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Research Article

Development and Nutritional Evaluation of Ready-to-Drink Beverage Using the Choongan (*Caralluma tuberculata* L.) Extract

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The present study aimed to develop ready-to-drink (RTD) beverage using the choongan (*Caralluma tuberculata* L.) extract and further to evaluate antioxidant potential and mineral estimation of the prepared RTD. The stem of *Caralluma tuberculata* was used to further isolate and purify the powder. Then, *C. tuberculata* powder was utilized in the formulation of beverages. All the treatments observed a decreasing trend for acidity, total soluble solids, total phenols, total antioxidant activity, total flavonoids, DPPH radical scavenging activity, and viscosity during storage whilst pH showed an increasing trend. It was observed that *Caralluma* RTD contained increasing trend of phytochemicals by increasing contents of the *Caralluma* extract. The same trend was also observed regarding all the mineral contents investigated in the present study. The treatment T_4 (*Caralluma* RTD having 1.5 g *C. tuberculata* powder) showed the maximum values regarding phytochemicals as well as minerals. The microbial counts ($\log_{10}$ CFU/mL) for all the treatments increased during storage. Even though T_4 showed promising results regarding phytochemicals and minerals, T_1 showed the maximum sensory score even after 21 days of storage.

1. Introduction

Fruits and vegetables play an important role in nourishing and healthy life due to the presence of antioxidant compounds like phytochemicals such as phenolic compounds, alkaloids, carotenoids (terpenes), tannins, and saponins [1]. Fruits and vegetables exhibit health-promoting properties by delaying the aging process and by reducing the risk of various diseases including cardiovascular disease, cancer, rheumatoid arthritis, cataract, Parkinson's, or Alzheimer's diseases [2]. The significance of medicinal plants is derived from their secondary metabolites, which produce unique physiological effects in plants and humans against a variety of infectious diseases and metabolic disorders [3]. *Caralluma* (*C.*) *tuberculata* is a perennial herb and is a member of the *Asclepiadaceae* family. It is typically found in Pakistan's mountainous regions, India (Andra Pradesh), the United Arab Emirates, Saudi Arabia, the south-eastern region of Egypt, Iran, and Nigeria [4]. *C. tuberculata*, *C. edulis*, *C. negevensis*, *C. sinaica*, *C. russeliana*, *C. stalagmifera*, *C. dalzielii N. E. Br*, *C. arabica*, etc. are members of this family but *Caralluma tuberculata* holds a prominent position among these. In the tropical regions of Punjab, Khyber Pakhtunkhwa, and Baluchistan provinces (Pakistan), roughly 23 genera and 40 species of *Asclepiadaceae* are present.

In traditional Arabic and Indo-Pakistani medicine, numerous *Caralluma* species can be used for the treatment

of diabetes, tumor, snake bites, redness, and skin problems [5, 6]. People usually use its leaves as fresh, as a salad, or with minced meat after boiling to eliminate their bitter taste. The succulent stem of *C. tuberculata* is used to treat rheumatism, febrifuge, stomachic, and carminative when cooked with minced meat [7]. In urban and rural Pakistan, this herb is usually used as a traditional antidiabetic treatment.

Wild *Caralluma* (Chungah) parts of the plant are obtained for consumption, medicinal use, and trading. *Caralluma* species have been neglected because they are difficult to cultivate and emit phenolics and latex, which make it difficult to form calluses and establish new shoots. *Caralluma* has anti-inflammatory, antitumor, antioxidant, anticancer, cytoprotective, and antiulcer properties, as well as antinociceptive properties [5, 8–10]. *Caralluma* species were found to have phytochemicals, such as flavonoids, catechic tannins, triterpenes, saponins, mucilages, coumarins, alkaloids, and quinones [11, 12]. Some polyphenols, for instance, ferulic acid, gallic acid, quercetin, hesperetin, and myricetin, were also observed to be present in the extract of *Caralluma europaea* [11]. In another study, it was observed that the extract of *C. tuberculata* contained phenolics (rutin and gallic acid) and flavonoids [13]. In addition, the findings also revealed some pharmacological features of *C. tuberculata* on the basis of promising results concerning its antileishmanial, in vitro antifungal, THP-1 leukemia cell line cytotoxicity, brine shrimp cytotoxicity, and protein kinase inhibition assays tuberculata which may authenticate its therapeutic role for the cure of various ailments [13]. Kebbou et al. [14] evaluated antioxidant, anti-inflammatory, and analgesic properties of ethanolic and ethyl acetate extracts of *Caralluma europaea*. They concluded that being rich in phenolic compounds, *Caralluma europaea* possessed antioxidant, antinociceptive, and anti-inflammatory activities. Adnan et al. [6] also reported efficacy of genus *Caralluma* concerning its pharmacological properties such as antioxidant, antimicrobial, and anticancer activity owing to the presence of compounds, for instance, flavonoid glycosides, pregnane glycosides, and flavones.

According to reports, *C. tuberculata* is severely endangered species [15]. To maintain plants with a high medicinal value, it is required to establish adequate conservation methods by promoting its cultivation. *Caralluma tuberculata* must be conserved by educating individuals on their sustainable utilization. So far, it has not yet been explored for its nutritional benefits, especially the presence of phytochemicals and minerals. Traditionally, people use *Caralluma tuberculata* in the preparation of a dish with some oil and spices. Such kind of dish is usually eaten along with chapatti (flat bread). *Caralluma tuberculata* is an endangered species in Pakistan and is seasonally available only in Sargodha city due to climatic and geographical locations. To the best of our knowledge, no study has been published regarding the use of *Caralluma* extract in drink. Therefore, the availability of *Caralluma* nutrients can be ensured by incorporating its extract into drink. Therefore, the study was planned to develop *Caralluma* ready to drink and further to evaluate it for physicochemical and sensorial characteristics, antioxidant potential, and mineral estimation.

