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Assessment of Anthocyanin Content and Consumer Acceptability of Dried Rose Herbal Tea Blended Infusions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Tea is a famous beverage that is used all over the world because of its refreshing characteristics. Herbal teas have recently gained popularity due to their therapeutic and antioxidant effects. The present investigation was carried out at Floriculture Laboratory, College of Horticulture, Sri Konda Laxman Telangana State Horticultural University, Rajendranagar, Hyderabad during the year 2022-23. The experiment was laid out in Completely Randomized Design (CRD) with seven treatments and each replicated thrice. They were evaluated for the antioxidant activity, total phenols, anthocyanin content and sensory analysis at period of 0, 30, 60, 90 days of storage. The study revealed that among different herbal teas, T_1 (Dried rose petals- Control) found to be best with total anthocyanin content (19.56 $mg.L^{-1}$). Antioxidant activity and total phenolics content was recorded highest in treatment T_4 (Dried rose petals and dried tulasi leaves in 1:1) with (92.70 %) and (140.68 $mg.100g^{-1}$ GAE) respectively. In respect with organoleptic scoring, T_2 (Dried rose petals and dried lemon grass in 1:1) was found to be best with flavor (8.50), taste (8.25), overall acceptability (8.60) and T_1 (Dried rose petals- Control) showed highest score (8.60) for colour.

Keywords: Dried rose herbal blends; tea infusions; bio-chemical parameters; sensory analysis.

1. INTRODUCTION

India is blessed with a rich heritage of traditional medical systems and is called as “Botanical Garden of the World”, with an extensive wealth of biodiversity. “In this modern era, the knowledge and experience of the usage of herbs were blend with advanced technologies to develop a safe and healthier herbal product” [1]. “The flower is an essential part of the plant that embraces a wide range of natural antioxidants like phenolic acids, flavonoids, and anthocyanins, as well as other nutrients such as minerals and vitamins. Edible flowers are emerging as new source of nutraceuticals due to their nutritional and medicinal value” [2]. “Roses are known as edible flowers and have been used for centuries as food components, either in the fresh form or in processed products, such as confectionary and beverages” [3].

“Rosa centifolia L. is having prominent nutritional qualities as the petals contain ingredients with an antioxidant effect and good organoleptic properties and microbial parameters. The flower is rich in sugars, organic acids, and potassium, all of which are ingredients that preserve product quality and have advantages in technological processes (syrups, juices, jam and tea)” [4]. “Rose- petal tea may serve as a caffeine-free beverage, either separately or in combination with other herbal materials which contains high antioxidant capacity. The radical- scavenging activity in rose tea is mostly due to the high content of phenolic compounds and free gallic acid” [5].

“Most herbal teas may consist of one main herbal ingredient or a blend of herbal ingredients, intended to bring about a specific purpose, such as relaxing mind and body, cardio protective, aiding with gastrointestinal problems, detoxification of body, immunostimulants, nourishing the nervous system, boosting energy levels and invigorating the body, relieving stress, helping to avoid colds, stimulating the internal organs, promoting a good night’s sleep, providing antioxidants and are usually caffeine free” [6].

2. MATERIALS AND METHODS

Experiment was carried at Floriculture Laboratory, College of Horticulture, Sri Konda Laxman Telangana State Horticultural University, Rajendranagar, Hyderabad, Telangana during the year 2022- 2023. The experiment was laid out in Completely Randomized Design (CRD) with seven treatments and each replicated thrice i.e., T_1: Dried rose petals- Control, T_2: Dried rose petals and dried lemon grass in 1:1, T_3: Dried rose petals and dried lemon grass in 2:1, T_4: Dried rose petals and dried tulasi leaves in 1:1, T_5: Dried rose petals and dried tulasi leaves in 2:1, T_6: Dried rose petals and dried ginger powder in 1:1, T_7: Dried rose petals and dried ginger powder in 2:1.

Source of experimental material: Rose flowers utilized in this experiment were collected from farmer’s field near shamshabad, Hyderabad. Lemon grass and tulasi leaves were procured from Medicinal and Aromatic Plants Research Station, Sri Konda Laxman Telangana State Horticultural University (SKLTSHU), Rajendranagar, Hyderabad. Dried ginger is purchased from local market, Rajendranagar, Hyderabad for carrying out the experiment.

