Research Article

Formulation and Evaluation of Functional Candies Fortified with Carrot Juice and Fennel Seed Extract: Nutritional and Sensory Properties

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Abstract | Carrot and fennel seeds are considered rich sources of antioxidants such as lutein, zeaxanthin, and β-carotene. The study aimed at formulating functional candies fortified with fennel seed extract and carrot juice. The candies can be used to improve the nutritive quality of confectionary products that could be healthy options for all age groups, particularly children. Fennel seed extract having the highest flavonoids and antioxidant capacity was selected to blend with carrot juice for hard candy formulation. Five different concentrations of fennel extract and carrot juice were added for the formulation of hard candy containing 30% (w/w) sugar, 30% (w/w) corn syrup, 0-40% (w/w) fennel seed extract, and 0 to 40% (w/w) carrot juice. The results showed the highest content of flavonoids in 40% (w/w) carrot juice formulation and the mixture of candies containing 10% (w/w) carrot juice and 30% (w/w) fennel extract, while the highest antioxidant activity was observed in candies containing 30% (w/w) carrot juice and 10% (w/w) fennel extract. The formulated candies showed a good sensory profile and can be utilized as a potential source of functional candies for improving the nutritional profile of children.

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Introduction

Plants are considered excellent sources of food, phytochemicals and herbal medicines. Some important drugs have been obtained from the plants directly or indirectly (Khalid *et al*., 2011). Fennel (*Foeniculum vulgare* L.) is an aromatic biennial herb belonging to the family of umbellifers with feathery leaves and hollow stems that can grow up to a height of 2.5 m (Akbar, 2018). In some regions of the world, it is well known for treating vision-related issues in human beings. Fennel seeds contain volatile oil (2.5-5%) and are majorly composed of trans-anethole (40-70%), fenchone (1-20%) and estragole (2-9%) (Samadi-Noshahr *et al*., 2021). Fennel seeds are nutritionally riched and have been reported to contain carbohydrates (42.3%), crude fiber (18.5%), minerals (13.4%), fat (10%), protein (9.5%) and moisture (6.3%) (Rather *et al*., 2016). It is also a potential source of flavonoids and antioxidants such as quercetin and kaempferol that protect the human body from infection, cancer, aging and degenerative neurological disorders (Das *et al*., 2013). Fennel seeds have anise-flavored and flora, can be grown in vegetable gardens and cultivated for cooking purposes as spice and condiments (Javed *et al*., 2020). Fennel seeds have been found with indigenous healing characteristics against infectious disorders associated with viral, mycobacterial, bacterial, and fungal nature (Javed *et al*., 2020; Miraj and Kiani, 2016). Due to its profound nutritional profile, fennel seed powders have been supplemented in bread at the concentration of 5-7%, to increase the crumb firmness, crumb moisture and antioxidant activity (Das *et al*., 2013). Another study reported the use of fennel seeds and fennel seed cake for the fortification of bread. The results of the study concluded that bread enriched with fennel seeds and cake observed higher antioxidant activity and total phenolic contents than the control group (Sayed-Ahmad *et al*., 2017).

Carrot (*Daucus carota* L.) is one of the utmost consumed root vegetables that belong to the family Umbelliferae (Apiaceae) and is famous for its profound nutritional and beneficial health impacts (Sharma *et al*., 2012). Carrots are also abundant in micronutrients primarily vitamins (thiamine, ascorbic acid, riboflavin, pyridoxine, niacin, vitamin A, vitamin K and folic acid) and minerals such as (iron, calcium, magnesium, potassium, phosphorous, zinc and sodium) (Ahmad *et al*., 2019). Carrots are a rich source of bioactive compounds such as carotenoids (β-carotene, α-carotene, zeaxanthin, and lutein), anthocyanins (cyanidin-3-O-xylosyl galactoside) and phenolic acids (ferulic, chlorogenic, caffeic, p-coumaric) that exhibit health advancing attributes (Bystrická *et al*., 2015). Carotenoids particularly, β-carotene (precursors of vitamin A) tend to perform the main role in improving the health (Coronel *et al*., 2019). Carrots also exhibit strong radical scavenging and antioxidant activities. Moreover, the frequent consumption of carrots helps to lower the risk of certain chronic diseases such as cataracts, atherosclerosis, cancer and diabetes (Sharma *et al*., 2012). Due to its exceptionally riched nutritional and phytochemical profile, carrots are known as a gold mine of antioxidants, polyphenols, vitamins, and carotenoids. Among all the varieties of carrots, orange carrots contain a persistent amount of antioxidants that help to neutralize the effect of free radicals (da Silva Dias, 2014).