2. Materials and Methods

2.1. Raw Material. Fresh and healthy *Caralluma tuberculata* were obtained from the local market of Sargodha, Pakistan. They were washed twice with clean and fresh water and were finely chopped. These were subjected to oven drying (70°C for 2 days) and then grinded finely into the powder form. After grinding, the powder was used in drink formulation (see treatment plan in Table 1). Some other raw materials such as preservatives, lemon juice, and plastic bottles were also purchased from the local market of Sargodha, Pakistan.

2.2. Preparation of Caralluma Drink. *C. tuberculata* powder was used in the drink according to treatment plan shown in Table 1. The powder of the *Caralluma* extract was bitter in taste. In order to mask the bitter taste of *Caralluma* powder, sorbitol was used for the preparation of its RTD. The samples of the prepared drink were analyzed at different storage period (1, 7, 14, and 21 days) at 20°C.

2.3. Physicochemical Analysis. Total soluble solids (TSSs) were determined by hand refractometer (model no. MASTER-53α, Atago, Japan). Reading was computed based on the blue color scale base, and sugar percentage was computed [16]. The pH of the samples was determined using a pH meter (AD 1040 Benchtop meter, Adwa, Hungary). Acidity was measured by the standard titration method as described in [16]. Viscosity measurements of juice were carried out using a rotatory viscometer (model DV- I, Brookfield, Stoughton, Mass, V U.S.A) with a precision cylindrical spindle rotating (UL) adapter. Experiments were conducted in a controlled condition (such as temperature, volume, and container) to determine the viscosity of the drink with varying compositions. The stationary rheometer cup was filled with 150–250 mL of drink. The viscosities of the samples were tested at temperatures between 25 ± 1°C.

2.4. Sensory Evaluation. The prepared fruit drinks in the present study were evaluated for sensory characteristics such as color, flavor, taste, texture, and overall acceptability by an untrained panel of 20 members including faculty and postgraduate students from the Institute of Food Science and Nutrition, University of Sargodha, by following the 9-point Hedonic scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = like extremely) [17].

2.5. Determination of Total Plate Counts (TPCs). The TPC was performed by the FDA's standard procedure outlined in the bacteriological analytical manual [18]. One mL of the sample was mixed with 9 mL of buffered phosphate diluent. Ten mL dilution was prepared by shifting 1 mL of the previous dilution to 9 mL of buffered phosphate diluents. One mL of the latter dilution was transferred into duplicate Petri dishes. Then, 12–15 mL plate count media was placed

Table 1: Treatment plan of *Caralluma* ready to drink (RTD).

Treatments	Formulation of *Caralluma* drink	Quantity of *Caralluma* extract
T_0	Lemon juice (10%), water (90 mL), sorbitol (20%), color (0.1 g), KMS (0.1 g)	0 g (control)
T_1	Lemon juice (10%), water (90 mL), sorbitol (20%), color (0.1 g), KMS (0.1 g)	0.5 g
T_2	Lemon juice (10%), water (90 mL), sorbitol (20%), color (0.1 g), KMS (0.1 g)	1 g
T_3	Lemon juice (10%), water (90 mL), sorbitol (20%), color (0.1 g), KMS (0.1 g)	1.5 g
T_4	Lemon juice (10%), water (90 mL), sorbitol (20%), color (0.1 g), KMS (0.1 g)	2 g

KMS: potassium metabisulphite.

into plates. Petri dishes were incubated at 35°C for 48 h. After incubation, colonies on those plates containing 30–300 colonies were counted and multiplied by the dilution factor. The results were expressed as Log_{10} CFU/g of sample.

2.6. Determination of Total Phenolic (TP) Contents. The TP contents were measured spectrophotometrically using the method described by Iqbal et al. [19]. Following dilution, 0.5 mL of the sample was taken for the experiment. Gallic acid was used as a standard to make the calibration curve. The total phenolic compounds were represented as mg of gallic acid equivalent (GAE) per 100 mL of juice.

2.7. Determination of Total Flavonoids (TFs). *Caralluma* drink was evaluated for TF by the method described by Malik et al. [20]. Catechinin ethanol was used as a standard, and the results were given in terms of mg of (+)-catechin equivalent (CE) per 100 g of fresh weight.

2.8. Determination of Total Antioxidant Capacity (TAC). Prieto et al. [21]'s method was used to analyze the total antioxidant activity. Standard calibration curves were made with ascorbic acid, and the results were given in terms of μg of ascorbic acid equivalent (AAE)/mL drink.

2.9. Determination of DPPH-Free Radical Scavenging Activity. *Caralluma* drink was evaluated for DPPH-free radical scavenging activities using the method described by Yi et al. [22]. One mL diluted sample was mixed with 1 mL of DPPH solution (60 μmol in ethanol). This solution was placed in the dark for 30 min before its absorbance at 517 nm was measured using a spectrophotometer. The preparation of the control (ethanol) sample followed a similar protocol. The results were given in terms of μg of ascorbic acid equivalent (AAE)/mL drink.