Drying of petals and herbal material: For preparing rose herbal tea blends, fresh petals of rose, leaves of herbs and dried ginger were collected. All plant materials were carefully inspected and all foreign materials are removed. Rose petals, lemon grass and tulasi leaves were gently rinsed in tap water and cleaned, lemongrass was cut into about 10 cm pieces using a stainless-steel kitchen knife and all the plant materials which were washed were spread thinly on paper and are shade dried until water evaporates. Later petals, leaves and dried ginger were mixed on weight basis in respective proportions according to the designed treatments and kept in hot air oven at 55℃ for 8 hours. After drying the dried ginger was grounded in an electric grinder and sieved through an aluminum sieve (2 mm). Then dried petals, leaves and dried ginger powder were stored in air tight glass jars and labelled were used for further analysis.

Preparation of herbal tea infusions: Tea blend infusions are prepared by an accurately weighed (3gms) samples in 200mL of boiling water at a temperature of 90ºC for 10 minutes.

Total moisture content (%): Each 100 gms of rose petals blended with different herbal material was collected and dried in a hot air oven at 55°C for 8 hours. The dried samples were removed then placed in a desiccator to get cooled, re-weighed, placed in the oven, heated, cooled, reweighed repeatedly until a constant weight was obtained. The moisture content was then determined by differences expressed as a percentage of the initial fresh sample weight [7].

$$Total\ moisture\ content\ \% = \frac{Fresh\ weight - dry\ weight}{Fresh\ weight} x\ 100$$

Total anthocyanin content (mg.L^{-1}): "Total anthocyanin content was measured by using a spectrophotometric pH differential protocol. The extracts (tea samples) were mixed thoroughly with 0.025 mol/L potassiumchloride pH 1 buffer in 1:3 or 1:8 ratio of extract to buffer andthe absorbance of the mixture was measured at 520 and 700 nm against distilled water as blank. The extracts were combined similarly with sodium acetate buffer pH 4.5 and the absorbance of these solutions were measured at the same wavelengths" [8]. The anthocyanin content was calculated by using the formula mentioned below and the results were expressed as cyanidin-3-glucoside equivalent in mg/L.

Calculation: Absorbance (A) = (A520nm – A700nm) pH 1.0 – (A520nm –A700nm) pH 4.5 The total anthocyanin content of samples was calculated using the formula given below:

$$Total\ anthocyanin\ content\ (mg/L) = \frac{A\ x\ MW\ x\ DF\ x\ 1000}{\varepsilon\ x\ 1}$$

Where,
A = absorbance
MW = Molecular Weight
DF = Dilution Factor
ε = molar extinction coefficient, L x mol^{-1} x cm^{-1}
l = Path length (1 cm)

Antioxidant activity (% inhibition) Sample extraction: Each 2 gms of dried sample was crushed in pestle and mortar with 20mL 80% methanol. The extract was then centrifuged at 10,000 rpm at 4°C for 20 minutes. The supernatant was taken for determination of antioxidant activity by DPPH method.

DPPH free radical scavenging activity: The DPPH assay method is based on the reduction of DPPH, a stable free radical. The antioxidant activity of the extracts was determined using DPPH assay described by Braca *et al*. [9]. Aqueous extract 0.1mL was added to 3.9mL of 0.0025 mol/L DPPH (2, 2-Diphenyl-1-picrylhydrazyl) in methanol (70%). The mixture was shaken and allowed to stand for 30 minutes in dark at room temperature. Absorbance was read at 517 nm using UV spectrophotometer. The percent inhibition of antioxidant activity was calculated by the formula:

Percent inhibition (%) = [(Ao-Ae)/Ao] ×100

(Ao = absorbance without extract; Ae = absorbance with extract).

Total phenolics content

Sample extraction: Each 2 gms of dried sample was crushed in pestle and mortar with 20mL 80% methanol. The extract was then centrifuged at 10000 rpm at 4ºC for 20 minutes. The supernatant was taken for determination of total phenolic content.