Keeping in view the nutritional profile of the aforementioned plants, this study was intending to add fennel seed extract to carrot juice in varying concentrations and then edible hard candies were formulated using this mixture. The plan behind the formulation of hard candies was to provide a healthier substitute of candies in the confectionery products compared to the conventional candies for all age groups, particularly children, young and old age people. People who particularly suffered from macular degeneration can take benefit if they consumed it on regular basis. Moreover, people of every age group can easily afford and consumed candies at any time of the day. This type of healthier substitute will not only easily available but can add a new and healthy variety to conventional processing lines of confectionery. Therefore, to evaluate the appealing attributes, an organoleptic or sensorial analysis was also performed.

Materials and Methods

Collection of samples and materials

The substrates used in the current study include fennel seeds, fresh orange carrots, refined sugar and corn syrup. The chemicals used for the formulation of candies and physicochemical characterization including organic solvents (methanol, ethanol and chloroform) and citric acid and were of analytical grade. After collecting, the dried fennel seeds were ground and stored in an airtight glass jar to avoid the entrance of air and any contaminant from the surrounding. For juice extraction, orange carrots were washed, peeled, cut into uniform-sized slices and blended in a Onex Juice Blender.

Fennel seeds extraction

The chloroform, methanol, ethanol and water were used as solvents to obtain fennel seed extracts followed the previous method (Anwar *et al*., 2009). For this purpose, 10 g of the homogenized fennel seeds powder was dissolved in each of the 100 mL organic solvents with a ratio of (1:10). Each mixture was incubated at ambient temperature for 24 h for efficient extraction from seeds. Afterward, the extract from each mixture was filtered using the muslin cloth

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and evaporated at 50°C using a rotary evaporator till desired consistency. The extraction procedure was performed in triplicate and the resulting extracts were stored in an air-tight plastic container at 4°C until further investigation.

Determination of total flavonoid contents

Total flavonoid contents (TFC) in fennel extract were determined by following the method reported by Hayat *et al.* (2019). For this purpose, 2 mL of fennel seed extract and 2 mL of 2% $AlCl_3$-ethanol solution were added to a glass beaker and well mixed. The prepared sample was incubated at ambient temperature for 1 h, afterwards, absorbance was measured at 420 nm. The extracted samples were evaluated for flavonoids at a concentration of 10 mg/mL. The development of yellow color in samples during incubation confirmed the presence of TFC. TFC was expressed in terms of quercetin (mg/g) and obtained results were used to prepare the calibration curve. The calibration curve showed a coefficient of determination (R^2) of 0.989, which indicates complete linearity.

Determination of radical scavenging activity

The radical scavenging activity by performing a 2, 2-diphenyl-1-picrylhydrazyl (DPPH) assay was measured following the method of Sun *et al.* (2012) with slight modifications. A solution of 0.135 mM DPPH in methanol was prepared and 1.0 mL of DPPH solution was added to 1.0 mL of fennel extract. The DPHH must be performed in the dark as light can disturb the curve. The radical scavenging activity of carrot juice was also measured by using the same protocol as mentioned for fennel seed extraction. All the reaction mixtures were shaken well and incubated for 30 mins at ambient temperature. Afterward, the absorbance of the reaction mixture was observed at 517 nm and a blank reading was also measured.

Formulation of carrot juice and fennel extract candies

Hard candies were prepared by adding corn syrup, sugar and water, and carrot juice into a boiling pan as per the set formulation. The glass thermometer was used to measured the temperature and the temperature was maintained between 100-110 °C. Cooking of the sugar syrup mixture was performed until 75° Brix and this stage fennel seed extract was added in varying concentrations and this candy mixture was continuously stirred to avoid sticking to the boiling pan. A few moments later, citric acid (food additive) was added to this mixture. Later, the mixture was poured into the candy mold and cooled until hard candies formed. For the final 100 g (w/w) formulation, a total of five candy treatments were prepared of varying concentrations. The detail of the treatment plan was presented in Table 1. A control group (T_o) was also prepared that contained only sugar, corn syrup, and water and served as a standard for comparison with other formulated candies.