2.10. Mineral Estimation of Caralluma Drink. The sample (1 mL) was prepared by the wet digestion method [23]. It was digested first with 10 mL HNO_3 at 60–70°C for 20 min. The sample was processed with $HClO_4$ at 190°C till a clear solution was obtained. The 100 mL volumetric flask containing the digested sample was topped up with distillation water and then filtered. The sample solution was put into the atomic absorption spectrophotometer. The standard curve for each mineral was created by running known concentration solutions. The contents of minerals in the samples were assessed by using the respective standard curve photometer according to the AACC [24] method.

2.11. Statistical Analysis. The data obtained were statistically analyzed using Statistix 8.1 software (Analytical Software, Tallahassee, FL, USA) by applying the two-way ANOVA test. Significant differences between mean values were determined by Tukey's pairwise comparison test at a significance level of $p < 0.05$.

3. Results and Discussion

3.1. Physicochemical Characteristics of Caralluma RTD. The results regarding physicochemical characteristics of *Caralluma* RTD are presented in Table 2. There was significant effect of the *Caramulla* extract and storage on TSS. In general, the TSS values of the *Caralluma* RTD (for all treatments) decreased significantly during storage. The highest TSSs were found in T_4 (15.10°Bx) having the maximum contents of the *Caralluma* extract and the lowest value was observed in T_0 (13.13°Bx) on day 1. The decrease in TSS might be the result of chemical interactions between organic beverage components [25]. Since vitamin C is water soluble and susceptible to oxidation, it is gradually reduced, which might be the primary reason for the decline in acidity and TSS [26].

There was a significant effect of the *Caralluma* extract and storage on pH of RTD. The highest pH value was found in T_0 (3.17) and the lowest was observed in T_4 (2.53) on day 1. The addition of more and more *Caralluma* extract might contribute to lower the values of pH. After 21 days of storage, the maximum value of pH was obtained in T_0 (4.01) and the least value was noted in T_4 (2.96). The pH values of the *Caralluma* RTD samples increased significantly during storage. Our results were concurrent with the findings of some previous studies who also observed a gradual increase in pH. This might be due to decrease in titratable acidity and Vitamin C during storage [27]. The increase in pH during storage of citrus juice might be due to acid hydrolysis of the polysaccharides into mono- and disaccharides [28]. Moreover, Singh et al. [29] manufactured ready-to-drink beverage by combining lemon and bitter gourd in various proportions (100 : 0, 75 : 25, and 50 : 50). They observed a decrease in acidity (5.03–4.167%) and a rise in pH (2.6–3.6) during a period of 60 days. The addition of the *Caralluma* extract into RTD caused decrease in pH which might be due to increase in its acidity.

During storage, highly significant variations of titratable acidity were observed between treatments of the *Caralluma* RTD. The titratable acidity values of *Caralluma* RTD decreased significantly during storage. The highest acidity value was found in T_4 (0.05%) and the least value in T_2 (0.04%) on day 1. After 21 days of storage, the maximum

Table 2: Physicochemical characteristics of *Caralluma* ready to drink (RTD) during storage.

Treatments	Days	pH	Acidity (%)	TSS (%)	Viscosity (cP)
T_0	1	3.17 ± 0.01h	0.05 ± 0.002ac	13.13 ± 0.06ef	0.35 ± 0.26b–e
	7	3.81 ± 0.01b	0.04 ± 0.001d–g	12.60 ± 0.10g	0.37 ± 0.01b–e
	14	3.82 ± 0.01b	0.03 ± 0.002fgh	12.13 ± 0.06hi	0.16 ± 0.01f
	21	4.01 ± 0.01a	0.03 ± 0.008hi	11.60 ± 0.10j	0.14 ± 0.01f
T_1	1	2.82 ± 0.01p	0.05 ± 0.001ab	14.01 ± 0.01b	0.41 ± 0.01a–d
	7	2.84 ± 0.01o	0.04 ± 0.001de	13.70 ± 0.10cd	0.41 ± 0.01a–d
	14	3.15 ± 0.01i	0.04 ± 0.001def	13.00 ± 0.10f	0.37 ± 0.01b–e
	21	3.20 ± 0.01g	0.03 ± 0.002gh	12.20 ± 0.10hi	0.22 ± 0.01ef
T_2	1	2.87 ± 0.01n	0.04 ± 0.002cd	13.80 ± 0.10bc	0.51 ± 0.01ab
	7	3.02 ± 0.01k	0.04 ± 0.001de	13.40 ± 0.10de	0.48 ± 0.01abc
	14	3.17 ± 0.01h	0.04 ± 0.001d–g	13.10 ± 0.10ef	0.41 ± 0.01a–d
	21	3.26 ± 0.01f	0.01 ± 0.001j	12.13 ± 0.15hi	0.28 ± 0.01d–f
T_3	1	3.07 ± 0.01j	0.04 ± 0.003b–d	13.40 ± 0.10de	0.56 ± 0.01a
	7	3.30 ± 0.01e	0.04 ± 0.002cd	13.20 ± 0.10ef	0.50 ± 0.01ab
	14	3.64 ± 0.01d	0.04 ± 0.001de	12.40 ± 0.10gh	0.42 ± 0.01a–d
	21	3.72 ± 0.01c	0.02 ± 0.002i	12.03 ± 0.12i	0.32 ± 0.02c–f
T_4	1	2.53 ± 0.02r	0.05 ± 0.003a	15.10 ± 0.10a	0.57 ± 0.01a
	7	2.63 ± 0.02q	0.04 ± 0.004b–d	14.10 ± 0.10b	0.52 ± 0.01ab
	14	2.92 ± 0.01m	0.04 ± 0.002bcd	13.70 ± 0.10cd	0.46 ± 0.01a–d
	21	2.96 ± 0.02l	0.03 ± 0.004e–h	13.20 ± 0.10ef	0.39 ± 0.01a–e

