The Total Monomeric Anthocyanin Content (TMAC) of the rose petal lipid extract was determined by the pH-differential method, employing two buffer systems: A 0.025 M potassium chloride buffer (pH 1.0) and a 0.4 M sodium acetate buffer (pH 4.5). This approach was described by Chew et al. [19]. An aliquot of 0.2 ml of the sample extract was diluted with 2.8 ml of potassium chloride buffer, with an absorbance of less than 1.2 at 510 nm. An additional dilution of the sample extract was prepared with sodium acetate buffer, employing the same dilution factor. All dilutions were allowed to be incubated for 15 min at room temperature. The absorbance of each dilution was measured at 510 nm against a blank, followed by a measurement at 700 nm, to subtract the absorbance of haze. The results were expressed as mg of monomeric anthocyanin pigment per g DW. The calculations were performed in accordance with the following equations:

A (Absorbance) = $(A_{510} - A_{700})$ pH 1.0 - $(A_{510} - A_{700})$ pH 4.5

Anthocyanin pigment (cyanidin-3-glucoside equivalent, mg/L) = AxMWxDFx10³ åx1

The symbols MW and ɛ correspond to the molecular weight and molar absorptivity of cyanidin-3-glucoside (MW=449.2 and ε =26,900), respectively. The symbol DF represents the dilution factor.

Determination of DPPH radical scavenging activity

The DPPH radical scavenging activities of the extract were conducted in accordance with the methodology described by Chew et al. [19], with modifications. A solution of 0.1 ml of rose petal lipid extract was prepared by adding it to 3.9 ml of a 0.2 M DPPH solution (dissolved in methanol), and the two were mixed thoroughly. The absorbance of the reaction mixture was determined at 517 nm against a blank sample, which was incubated in a dark room for 40 min. The results were expressed as DPPH free radicals radical scavenging activity (%), as calculated using the following equation: $A_0 - A_c$

DPPH radical scavenging activity (%) =

The A0 value represents the absorbance of the control sample without the addition of the extract, while the Ac value represents the absorbance of the extract.

 $\boldsymbol{0}$ A

Determination of ABTS radical scavenging activity

The ABTS radical scavenging activity was performed in accordance with the methodology described by Chew et al. [19]. The ABTS reagent was prepared by reacting 5.0 ml of 7 mM ABTS with 88.0 μl of 140 mM of potassium persulfate solution, and the mixture was then allowed to stand at room temperature in the dark for 12-16 h. After the incubation period, the ABTS reagent was prepared by adding 95% ethanol to obtain an absorbance of 0.7±0.05. An aliquot (0.1 ml) of the rose petal lipid extract, appropriately diluted, was mixed with 1.0 ml of the ABTS reagent. Absorbance was measured against a blank at 734 nm after a 6-min incubation period. A standard curve was constructed using Trolox (0-0.2 mg/ml) was used to construct a standard curve for quantification purposes, with the results expressed as mg of Trolox Equivalent (TE) per g DW.

Statistical analysis: The data were expressed as mean ± standard deviation. The statistical analyses were conducted using a one-way analysis of variance, followed by Duncan's multiplerange test, with the IBM software SPSS version 21.0. A p-value of less than 0.05 was considered statistically significant.

Results

Proximate analysis

The nutritional composition of the flower petals of four distinct cultivars of *R. hybrida*, sourced from Cameron Highland, Malaysia, is presented in (Table 1). The petals of *R. hybrida* were found to contain a significant proportion of carbohydrates, representing approximately 70% of the macronutrients. The carbohydrate contents determined for *R. hybrida* exhibited no statistically significant difference (p>0.05) between cultivars 201 and 302, and between cultivars 402 and M301. The secondhighest macronutrient in the sample was protein, with a range of 12.65% to 14.23%. No significant differences were observed between the cultivars in this regard.