Method: The total phenol content was estimated based on Folin– Ciocalteau (FC) method [10] with some slight modifications. 0.1mL of the aliquot was diluted with 1.4mL of Distilled water to which 0.5mL of Folin–Ciocalteau (FC) was added and test tubes were shaken well. Then 10mL of sodium carbonate (20%, w/v) was

added. Similarly, this process was done for 0.2, 0.3, 0.4mL aliquots and sample. These solutions were mixed well and incubated at room temperature for 60 minutes. After incubation, the absorbance was measured at 760nm. A suitable calibration curve was prepared using gallic acid and the results are expressed in milligram per gram (mg/g) gallic acid equivalent.

Mg gallic acid dilution equivalent per gram= (O.D x Standard curve factor x volume made up/ Aliquot taken x weight of sample)

Organoleptic scoring: The tea infusions were subjected to sensory evaluation for their acceptability using 9-point hedonic scale for colour, flavour, taste and overall acceptability by 10 members of panelists.

List 1. 9-point hedonic scale

Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

Statistical analysis: The experiment was carried out with completely randomized design consisting of seven treatments and three replications. The variability was estimated as per the procedure for analysis of variance (ANOVA) suggested by Panse and Sukhatme [11].

3. RESULTS AND DISCUSSION

Total moisture content (%): The results showed that there were significant differences among the treatments for total moisture content. The lowest total moisture content (6.95 %) was recorded in T_2 (Dried rose petals and dried lemon grass in 1:1). Whereas, the maximum total moisture content (9.96 %) was observed in T_5 (Dried rose petals and dried tulasi leaves in 2:1). Moisture content is an important parameter to be taken in consideration. Low moisture content indicates that the product is more resistant to deterioration and high moisture content may increase the susceptibility of product to microorganisms [12]. The results of the study conducted by [13] showed similar trend with *Cymbopogon citratus* (lemongrass) having the least moisture content (6.17 %), in their study they reported that the minimum moisture retention in lemongrass is attributable to longer leaf length and wider leaf surface area. The high moisture content of the teas is not recommended for their storage, because higher moisture content might lead to lower the shelf period [14]. The higher the moisture content, higher is the chances of microbial susceptibility in the herbal products, which may cause the enzymatic activation and loss of the active chemicals of the herbal products [15]. Slight increase in the moisture content of dry petals with the advancement of storage might be due to absorption of moisture by dry petals from the external environment (temperature and humidity). Similar kind of results were also reported by Amol *et al.* [16] in rose tea and hibiscus tea during the storage period of 60 days.

Total anthocyanin content ($mg.L^{-1}$): The treatments differed significantly with respect to total anthocyanin content. Among all the treatments, T_1 (Dried rose petals- Control) significantly recorded the maximum total anthocyanin content (19.56 $mg.L^{-1}$), followed by T_5 (Dried rose petals and dried tulasi leaves in 2:1) (18.00 $mg.L^{-1}$). Significantly, the lowest total anthocyanin content (14.69 $mg.L^{-1}$) was registered in T_6 (Dried rose petals and dried ginger powder in 1:1). In the present study, rose petal tea had recorded highest anthocyanin content among all the other herbal teas, might be due to the bright red colour. Ramya *et al*. [17] observed that T_2 which contains hibiscus tea (freshly prepared, 0-day storage), exhibited the highest anthocyanin content of 72.13 mg c3g eq/L while T_3 which comprises of green tea + hibiscus tea (freshly prepared, 0-day storage), reported an anthocyanin content of 37.14 mg c3g eq/L and concluded that the anthocyanin content was found to decrease gradually when dried petals were stored longer. A similar decrease trend of total anthocyanin content was observed by Sharma *et al*. [18] and also by Amol *et al*. [16] in dried hibiscus and rose tea where the anthocyanin content of hibiscus and rose tea prepared decreased from (22.27-8.73 $mg.L^{-1}$) at 0 days to (13.93-5.23 $mg.L^{-1}$) at 60 days of storage period respectively.