T_0 = control (without the *Caralluma* extract) RTD, T_1 = 0.5 g *Caralluma* extract RTD, T_2 = 1.0 g *Caralluma* extract RTD, T_3 = 1.5 g *Caralluma* extract RTD, and T_4 = 2.0 g *Caralluma* extract RTD. Means with different letters show significant ($p < 0.05$) variations between treatments and storage.

value of acidity was obtained in T_0 (0.03%) and the least value was observed in T_2 (0.01%).

The decrease in titratable acidity during storage of citrus juice might be due to the acid hydrolysis of the polysaccharides into mono- and disaccharides [28]. The addition of the *Caralluma* extract into RTD caused an increase in its acidity. Gutam et al. (2021) also found that the ascorbic acid contents of blended nectar decreased significantly from 9.35 mg/100 g to 7.76 mg/100 g after 6 months. The viscosity of *Caralluma* RTD varied significantly among treatments but nonsignificant effect was observed during storage. The viscosity of the *Caralluma* RTD decreased significantly during storage. The decrease in viscosity of *Caralluma* RTD during storage might be due to the degradation of sugars and pectin of lemon juice used in the formulation of the drink. Our results were in conformity to the findings of some researchers who also suggested precipitation of pulp and degradation of pectin during storage [30, 31]. Moreover, orange juice has shear thinning behavior [32]. The highest viscosity value was found in T_4 (0.57 cP) and the lowest was observed in T_0 (0.35 cP). The addition of more and more *Caralluma* powder in subsequent treatments caused the increase in viscosity in drinks. It might be assumed that the addition of *Caralluma* powder contributed to increase in total solids of *Caralluma* RTD, thereby caused an increase in viscosity. After 21 days of storage, the maximum value of viscosity was obtained in T_4 (0.39 cP) and the least value was found in T_1 (0.14 cP).

3.2. Sensory Analysis. The results relating to sensory parameters are presented in Figure 1. The treatment T_1 was highly acceptable according to the sensory panel's opinion even after 21 days of storage. The results indicated that the sensory parameters of *Caralluma* RTD reduced during storage. The highest color of the drink was found in T_1 (7.90) and the lowest score was obtained by T_4 (6.50) on day 1. The color of *Caralluma* RTD became more and more darker in color due to the addition of more *Caralluma* extract powder in subsequent treatments. The decreasing trend of color perception of the *Caralluma* RTD during storage might be due to the degradation of some compounds, such as flavonoids, catechic tannins, triterpenes, saponins, coumarins, alkaloids, and quinones [12, 33]. In addition, enzymatic (due to the activities of polyphenol oxidase and peroxidase) [34] and nonenzymatic browning (due to the Maillard reaction, caramelization, and ascorbic acid degradation) [35] during the storage of *Caralluma* RTD might be responsible for the deterioration of its color.

The highest flavor score of *Caralluma* RTD was obtained by T_1 (7.60) and the lowest was observed in T_4 (7.05) on day 1. The reduction in flavor during storage may be related to the loss of volatile aromatic compounds [36]. Decrease in flavor was also observed by several researches [37–39]. Din et al. [40] also noted decreasing trend of the flavor score of bitter gourd RTS (from 7.5 to 6.1) during 90 days of storage.

The highest taste score of *Caralluma* RTD was found in T_1 (7.60) and the lowest was observed in T_4 (7.05) on day 1. The change in taste during storage might be due to the degradation of ascorbic acid and some other compounds, such as flavonoids, catechic tannins, triterpenes, saponins, coumarins, alkaloids, and quinones [12, 33]. The reduction in flavor during storage may be related to the loss of volatile aromatic compounds. Ahmed et al. [37] observed loss of flavor during storage which is similar to the current study. Their findings showed that the taste score in freshly made bitter gourd RTS decreased from 7.5 to 6.1 after 90 days of