Table 1: *Treatment plan for formulation of hard candy containing fennel seed extracts and carrot juice.*

Treat-ments	Corn syrup (mL) + sugar (g)	Water (mL)	Carrot juice (mL)	Fennel extract (mL)
T_o	60%	40%	-	-
T_1	60%	-	40%	-
T_2	60%		30%	10%
T_3	60%		20%	20%
T_4	60%		10%	30%
T_5	60%		-	40%

Physicochemical characterization of candies

Color plays a significant role in determining the overall acceptability and quality of candies. Color parameters of hard candy treatments on L*a*b scale, L*: brightness, a*: ± red-green, and b*: ± yellow-blue were determined using a colorimeter (Chroma Meter, Konica Minolta, Japan) . The total soluble solids (TSS) were estimated by using a handheld refractometer following the method reported by Nath *et al.* (2013) and values were expressed as °Brix at 20°C. The pH of formulated candies was determined using a digital pH meter after two-point calibration with a buffer solution of pH 4.0 and 7.0 (Mutlu *et al.*, 2018).

Sensory analysis of formulated hard candies

Candy samples were subjected to sensory analysis i.e. texture, color, flavor and taste using a 9-point hedonic scale by 10 trained and non-trained panelists. The training of panelists was performed by providing them training guides by a professional trainer and conducting hands-on practices on sensory stimulus tests during which they were able to develop skills of perceiving, recognizing, describing, and distinguishing sensory stimuli. The randomly coded candy samples were evaluated in an ordered manner for appearance for color, odor, texture, taste and after-taste by panelists.

Statistical analysis

All the test analyses were performed in triplicate.

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The collected data were statistically analyzed using IBM (SPSS 21). For the estimation of significant differences, the one-way ANOVA was performed at a 95% probability level to determine the differences among samples. For a comparison of mean differences in population, Tukey's test was performed at (p < 0.05).

Results and Discussion

Fennel seeds are well known due to their phytochemical attributes, aromatic smell, flavor and extensive use as a medicinal plant. Its seeds are also used as flavoring agents, and preservatives, for the formation of essential oils in perfumery and the preparation of medicines. Its medicinal properties are usually attributed due to its antioxidant activity (Das et al., 2013).

Antioxidant activity of fennel seed extracts

The antioxidant activity of fennel seed extract was determined by performing a DPPH assay and was presented in Figure 1. Different solvents including distilled water, chloroform, ethanol and methanol solutions were used to check the antioxidant activity. Figure 1 showed that the methanolic fennel extract showed the highest inhibition percentage (p = 0.00) as compared to other solvents. Among all the solvents used, methanol shows the highest extraction yield (86.21%), followed by chloroform (65.89%), ethanol (41.35%), and water (4.430%). Figure 1 indicates that extraction efficiency favors the highly polar solvent and that might be due to the nature and polarity of methanol since it acts as a very good solvent to extract the secondary plant metabolites. Rodríguez *et al.* (2014) showed that methanol resulted to be the most suitable extracting solvent for the target volatile compounds including estragole and trans-anethole in the essential oil extracted from fennel seeds. Fennel seeds are naturally occurring antioxidants that provide protection against oxidative stress damage and exhibit the highest radical scavenging activity (Rathore *et al*., 2013). This study is in with strong argument with the study presented by Sultana *et al.* (2009) that antioxidant components can be extracted in higher amounts in methanol compared with ethanol and water. Another study also reported that cultivated fennel seeds and methanol extract showed a maximum antioxidant activity with a DPPH value of 83-88 µg/m (Anwar *et al*., 2009). Therefore, For the extraction of volatile compounds from the fennel seeds, methanol as an effective solvent can be used. However, the effective concentration of methol as a solvent needs to be defined.

