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The influence of chocolate fortification with *Centella asiatica*, *Abelmoschus esculentus*, and *Psidium guajava* on the content of biologically active substances

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Abstract

Herbal-based chocolate consists of micro-nutrients and spices, which can be consumed in infinite combinations. This research examines the combination of *Centella asiatica*, *Abelmoschus esculentus*, *and Psidium guajava* in the process of chocolate formulation. The concentration of the product was optimized based on taste, flavor, texture, and stability. The optimized concentration was subjected to Nutritional, Stability, Sensory, Anti-oxidant, Antimicrobial, and shelf life analysis. The Nutritional analysis shows an increased level of calcium (67.8 mg), iron (2.34 mg), and sodium (355 mg) per 100 g in the formulated chocolate in comparison to the conventionally available chocolates. The antioxidant activity was determined to be 52.15% by DPPH assay. The product has antimicrobial activity against tooth decaying organisms, *Streptococcus mutans* and *Lactobacillus acidophilus*. The shelf life of the product was found to be around thirty days. The observations made from various analyses conclude that the formulated product Phytolate has enriched micronutrients and medicinal value.

Practical applications

The novel product synergizes phytomedicine and chocolate. Increase in the vital nutrients such as calcium, sodium and iron was observed. Antimicrobial activity of novel chocolate towards organisms responsible for tooth decay.

1 | INTRODUCTION

Over several decades, chocolates are consumed by humans solely for pleasure. Chocolates are one of the most popular food worldwide due to its high calorific value and it is also promoted to be a functional food due to its rich profile of bioactive compounds (Belscak-Cvitanovic et al., 2015; Godocikova et al., 2017). The progressions of medical technology in the last two decades have unraveled its medicinal benefits on human health due to high percent of poly-phenols. The most essential sources of poly-phenols include tea, chocolate, vegetables, and nuts (Quinones et al., 2013). The cacao has a profuse amount of phenol and flavanols of all foods on per weight basis (Martín & Ramos, 2016). In human diet, a significant amount of the mineral content can be provided by chocolates, in particular, cacao is the rich source of essential minerals required (Cinquanta et al., 2016; Paoletti et al., 2012). Chocolate is predominantly used for weight gain in emaciated patients, invigorating the nervous system and stimulating digestion (McShea et al., 2009). Polyphenols hinder biocatalyst that increases oxidative stress, chelate metals, affine carbohydrates, and proteins (Heim et al., 2002). These properties facilitate them to act as a barrier toward a carcinogen, inflammation, hepatotoxicity, allergic compounds, bacteria, and virus (Zaveri, 2006).

Centella asiatica (CA) is a perennial herbaceous creeper, extensively used as a blood purifier and also as a memory enhancer. CA is one of the indispensable herbs for revitalizing the nerves and

TABLE 1 The list of questions and the options of the E-survey form circulated among various age groups of people

1	Why do you prefer chocolates? **Options:** Tastes good, Feels energetic, Aroma, Stress relief
2	Pick your chocolate **Options:** Molded chocolates, Candies, Brownies, Jellies
3	Expected nature of chocolate **Options:** Soft, Crunchy
4	Do you consume spinach **Options:** Yes, No
5	Reasons to avoid CA in food items **Options:** Bitterness, Aroma, Digestive problems, Allergy, None of these
6	How would you like to have CA? **Options:** Chocolates, Bakes items, Smoothie

brain cells. Crude extract containing glycosides, isothankuniside, and thankuniside are responsible for action against fertility in mice (Heidari et al., 2007). CA effect on neuronal health was studied in the animal models, and the result obviously shows improvement in neuronal morphology, learning performance, and memory retention (Rao et al., 2005). Furthermore, CA has also shown anti-stress, antidepressant, anxiolytic, and anti-seizure properties in pre-clinical studies. In animal models, asiaticoside and asiatic acid showed neuroprotective, anti-depressive, and anxiolytic effects (Wijeweera et al., 2006). The herb has psychotropic, sedative, and anticoagulant effects. Some commercial Products such as Mandukaparni, Abana (Heart Care), and Weight Loss Tea were launched in the market, containing *C. asiatica* (Sakshi et al., 2010).