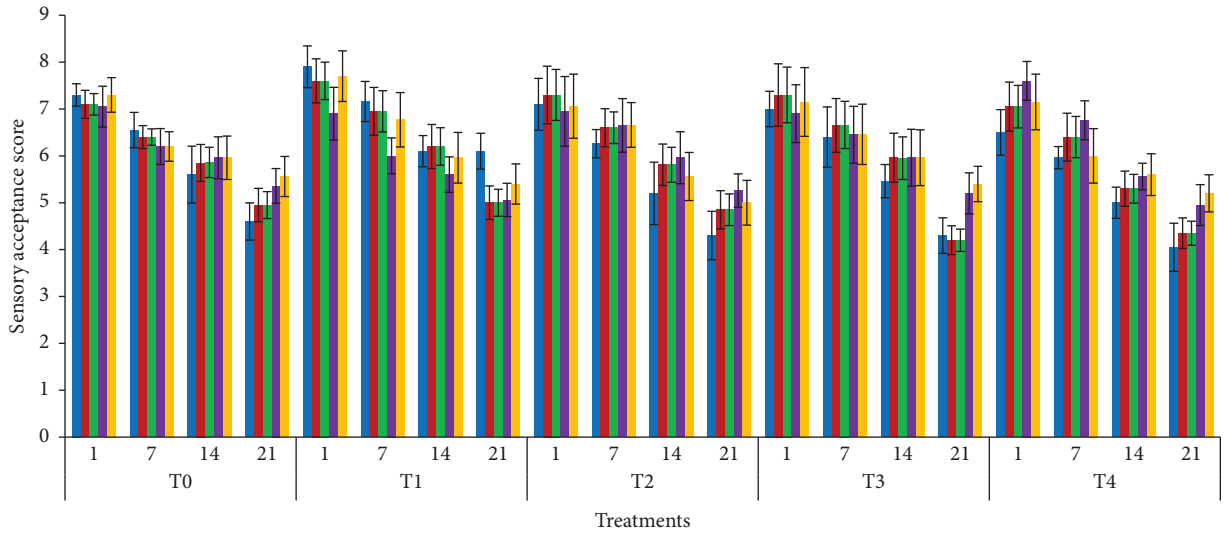

Figure 1: Sensory scores (means ± SD, color (blue bars), flavor (red bars), taste (green bars), texture (purple), and overall acceptability (yellow bars)) of *Caralluma* ready to drink (RTD) (T_0 = control (without the *Caralluma* extract) RTD, T_1 = 0.5 g *Caralluma* extract RTD, T_2 = 1.0 g *Caralluma* extract RTD, T_3 = 1.5 g *Caralluma* extract RTD, and T_4 = 2.0 g *Caralluma* extract RTD) during storage.

storage. Similar results were observed by Gaikwad et al. [39] in low-calorie herbal aonla-ginger RTS.

The highest texture score of *Caralluma* RTD was found in T_4 (7.60) and the least was observed in T_1 (6.90) on day 1. The texture score of RTD reduced during storage. The highest overall acceptance score of *Caralluma* RTD was found in T_1 (7.70) and the least was obtained by T_3 (7.06) on day 1. The overall acceptance score of RTD was reduced gradually during storage. Thakur et al. [36] showed a reduction in overall acceptability (7.6–5.7) for the bitter gourd RTS beverage during storage. Ahmed et al. [37] and Gaikwad et al. [39] observed a decreasing tendency in overall acceptability during the storage of functional and dietetic beverages manufactured from varying ratios of bitter gourd and herbal aonla-ginger RTS beverages.

3.3. Total Plate Counts (TPCs). Nonsignificant differences of TPC were observed among treatments during storage condition as shown in Figure 2. The TPC of *Caralluma* RTD increased significantly during storage. The highest TPC was found in T_0 (1.75 $\log_{10}$ CFU/mL) and the lowest was observed in T_4 (1.60 $\log_{10}$ CFU/mL). The highest TPC was found in T_0 (2.00 $\log_{10}$ CFU/mL) and the lowest was observed in T_4 (1.90 $\log_{10}$ CFU/mL) after 21 days of storage. This might be due to antimicrobial activities of *Caralluma* powder. The addition of more and more *Caralluma* powder might cause reduction in microbial counts during storage.

3.4. Total Phenolic (TP) Contents. The results regarding TP contents of *Caralluma* RTD are presented in Figure 3. Highly significant differences were observed in TP contents among treatments during storage. The highest TP values were found in T_4 (599.9 mg GAE/100 mL) and the lowest were observed in T_0 (182.42 mg GAE/100 mL) on day 1. Amrati et al. [33] observed polyphenols as 55 mg/g of the *Caralluma europaea* hydroethanolic extract. Therefore, addition of more and more *Caralluma* extract caused increased contents of total phenolics in the present study. After 21 days of storage, there was decrease in the phenolic content in all treatments and this decrease was more pronounced in T_0 (84.77 mg GAE/100 mL). It might be assumed that due to the addition of *Caralluma* powder, change in total phenolic compound was less in treatments as compared to the control. The value of TP of *Caralluma* RTD decreased significantly during storage. Our results were confirmed by the study of Liu et al. [41] who reported that many phenolic compounds (ferulic acid, cryptochlorogenic acid, erucic acid, catechin, heperetin, and baicalein) were degraded in lemon juice during storage. Similar decreasing trend of TP contents of bitter gourd kiwi squash was observed by Sharma et al. [42].

The present study's findings are also consistent to those of Siah et al. [43], who showed that the polyphenol contents of herb *Centella asiatica*-supplemented citrus beverages steadily dropped after a four-month storage period. Due to their utilization in the synthesis of protein-complexed polymeric molecules, the overall number of phenols that have been stored could undergo a significant decrease [44].