Antioxidant activity (% inhibition): Among the different treatments, the antioxidant activity (%) showed significant difference. T_4 (Dried rose petals and dried tulasi leaves in 1:1) significantly recorded the maximum antioxidant activity (92.70 %), which was statistically at par with T_2 (Dried rose petals and dried lemon grass in 1:1) (91.00

%), T_5 (Dried rose petals and dried tulasi leaves in 2:1) (90.98 %), T_3 (Dried rose petals and dried lemon grass in 2:1) (89.70 %), and followed by T_1 (Dried rose petals-Control) (88.64 %). Whereas, significantly lowest antioxidant activity (85.00 %) was registered in T_6 (Dried rose petals and dried ginger powder in 1:1). Combining different medicinal plants showed higher antioxidant potential than using an individual plant [19]. These results are in accordance with research findings of Amol *et al*. [6] who reported that decrease in the antioxidative property during storage period may be due to degradation in anthocyanin, total phenols, change in the pH composition, which are responsible for stability of antioxidant properties. Similar results were reported by Naithani *et al*. [20] and Ramya *et al*. [17].

Plate 1. Herbal tea prepared from different blends of rose

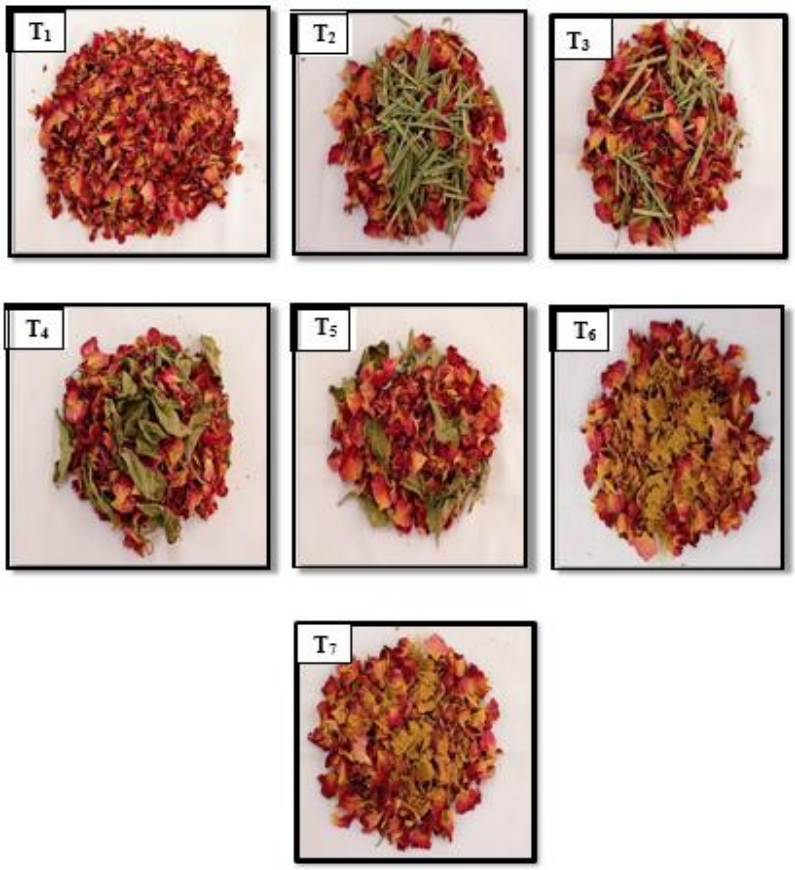

Plate 2. Dried rose herbal tea blended product

Table 1. Bio-chemical parameters of dried rose herbal tea blends

Treatments	Total moisture content (%)	Total anthocyanin content (mg.L^{-1})	Antioxidant activity (% inhibition)	Total phenolics content (mg.100g^{-1} GAE)
T1: Dried rose petals- Control	7.92^{c}	19.56^{a}	88.64^{b}	134.90^{b}
T2: Dried rose petals and dried lemon grass in 1:1	6.95^{e}	15.08^{d}	91.00^{ab}	138.72^{ab}
T3: Dried rose petals and dried lemon grass in 2:1	7.03^{d}	17.82^{bc}	89.70^{ab}	136.82^{ab}
T4: Dried rose petals and dried tulasi leaves in 1:1	9.85^{ab}	15.98^{cd}	92.70^{a}	140.68^{a}
T5: Dried rose petals and dried tulasi leaves in 2:1	9.96^{a}	18.00^{b}	90.98^{ab}	137.54^{ab}
T6: Dried rosepetals anddried ginger powder in 1:1	8.88^{b}	14.69^{e}	85.00^{c}	132.78^{c}
T7: Dried rosepetals anddried ginger powder in 2:1	8.75^{bc}	16.84^{c}	87.82^{bc}	133.00^{bc}
SEm±	0.09	0.28	1.01	1.60
CD at 5%	0.29	0.87	3.09	4.87