In contrast, lipid content exhibited significant inter-cultivar variation (p<0.05) between the rose petals of different cultivars, with the highest lipid content observed in cultivar 402 (4.59±0.11%) and the lowest lipid content in cultivar M301 (3.59±0.13%). The crude fiber content was found to be similar among the rose samples of different cultivars, with the exception of cultivar 201, which exhibited a significantly higher (p<0.05) crude fiber content (6.19±1.38%) compared to the other cultivars. The ash content exhibited variability among the samples of different cultivars, with the exception of cultivars 201 and M301.

Antioxidants content of flower petals

(Table 2) presents the TPC, TFC, and TMAC of a lipid extract of *R. hybrida*'s flower petals from Cameron Highland, Malaysia. A significant difference (p<0.05) was observed in the TPCs of the rose petals among the various cultivars of *R. hybrida*. It was observed that cultivar 302 exhibited the highest TPC (82.32±0.67 mg GAE/g DW), which was more than twofold greater than the TPC of cultivar M301 (36.65±2.28 mg GAE/g DW).

The TFC of the rose petal lipid extract of cultivar 302 (23.80±2.10 mg RE/g DW) was significantly higher (p<0.05) than that of the other cultivars, with cultivars 201 (20.23±3.39 mg RE/g DW) and cultivar 402 (15.14±0.65 mg RE/g DW) exhibiting intermediate levels. The lowest TFC was observed for cultivar M301, with a value of 11.10±0.46 mg RE/g DW. The trend of TMAC in the rose petal lipid extracts differed from the trends observed for TPC and TFC. The results demonstrated that the TMAC of cultivar 302 (0.44±0.01 μmol TE/g DW) and cultivar M301 (0.48±0.10 μmol TE/g DW) was not statistically significant (p<0.05). However, cultivar 201 exhibited the most significant TMAC (0.72±0.01 μmol TE/g DW) in comparison to the other cultivars of *R. hybrida* (p<0.05).

Antioxidant activities of flower petals

(Table 2) illustrates the DPPH and ABTS radical scavenging activities of the lipid extracts derived from four distinct cultivars of *R. hybrida*'s flower petals sourced from Cameron Highland, Malaysia. A slight variation in DPPH radical scavenging activities was observed among the lipid extracts, with values ranging from 78.58% (for cultivar 201) to 82.21% (for cultivar 402). Cultivar 402 exhibited a significantly higher DPPH radicals scavenging activity significantly higher than cultivar 201 (p<0.05), though not to a degree that was significantly higher than that observed in cultivar 302.

In contrast, the lipid extract of cultivar 402 of *R. hybrida*'s flower petal exhibited the lowest ABTS radical scavenging activity, with a significantly lower TE value (p<0.05) compared to cultivars 302 and 201. The TE values of the rose petal lipid extracts exhibited a range of 0.78 to 0.98 mg TE/g DW. The results demonstrated that cultivar 302 exhibited the most significant TE value, while cultivar M301 exhibited the lowest TE value.

Correlation between antioxidant component and antioxidant activity

The relationships between antioxidant components (TPC, TFC, and TMAC) and antioxidant activities (DPPH and ABTS assays) of lipid extracts of *R. hybrida*'s flower petal obtained from Cameron Highland, Malaysia are illustrated in (Table 3). As in-

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dicated by Guildford's Rule of Thumb [20], a highly significant and positive correlation was identified between TPC and ABTS (r=0.98, p<0.01), and a strong and positive correlation was observed between TFC and ABTS (r=0.85, p<0.01).

The findings of this study demonstrate that the phenolic and flavonoid compounds present in the rose petal lipid extract exert a considerable influence on the ABTS radical scavenging activity. Conversely, a moderate and negative correlation was observed between TMAC and DPPH (r=-0.60 and p<0.01), indicating that anthocyanins in the lipid extract exert a moderate

The values marked with different lowercase superscript letters are significantly different at the p<0.05 level within the same row.

The values marked with different lowercase superscript letters are significantly different at the p<0.05 level within the same row.

Note: ND*: Not Determined; TPC: Total Phenolic Content; TFC: Total Flavonoid Content; TMAC: Total Monomeric Anthocyanin Content.