Total flavonoid contents in fennel seed extracts

In this study, fennel seeds extract showed a TFC of 22.8 ± 0.79 µg/mL. A past study also reported that the fennel seed extract in methanol exhibited the highest amount of flavonoid (38.98 ± 0.96) mg/g quercetin equivalent (Pérez-Jiménez and Saura, 2006). A study by Anwar *et al.* (2009) was in strong argument with the current study, which reported 374.88–681.96 quercetin equivalent concentration of TFC in fennel seeds extract. A recent study reported the maximum extraction of phytochemicals and TFC in the fennel seeds by using varying concentrations of methanol as a solvent (Mehra *et al*., 2022). A study was conducted to determine the TFC in different herbs where the methanolic extract of fennel exhibited stronger flavonoid activity as compared to other herbs. It was concluded that higher value was due to the presence of linalool, eugenol, anethole, estragole and tannins (Gupta, 2013). The polyphenolic contents present in fennel seeds are responsible for the decrease in oxidative stress of free radicals that make them suitable as a strong free radical scavenger and for pharmaceutical products (Khan *et al*., 2010).

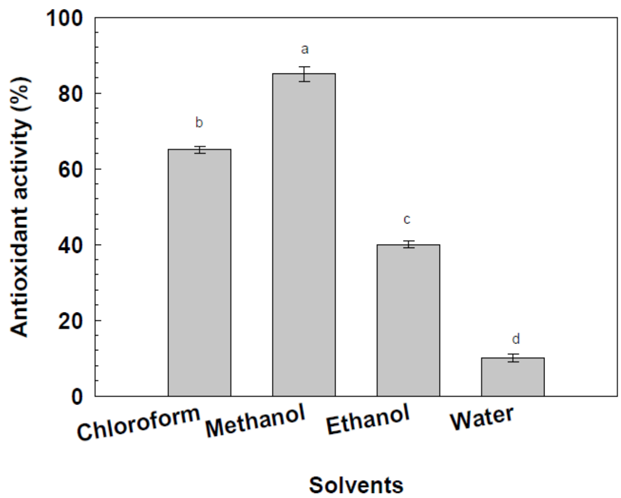

Figure 1: *DPPH radical scavenging of fennel seed extract in different solvents.*

Flavonoid contents in different candy treatments

Figure 2 shows the different concentrations of flavonoids among all the candy treatments. The highest concentration of flavonoids was present in T_1 and T_4. Whereas T_2 and T_3 showed the combined response having no significant difference among treatments (p = 0.054). While the lowest concentration of flavonoids was present in T_o as well as T_5, and there was a non-

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significant difference observed in the mean values (p = 0.055) between T_o and T_5. A previous study reported the value of TFC carrot extract (20.48 ±28 mg QE/g) dry weight material (Nadeem *et al*., 2018). Some recent studies have also shown that the decrease in TFC during thermal processing is particularly associated with the extraction technique, time of processing, and solvent used (Khammassi *et al*., 2022; Ben Abdesslem *et al*., 2021). This study was in strong evidence with the study conducted on the basil seeds that determined the effect of boiling temperature (>100 °C) on the TFC and TPC. It was concluded that with the increase in the boiling temperature from 5-15 °C, there was a clear decrease in the flavonoid contents (Salamatullah *et al*., 2021). Furthermore, there was an increase in TFC during thermal processing such as roasting and microwave drying (Babiker *et al*., 2021; Das *et al*., 2013). It was evident from the previous studies that the concentration of flavonoids can be affected by some extrinsic and intrinsic factors such as temperature, the solvent used, seed variety, and concentration that are responsible for the increase or decrease in their final concentration (Granato *et al*., 2018; Nadeem *et al*., 2018).

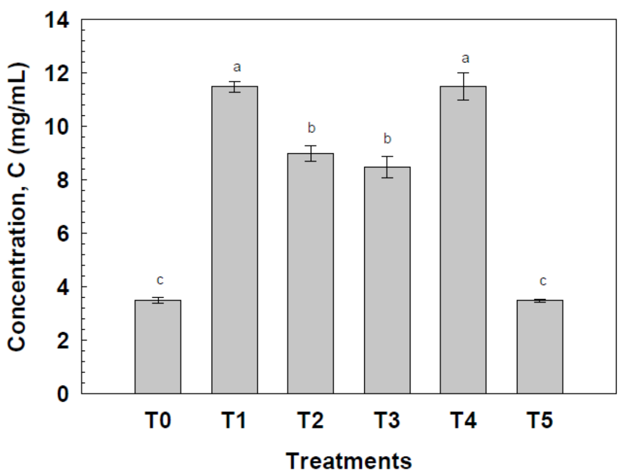

Figure 2: *Total flavonoid contents in hard candies formulated with fennel seed extract and carrot juice. The different letters show significant differences among all treatments.*