**Values expressed in alphabets indicate the priority order of the respective parameter*

Table 2. Organoleptic scoring of dried rose herbal tea blends

Treatments	Colour	Flavor	Taste	Overall acceptability
T1: Dried rose petals- Control	8.60^{a}	6.00^{e}	6.00^{e}	6.00^{f}
T2: Dried rose petals and dried lemon grass in 1:1	7.90^{bc}	8.50^{a}	8.25^{a}	8.60^{a}
T3: Dried rose petals and dried lemon grass in 2:1	8.15^{bc}	7.00^{cd}	7.25^{bc}	7.25^{cd}
T4: Dried rose petals and dried tulasi leaves in 1:1	7.95^{bc}	7.75^{b}	8.00^{ab}	8.00^{b}
T5: Dried rose petals and dried tulasi leaves in 2:1	8.20^{b}	6.75^{d}	7.00^{c}	7.00^{d}
T6: Dried rose petals and dried ginger powder in 1:1	7.80^{c}	7.25^{c}	7.50^{b}	7.50^{c}
T7: Dried rose petals and dried ginger powder in 2:1	8.00^{bc}	6.50^{de}	6.20^{d}	6.50^{e}
SEm±	0.11	0.08	0.12	0.08
CD at 5%	0.34	0.26	0.38	0.26

**Values expressed in alphabets indicate the priority order of the respective parameter*

Total phenolics content (mg.100g^{-1} GAE): There were significant differences among the treatments for total phenolics content. Among all the treatments, T_4 (Dried rose petals and dried tulasi leaves in 1:1) significantly recorded the maximum total phenolics content (140.68 mg.100g^{-1} GAE), which was statistically at par with T_2 (Dried rose petals and dried lemon grass in 1:1) (138.72 mg.100g^{-1} GAE), T_5 (Dried rose petals and dried tulasi leaves in 2:1) (137.54 mg.100g^{-1} GAE), T_3 (Dried rose petals and dried lemon grass in 2:1) (136.82 mg.100g^{-1} GAE), and followed by T_1 (Dried rose petals- Control) (134.90 mg.100g^{-1} GAE). While, significantly lowest (132.78 mg.100g^{-1} GAE) was registered in T_6 (Dried rose petals and dried ginger powder 1:1). In the present study the combination which contained more amount of tulasi recorded highest total phenolics content and the result can also be correlated with antioxidant activity. The results are in accordance with the findings reported by Thakur [21] in Studies on development and standardization of herbal tea, where *Ocimum sanctum*, *Cymbopogon citratus* and *Zingiber officinalis* showed the total phenolic content of 86 mg.g^{-1} GAE, 85 mg.g^{-1} GAE and 80 mg.g^{-1} GAE respectively. Similar result was produced by Ramya *et al*. [17] in hibiscus and green tea infusions who reported that T_3 which comprises of green tea + hibiscus tea (freshly prepared, 0- day storage), exhibited the highest total phenol content (82.57 mg.g^{-1} GAE) while T_2

which contains only hibiscus tea (freshly prepared, 0-day storage), showed a total phenol content of (64.79 $mg.g^{-1}$ GAE). The findings of their work concluded that the combined effects of green tea and hibiscus tea extracts enhanced the total phenolic contents and thereby increased the antioxidant potential in freshly dried petals of green tea + hibiscus tea infusions and also observed that there was a gradual decline in phenol content with respect to storage days.