The values marked with different lowercase superscript letters are significantly different at the p<0.05 level within the same row.

Note: ABTS: ABTS assay; DPPH: DPPH radical scavenging activity.

Table 4: Correlation coefficient (r) between antioxidant components and antioxidant capacities of four different cultivars of *Rosa hybrid* from Malaysia.

A significant correlation was observed at a p-value of less than 0.01.

influence on the DPPH radical scavenging activity.

Discussion

The consumption of edible flowers by humans has been the subject of increasing interest for several decades. The global market for fresh, premium-quality flowers is currently experi-

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encing a period of growth [2]. These products, are packaged in bunches or boxes and are sold either directly at farm shops or through various specialized outlets. Edible flowers are typically consumed in their entirety. However, rose (Rosa spp.) petals are commonly consumed fresh, imparting a sweet and aromatic flavor [1]. It is of great importance to ascertain the nutritional value of roses, which provides a compelling rationale for their consumption. However, the existing literature on the nutritional composition of flower petals of *R. hybrida* from Malaysia is insufficient.

In this study, carbohydrates constituted the predominant macronutrient in all rose petals, representing 73.31%-75.83% of the total macronutrient content. Additionally, the petals were found to contain a fat content of between 3% and 5%. The proximate compositions of the *R. hybrida* flower petals were found to be comparable among the various cultivars. As was the case in a previous study, the protein and carbohydrates of edible flowers from wild Mexican plants were identified as the primary macronutrients on a dry weight basis [21]. However, the carbohydrate content of all rose petal samples is higher than that of edible flowers, including *Agave salmiana, Aloe vera, Arbutus xalapensis, Cucurbita pepo, Erythrina americana, Erythrina caribaea, Euphorbia radians, and Yucca filifera*, which ranged between 42.40% and 66.70% [21]. Additionally, the edible flowers exhibited comparable levels of lipid (1.5-5.0%), yet displayed higher contents of fiber (8.5 to 17.7%) and ash (5.8 to 15.9%) than the rose petal samples under investigation. In addition to the macronutrients that have been identified, flower petals also provide essential vitamins, particularly vitamin A, minerals, and antioxidants [1]. It is nevertheless inadvisable to consume edible flowers originating from untested cultivars or florists' shops, as some flowers may contain toxic substances resulting from the use of fertilizers, herbicides, and pesticides [1].

Phenolic compounds have been the subject of considerable research interest due to their biological properties, including anti-mutagenic, anti-carcinogenic, and antioxidant activities [5]. The findings of the present study indicate that cultivar M301 of R. hybrida flower petals exhibits the lowest TPC value among the various cultivars examined. The TPC value (36.65 mg GAE/g DW) is higher than the TPC values reported for five different species of rose hips (31.08-52.94 mg GAE/g DW) reported by Demir et al. [22]. The most prevalent phenolic acids in the rose hips of *Rosa canina* L. and *Rosa arvensis* Huds. are gallic and protocatechuic acids [23]. Additionally, flavonols, including quercetin, and quercetin or kaempferol monoglycosides (e.g., quercetin or kaempferol glucosides, rhamnosides, arabinosides, and their diglycosides, such as rutinoside), have been identified in the dried Rosa chinensis flowers [9].

The flavonoid group includes anthocyanins, which have been shown to have a protective effect against a number of diseases, including those associated with atherosclerosis, cancer, diabetes, and inflammation [13,24]. Anthocyanins are present in the majority of flowers, with red and orange-colored roses being particularly rich sources. With the exception of yellow roses, roses contain a substantial quantity of anthocyanins, which serve as antioxidants [9].

The findings of the present study indicate that the flower petals of the *R. hybrida* cultivar 201 exhibited the highest TMAC value. The primary anthocyanidins in roses include cyanidin, pelargonidin, and petunidin [25]. In their study, Lee et al. [13] identified the anthocyanidins cyanidin 3,5-di-O-glucoside and pelargonidin 3,5-di-O-glucoside in the red petals of the Korean edible rose (*R. hybrida* cv. Noblered). Our findings are also corroborated by previous reports [26,27].