3.5. Total Flavonoid (TF) Contents. The results concerning the TF contents are presented in Figure 3. The major flavonoid identified in *Caralluma* species was luteolin-4-O-neohesperidoside [45]. During storage, highly significant variations of TF contents were observed between treatments. The TF contents of *Caralluma* RTD decreased significantly during storage in all the treatments. Our results were in accordance to the findings of Liu et al. [41] who reported that total flavonoids were degraded in lemon juice during storage. Porto et al. [46] also observed that the total flavonoid content of beet orange juice decreased during storage. The highest TF values were found in T_4 (212.25 mg

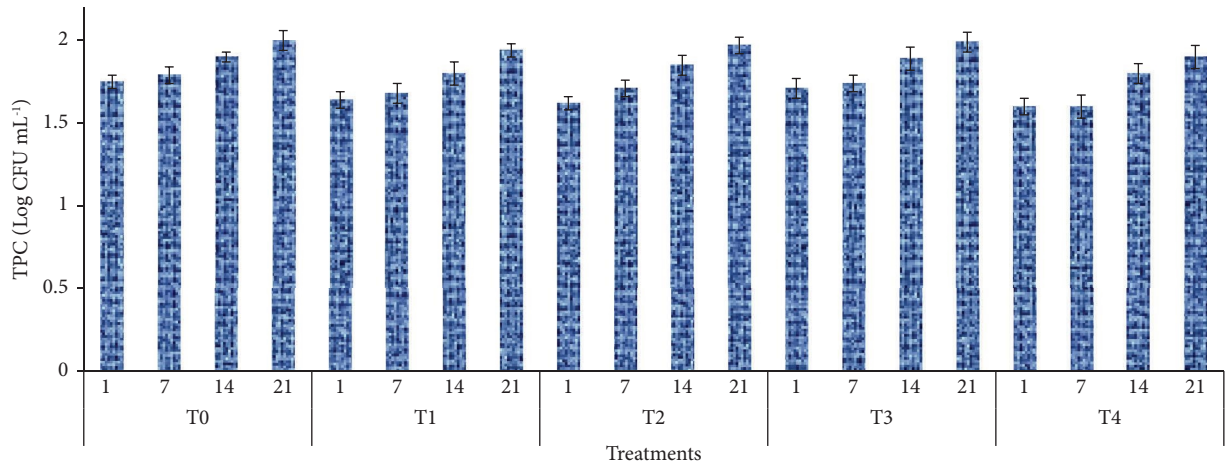

Figure 2: Total plate counts (TPC, means ± SD) of *Caralluma* ready to drink (RTD) (T_0 = control (without the *Caralluma* extract) RTD, T_1 = 0.5 g *Caralluma* extract RTD, T_2 = 1.0 g *Caralluma* extract RTD, T_3 = 1.5 g *Caralluma* extract RTD, and T_4 = 2.0 g *Caralluma* extract RTD) during storage.

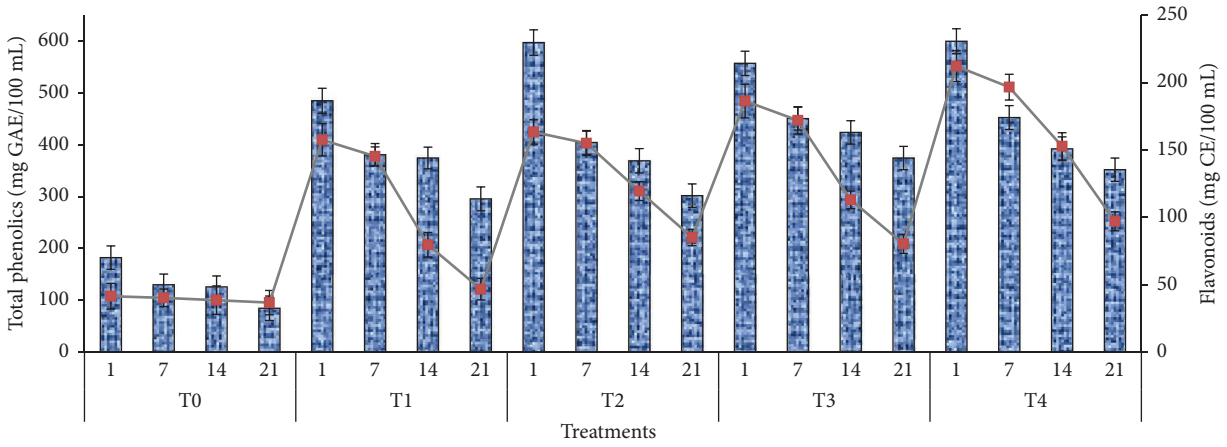

Figure 3: Phytochemicals (total phenolics (bars, means ± SD) and flavonoids (lines, means ± SD)) of *Caralluma* ready to drink (RTD) (T_0 = control (without the *Caralluma* extract) RTD, T_1 = 0.5 g *Caralluma* extract RTD, T_2 = 1.0 g *Caralluma* extract RTD, T_3 = 1.5 g *Caralluma* extract RTD, and T_4 = 2.0 g *Caralluma* extract RTD) during storage.

CE/100 mL) and the lowest was observed in T_0 (41.55 mg CE/100 mL) on day 1. After 21 days of storage, the maximum values of TF contents were observed in T_4 (97.10 mg CE/100 mL) and the least values were observed in T_0 (36.78 mg CE/100 mL). Amrati et al. [33] observed total flavonoids as 40 mg/g of the *Caralluma europaea* hydroethanolic extract. Therefore, addition of more and more *Caralluma* extract caused increased contents of total flavonoids in the present study.