Organoleptic scoring:

Colour: The treatments differed significantly with respect to organoleptic scoring (colour). Among all the treatments, T_1 (Dried rose petals- Control) registered the highest color score (8.60), followed by T_5 (Dried rose petals and dried tulasi leaves in 2:1) (8.20). The lowest colour score (7.80) was registered in T_6 (Dried rose petals and dried ginger powder in 1:1). T_1 (Dried rose petals- Control) scored higher value because of its bright appetizing red color when compared to other treatments. There was colour reduction observed in herbal tea with an increase in storage period might be due to oxidation and enzymatic browning of the stored product.

Flavor: A significant difference was observed in the taste of herbal tea made from different treatment combinations. Among all the treatments, T_2 (Dried rose petals and dried lemon grass in 1:1) recorded the highest score (8.50), followed by T_4 (Dried rose petals and dried tulasi leaves in 1:1) (7.75). Whereas, the lowest score (6.00) was registered in T_1 (Dried rose petals- Control). The present results are in accordance with Singh *et al*. [22] where it was concluded that (Corn Silk with Dried Lemon grass) blend obtained highest flavor score of 7.99±0.95 while with Tulsi blend it scored 7.44±1.21. A similar variation in the appeal of judges was reported by Oduro *et al*. [23] their findings reported score favored by high blends of *Cymbopogon citratus* because of high essential oil concentration in *Cymbopogon citratus*. The decrease in flavour score during storage might be due to loss of volatile aromatic substances [24]. The developed herbal teas have shown quiet high scores for the flavor preference. This might be due to the blend of volatile oils present in the different herbal ingredients; the herbal ingredients used in the preparation of the herbal teas are very active in volatile components which might have appealed the senses of judges to give them best score.

Taste: The treatments differed significantly with respect to organoleptic scoring (taste). The highest score (8.25) for taste was registered in T_2 (Dried rose petals and dried lemon grass in 1:1), which was at par with T_4 (Dried rose petals and dried tulasi leaves in 1:1) (8.00), followed by T_6 (Dried rose petals and dried ginger powder in 1:1) (7.50). The significantly least score was recorded in T_1 (Dried rose petals- Control) (6.00). All the herbal tea samples were given acceptable scores greater than 5, this might be due to effect of interaction between different acids, sugars and volatile components in the formulations. The herbal tea T_2 (Dried rose petals and dried lemon grass in 1:1) appealed most to the judges. Infusion of lemon grass leaf gives an aromatic drink with a characteristic lemon flavor Figueirinha *et al*. [25]. The lemon taste in the herbal tea T_2 might have forced the judges to assign it best score. The less astringent properties of the tea might led to score high for taste reported by Moodley *et al*. [26].

Overall acceptability: There were significant differences among the treatments for overall acceptability of prepared herbal tea. Among all the treatments, T_2 (Dried rose petals and dried lemon grass in 1:1) significantly recorded the maximum score for overall acceptability (8.60), followed by T_4 (Dried rose petals and dried tulasi leaves in 1:1) (8.00) and significantly lowest score (6.00) was registered in T_1 (Dried rose petals- Control). The results are in agreement with Singh *et al*. [21] in herbal tea formulation using different flavoured herbs with dried corn silk powder on sensory evaluation (overall acceptability) where it was indicated that blend with dried lemon grass flavour obtained highest score (7.92±0.94) whereas with tulsi blend it was 7.64±1.11. The degradation in overall acceptability with an increment of storage might be due to the decline of color, flavor, texture, and taste with an increased storage period.

4. CONCLUSION

From the present study it can be concluded that T_4 (Dried rose petals and dried tulasi leaves in 1:1) exhibited high radical scavenging activity (92.70 %) and total phenol content (140.68 $mg.100g^{-1}$ GAE) due to the presence of catechins, while T_1 (Dried rose petals- Control) possesses highest anthocyanin content (19.56 $mg.L^{-1}$) and sensory evaluation- colour score (8.60) with an appreciable level of anti-oxidant

value (88.64 %). The consumer preference-flavor (8.50), taste (8.25) and overall acceptability (8.60) was highest for T_2 (Dried rose petals and dried lemon grass in 1:1) tea infusion. Considering the above facts, aqueous infusions of dried rose petals with herbal blends are showcased as potential caffeine-free beverage with enhanced health benefits when used in combination. Hence this paper opens up the real scope of need to commercialize the combination of dried rose herbal tea infusions as a health drink in addition to other beverages.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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