Antioxidant activity in different candy treatments

Figure 3 showed significant difference among all the treatments (p= 0.00), the highest antioxidant activity was observed in T_2, formulation with a mean concentration of 30% carrot juice and 10% fennel extract. However, the second highest antioxidant was presented by T_5 with a mean concentration of 40% fennel extract. The reduced antioxidant activity was observed in T_3 with a 42.43% inhibition percentage. The T_4 showed inhibition of 36.84%, where 30% (w/w) fennel extract and 10% (w/w) carrot juice were used to formulate hard candy. Whereas, T_1 containing a whole percentage of carrot juice showed decreased antioxidant activity of 20.96%. While the least antioxidant activity was observed in T_o (4.67%) which might be due to the composition of the candy recipe that was formulated using 40% (w/w) water and other constituents were sugar and corn syrup. The results showed all the treatments have significant effect (p = 0.000) on the antioxidant contents of formulated candies. Goswami and Chatterjee (2014) explained that the total antioxidant activity of fennel extract in methanol solvent, while the orange carrots observed antioxidant activity of 28.27% (Owolade *et al*., 2017). Another study also revealed that antioxidant activity can be affected by thermal processing, and intrinsic and extrinsic factors during cooking (Gunathilake *et al*., 2018).

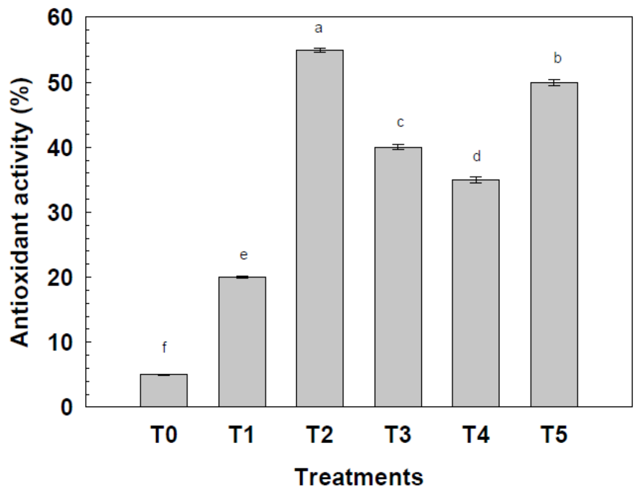

Figure 3: *Total antioxidant contents in hard candies formulated with fennel seed extract and carrot juice. The alphabetic letters show significant differences among treatments.*

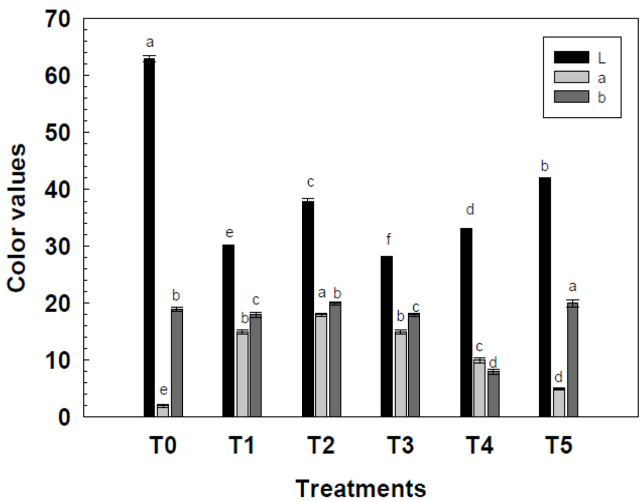

Figure 4: *Color estimation of hard candies formulated with fennel seed extract and carrot juice.*