3.6. Total Antioxidant Capacity (TAC). The results concerning the TAC are presented in Figure 4. Regarding TAC, highly significant variations were seen between treatments and storage of the *Caralluma* RTD. The TAC values of *Caralluma* RTD decreased during storage. Liu et al. [41] observed degradation of total phenolics and flavonoids in lemon juice during storage which might have influenced the total antioxidant activity of juice. There might be the possibilities of degradation of phytochemicals in *Caralluma* RTD which ultimately influenced total antioxidant activity. The highest TAC values were found in T_4 (306.00 μg AAE/mL) and the lowest values were observed in T_0 (129.2 5 μg AAE/mL). After 21 days of storage, the maximum values of TAC were obtained by T_3 (282.67 μg AAE/mL) and the least values were noted in T_0 (118.67 μg AAE/mL). It might be assumed that the behavior of *Caralluma* powder would be changed in the drink, i.e., different other compounds would be produced due to the reactions of powdered compounds and lemon juice RTD contents. There might be possibilities that the degradation of phenolics and flavonoids occurred more at the highest concentrations of the *Caralluma* extract in T_4.

3.7. DPPH Radical Scavenging Activity. The results concerning to DPPH radical scavenging activity are presented in Figure 4. There were significant variations of DPPH radical scavenging activity between treatments and storage of *Caralluma* RTD. DPPH radical scavenging activity values of

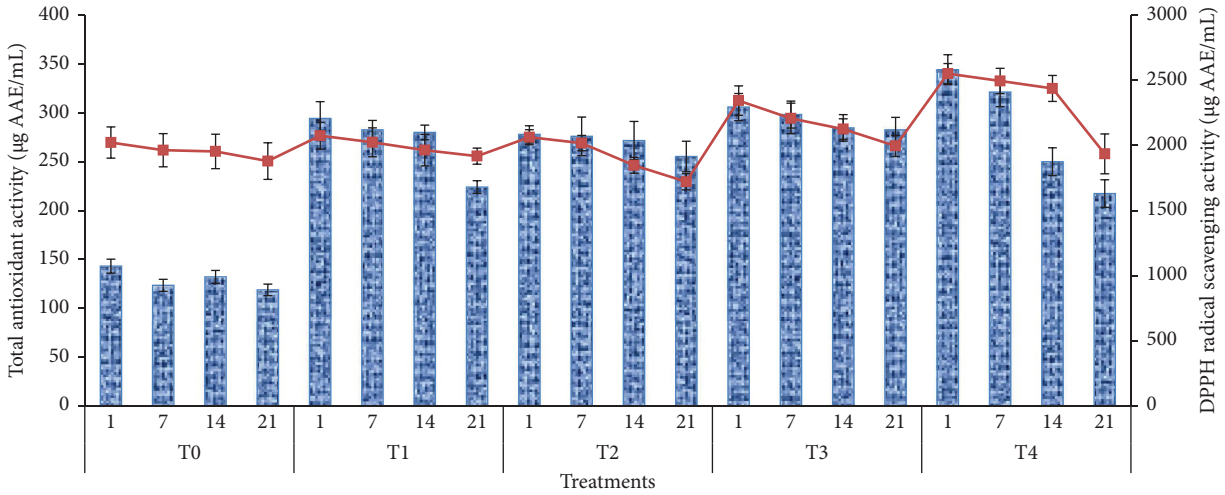

Figure 4: Total antioxidant capacity (TAC (bars, means ± SD)) and DPPH radical scavenging activity (lines, means ± SD) of *Caralluma* ready to drink (RTD) (T_0 = control (without the *Caralluma* extract) RTD, T_1 = 0.5 g *Caralluma* extract RTD, T_2 = 1.0 g *Caralluma* extract RTD, T_3 = 1.5 g *Caralluma* extract RTD, and T_4 = 2.0 g *Caralluma* extract RTD) during storage.

the *Caralluma* RTD decreased significantly during storage. The highest DPPH activity values were found in T_4 (2552.00 μg AAE/mL) and the lowest values were observed in T_0 (2022.3 μg AAE/mL) on day 1. The phytochemicals, such as flavonoids, catechic tannins, triterpenes, saponins, coumarins, alkaloids, and quinones [12, 33], and some polyphenols, for instance, ferulic acid, gallic acid, quercetin, hesperetin, and myricetin, have been reported in the extract of *Caralluma europaea* [33]. The aforementioned phytochemicals present in *Caralluma* species might be responsible for DPPH radical scavenging activities in *Caralluma* RTD. After 21 days of storage, the maximum value of DPPH activity was obtained in T_3 (1996.30 μg AAE/mL) and the lowest values were observed in T_2 (1720.7 μg AAE/mL).

3.8. Minerals in Caralluma RTD. The results regarding minerals in *Caralluma* RTD are presented in Table 3. The minerals were estimated on day 1 and after 21 days of storage in all the samples. The potassium concentrations of *Caralluma* RTD decreased significantly after 21 days of storage. The highest potassium concentration was found in T_4 (1452.0 mg/100 g) and the lowest was found in T_0 (775.00 mg/100 g) on day 1.

There were significant differences regarding sodium concentrations among treatments and storage. The sodium concentrations of *Caralluma* RTD decreased significantly after 21 days of storage. The highest sodium concentration was found in T_4 (2353.33 mg/100 g) and the lowest was found in T_0 (937.70 mg/100 g) on day 1.

There were significant differences regarding calcium concentrations among treatments and storage. The calcium concentrations of *Caralluma* RTD decreased significantly after 21 days of storage. The highest calcium concentration was found in T_4 (6751.34 mg/100 g) and the lowest was found in T_0 (251.67 mg/100 g) on day 1.

There were significant differences regarding magnesium concentrations among treatments and storage. The magnesium concentrations of *Caralluma* RTD decreased significantly after 21 days of storage. The highest magnesium concentration was found in T_4 (320.07 mg/100 g) and the lowest was found in T_0 (67.51 mg/100 g) on day 1.