In this study, the antioxidant activities of *R. hybrida* flower petals were determined through the use of DPPH and ABTS assays. The results of the DPPH assays conducted in the present study indicate that the antioxidant activities (78.6-82.2%) of the lipid extracts of *R. hybrida* flower petals are significantly higher than those of the aqueous extracts of a few cultivars of *R. hybrida*. The cultivars in question are Belami (13.82%), Sunlight (16.99%), Tinke (26.06%), Golden (38.48%), Calora (36.24%), Bingo (45.43%), Cardinal (54.41%), Dalares (60.50%), Egene (60.31%), and Samantha (71.26%) [28].

Among the phenolic compounds present in the rose petal, cyanidin 3,5-di-O-glucoside demonstrated a moderate capacity to scavenge the DPPH radical, with an IC50 value of 55.2 μg/ml [13]. Therefore, the antioxidant activities of the rose petal lipid extract under investigation may be associated with the anthocyanin content. This observation is further corroborated by our findings that the DPPH radical scavenging activity of the rose samples is moderately correlated with the anthocyanin content.

As previously demonstrated, a plant's high antioxidant potential is primarily attributable to its elevated phenolic content [29]. The relationship between antioxidant potential and phenolic compounds has been demonstrated by a robust correlation between TPC and antioxidant activity (ABTS assay), with a coefficient of determination (r) exceeding 0.98 and a p-value less than 0.01. Similarly, the TFC of the rose petal lipid extracts exhibited a strong correlation with its ABTS radical scavenging activity (r>0.85 and p<0.01). These findings suggest that the TPC and TFC of the rose petal lipid extracts are attributed to the ABTS radical scavenging activity of the extract under investigation.

The correlation between antioxidant components and antioxidant activities represents a pivotal factor in the prevention of disease. A positive correlation between the levels of phenolic compounds and the ability to scavenge free radicals indicates that an increase in the former elevates the latter. Therefore, a high level of antioxidants may assist in reducing oxidative stress and preventing the onset of several chronic diseases. Nutritional antioxidants, such as phenolic compounds, are essential for maintaining optimal health. In addition to the macronutrients, phytochemicals in plants represent a physiological necessity. Rose petals are a rich source of phytochemicals, particularly phenolic compounds. Consequently, an evaluation of the antioxidant properties of rose petals is essential for a comprehensive nutritional assessment.

Conclusion

This study presents data on the nutritional composition and antioxidant properties of the flower petals of four distinct cultivars of *R. hybrida* from Cameron Highland, Malaysia. The major macronutrients in the rose petals were carbohydrates and proteins. The lipid extracts of the four cultivars of *R. hybrida* demonstrated moderate to high antioxidant capacities. However, cultivars 201 and 302 exhibited higher antioxidant contents than the other two cultivars. The findings of this study provide scientific evidence for the importance of consuming rose petals and the usage of rose petals as a new and prospective source of functional food, nutraceuticals, and also for the food industry. Nevertheless, additional research is required to ascertain the presence of other phytochemicals in this species and their associated health benefits, as well as their potential for the prevention of chronic diseases.

Abbreviations: ABTS: [2,2'-Azinobis(3-Ethylbenzothiazoline-6-Sulfonic Acid)] diammonium salt; DPPH: 2,2-Diphenyl- 1-Picrylhydrazyl; DW: Dry Weight; GAE: Gallic Acid Equivalent; RE: Rutin Equivalent; TE: Trolox Equivalent; TFC: Total Flavonoid Content; TMAC: Total Monomeric Anthocyanin Content; TPC: Total Phenolic Content; UV-Vis: Ultraviolet-Visible.

Acknowledgments: The authors would like to express their gratitude to the Faculty of Applied Sciences at UCSI University for their invaluable support in the realization of this project. Furthermore, the authors express their gratitude to the technical staff of the laboratories at UCSI University for their invaluable assistance in this project.

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