Color analysis in different candy treatments

A portable colorimeter was used to determine the color in formulated carrot juice-based hard candies enriched with fennel seed extract. The white calibration of the calorimeter was performed before measuring the sample readings. The results were determined using L* a* b* uniform color space (Lab), where L represents luminosity, a relates to the change in color from green (-) to red (+), whereas b corresponds to change in color from blue (-) to yellow (+). Figure 5a showed the representation of color on different candies. Figure 4 showed the difference in color after the formulation of candies, control treatment T_o showed a significantly higher L value (lightness) as compared to other treatments. A significant difference between (p = 0.000) the L value of T_o, whereas the least L value was observed in T_3 where 20% (w/w) carrot juice and an equal amount of fennel extract were used for candy formulation. T_2 exhibited a significantly higher (p = 0.000) a value (redness) as compared to other treatments whereas, T_o exhibited a lesser a* value means more orange color. A significant difference was also observed in T_5 and T_2, whereas there was no significant difference between T_3 and T_1, the least difference was observed in T_4. A past study also showed that a change in the color of the carrot was observed by applying heat at > 60 °C, the possible reason is that β-carotene in carrots started to degrade which imparts the color changes (Araya *et al*., 2009). Loss of carotenoids, particularly, β-carotene causes the degradation in color at a higher temperature. Some studies also showed that heat also affects the composition of fennel due to which color imparting compounds started to degrade (Anwar *et al*., 2009; Oktay *et al*., 2003).

Sensory evaluation of hard candies

The sensory evaluation was performed using the 9-point hedonic scale method. Figure 5 shows the sensory attributes of formulated candies as well as the comparison of sensory attributes. In this research, the acceptability in terms of appearance, color, flavor, taste and overall acceptability was quite similar in all the treatments and shows the significant difference in the color of the formulated candy. Among all the treatments (Figure 5B), the flavor is one of the critical factors that vary among all the evaluated sample candies. For the flavor, T_5 was ranked highest, that might be due to the presence of a high concentration of fennel extract. In terms of color and appearance, T_5, ranked higher among all the treatments due to the higher concentration of fennel extract. While in terms of taste T_3 and T_5 were ranked equally preferable. Moreover, among all the treatments, the overall acceptability score was significantly higher in T_5. Previous studies showed that *F. vulgare* gives anise flavor and a delicious taste when combined with carrots (Rana and Vilas, 2017). While the terpenoids, the volatile compounds of carrots are responsible to give sweet flavor. When carrot is cooked with sugar it imparts a good flavor and taste (Rosenfeld *et al*., 2002).

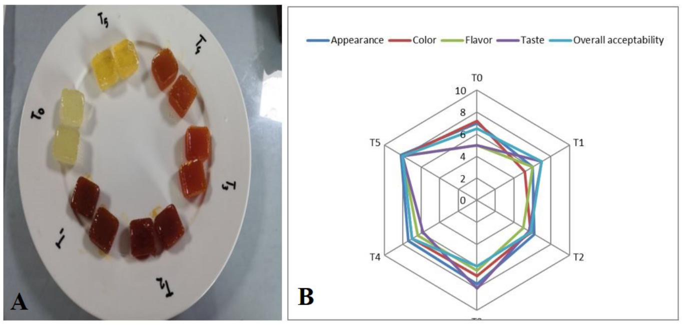

Figure 5: *Sensory evaluation of hard candies containing fennel seed extract and carrot juice. (a) visual outlook of candies and (b) spider web showing sensory attributes of hard candies.*

Conclusions and Recommendations

Carrot is a root vegetable and exhibits multiple health benefits. It contains a significant amount of bioactive compounds, carotenoids, vitamins, and minerals. Fennel is a persistent herbaceous plant that is widely used as a medicinal herb by most people worldwide. It is considered a rich source of antioxidants with a considerable amount of flavonoids and carotenoids. The current research study focuses on the benefits of carrot and fennel to improve the nutritive profile of conventional hard-boiled candies. Candies were formulated by incorporating fennel extract with different combinations. The current study suggests that T_2 presented the highest antioxidant value, whereas T_1 and T_4 showed higher flavonoid contents. Further candies are assessed for overall quality, taste, flavor, and color, where T_5 gained the highest acceptability by consumers. The present research study suggests that fennel-based carrot candies are acceptable for sensory, and physicochemical characteristics and contain bioactive compounds that are proven to possess several health-promoting effects.

Novelty Statement

Candies are a popular confectionery product among children and contain an ample amount of sugar that leads to the development of dental caries and other nutritional disorders. The research provided insight into the development of nutraceutical candies that have abilities to provide nutrition and protection against various dental issues.

Author's Contribution

Maham Tariq: Conducted the research work as part of MS Thesis.
Aqsa Akhtar: Cross verified the results and performed additional experiments.
Maham Tariq, Aqsa Akhtar and Nauman Khalid: Drafted the initial draft.
Nauman Khalid: Planned the study and finalized the submission.

Conflict of interest

The authors have declared no conflict of interest.

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