There were significant effects of treatments and storage on zinc concentrations. The zinc concentrations of *Caralluma* RTD decreased significantly after 21 days of storage. The highest zinc concentration was found in T_4 (25.07 mg/100 g) and the lowest was found in T_0 (0.75 mg/100 g) on day 1. The mineral content in juices and drinks may change during storage [47]. Rajauria and Tiwari [48] narrated that the stability of drinks may be affected during storage which might be due to the interaction of the mineral content with packaging.

There were significant effects of treatments and storage on copper concentrations. The copper concentrations of *Caralluma* RTD decreased significantly after 21 days of storage. The highest copper concentration was found in T_4 (12.52 mg/100 g) and the lowest was found in T_0 (0.22 mg/100 g) on day 1.

There were significant effects of treatments and storage on copper concentrations. The copper concentrations of *Caralluma* RTD decreased significantly after 21 days of storage. The highest copper concentration was found in T_4 (83.33 mg/100 g) and the lowest was found in T_0 (3.42 mg/100 g) on day 1.

There were significant effects of treatments and storage on manganese concentrations. The manganese concentrations of *Caralluma* RTD decreased significantly after 21 days of storage. The highest manganese concentration was found in T_4 (2.08 mg/100 g) and the lowest was found in T_0 (0.39 mg/100 g) on day 1.

Table 3: Mineral concentrations (mg/100 g) of *Caralluma* ready to drink (RTD) during storage.

Treatments	Days	K	Na	Ca	Mg	Zn	Cu	Fe	Mn
T_0	1	0775.00 ± 22.00g	937.17 ± 31.04g	251.67 ± 11.08f	67.51 ± 1.01g	0.75 ± 0.01e	0.22 ± 0.01h	3.42 ± 0.02e	0.39 ± 0.01d
	21	652.00 ± 21.11h	717.53 ± 20.04i	15.50 ± 1.02j	37.50 ± 1.02i	0.56 ± 0.02e	0.062 ± 0.02i	2.75 ± 0.01e	0.43 ± 0.01d
T_1	1	1074.67 ± 25.53f	1058.33 ± 33.7d	336.33 ± 9.53e	85.00 ± 2.10e	1.65 ± 0.32 d	0.33 ± 0.02g	5.45 ± 0.15d	0.42 ± 0.01d
	21	0602.33 ± 12.52i	636.33 ± 9.53j	197.53 ± 3.03h	72.513 ± 3.01f	0.84 ± 0.01e	0.055 ± 0.01i	3.75 ± 0.03e	0.152 ± 0.01f
T_2	1	1375.09 ± 30.08b	1082.00 ± 25.0c	552.33 ± 12.52d	172.51 ± 6.01c	1.77 ± 1.32d	6.16 ± 1.01c	5.81 ± 0.01d	0.69 ± 0.01c
	21	1251.67 ± 21.53c	871.67 ± 21.08h	37.52 ± 1.03i	28.75 ± 1.02j	1.69 ± 0.42 d	0.93 ± 0.03e	2.75 ± 0.02e	1.11 ± 0.01b
T_3	1	1376.33 ± 31.53b	1253.00 ± 34.0b	1251.00 ± 14.0c	182.52 ± 2.020b	23.51 ± 1.01b	6.16 ± 1.01c	33.50 ± 10.06b	0.72 ± 0.01c
	21	1125.00 ± 28.00e	151.33 ± 6.53e	207.52 ± 5.02g	142.513 ± 2.01d	2.41 ± 0.01c	0.93 ± 0.03e	3.81 ± 0.01e	0.47 ± 0.02f
T_4	1	1452.0 ± 29.20a	2353.33 ± 57.08a	4075.33 ± 22.53a	320.07 ± 11.06a	25.07 ± 2.06a	12.52 ± 1.02b	83.33 ± 1.52a	2.08 ± 0.07a
	21	1175.07 ± 36.4d	1024.7 ± 24.52f	2550.33 ± 16.51b	142.513 ± 4.01d	2.37 ± 0.45c	0.78 ± 0.01f	11.52 ± 0.02c	0.25 ± 0.02e

T_0 = control (without the *Caralluma* extract) RTD, T_1 = 0.5 g *Caralluma* extract RTD, T_2 = 1.0 g *Caralluma* extract RTD, T_3 = 1.5 g *Caralluma* extract RTD, and T_4 = 2.0 g *Caralluma* extract RTD. Means with different letters show significant ($p < 0.05$) variations between treatments and storage.

4. Conclusion

On the basis of our findings in the present study, it was concluded that T_4 exhibited higher values of TSS, acidity, antioxidant activity, DPPH activity, flavonoids, and phenolic contents. In addition, T_4 also showed the highest concentrations of all the minerals.

The results of the present study suggested that *Caralluma* RTD may be developed by the food industry in order to enhance the utilization of phytochemicals found in underutilized *Caralluma tuberculata* plant.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

N.N., G.M.U., and M.N contributed to conceptualization. T.M.Q., W.K., M.A.N., and F.A contributed to data curation. A.I., F.M., and A.A contributed to formal analysis. G.M.U. and A.A contributed to investigation. N.N., A.I., and A.A contributed to the methodology. M.N. and G.M.U. contributed to writing the original draft. T.M.Q., N.N., A.I., and A.A contributed to reviewing and editing. All the authors have discussed the results and commented on the manuscript.

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