Abelmoschus esculentus L. (AE) belongs to the *Malvaceae* family that is more generally known in several other vernacular names as lady's finger, okra, bhindi, or gumbo (Kumar et al., 2013). Generally, okra has a rich source of nutrients that are vital to human health, for example, vitamins, potassium, calcium, carbohydrates, dietary fiber, and unsaturated fatty acids and also of bio-stimulatory chemicals (Wannisa & Preecha, 2013). The bioactive constituents of okra (quercetin and rutin) shield neuronal function and improve learning and memory deficits in dexamethasone-treated mice (Mathew & Subramanian, 2014). Some of the commercial products from okra are pickles, ice-cream, potato chips, and baked goods, providing a healthy dietary option and more stable shelf-life (Hu & Lai, 2016). The fruit extract obtained from this herb is used in different recipes as a thickener to increase its consistency.

Psidium guajava (common name-guava) leaves are wide and clear green and have prominent veins. The leaves have a profuse amount of limonene and caryophyllene (Shao et al., 2012). Guava extracts have hepato-protective activity and have potent antioxidants substances which include beta-carotene, lycopene, vitamin C, E, and A. Aqueous and organic solvents extracts of guava have shown a large quantity of antioxidant compounds (Musa et al., 2011; Thuaytong & Anprung, 2011). The anti-oxidative potential of guava extracts has gained promising attention for treating various diseases and complications. Guava act as a barrier toward virus, inflammation, plaque, and aberration.

The addition of herbal additives in conventional food products such as juices, candies, tea, snacks, and nutritive bars has begun way earlier (Belscak-Cvitanovic et al., 2015). The extracts of a variety of medicinal plants have been used towards improving the nutritional profile and the antioxidant capacity of the food products such as cheese (El-Aziz et al., 2012) and candies (Skouroliakou et al., 2009). Numerous research works have already been carried out en route for improving the content of bioactive substances in dark and compound chocolates with natural plant extracts and phytosterols (Godocikova et al., 2017; Tolve et al., 2018).

Considering the beneficial effects of the above-listed phytomedicines, nutritive-rich chocolate was formulated followed by performing the nutritional analysis, sensory analysis, antioxidant analysis, antimicrobial activity against tooth decaying organisms, and shelf life analysis.

2 | MATERIALS AND METHODS

C. asiatica (leaves), *A. esculentus* (powder), and *P. guajava* (leaves) were procured from T. Nagar market, Chennai, TN and certified by a botanist. The branded compound chocolate was purchased from the shopping mall, Chennai. The bacterial cultures *Streptococcus mutans* and *L. acidophilus* bacterial culture were purchased from Life Teck Research Center, Arumbakkam, Chennai, TN.

2.1 | e-Survey

Initially, an e-survey was conducted with a set of six questions provided with multiple choices of answers to study the temperament of the consumers toward chocolates and the idea of the incorporation of medicinal herbs in chocolates. The e-survey form with the questions and their relevant answers was circulated among various age groups of people in order to collect a complete data profile stating their necessity and choice for the chocolates. The questions and options given are listed below (Table 1).

2.2 | Preparation of *C. asiatica* extract

Twenty-five grams of fresh and clean *C. asiatica* leaves were cleaned with distilled water. The leaves were then crushed using Mortar and pestle for obtaining crude extract. The crude extract was collected in a centrifuge tube and allowed to centrifuge at 3,000 rpm for 5 min. The

pellet was discarded and the supernatant was collected and packed in a zip lock cover and stored in the refrigerator until further use.

2.3 | Preparation of *A. esculentus* extract

Ten grams of the *A. esculentus* powder was transferred to a beaker containing 50 ml of water. The beaker containing the sample was heated up to 100°C for a period of 5 min. The obtained extract was then filtered using filter paper (Whatman No.1) and transferred into a centrifuge tube. The centrifuge tube was then stored in the refrigerator for further use.

2.4 | Preparation of *P. guajava* extract

Twenty grams of the *P. guajava* leaves were washed with distilled water and allowed to dry. The dried guava leaves were mashed and transferred to a beaker containing 50 ml of distilled water. The beaker containing the sample was heated up to 100°C for a period of 5 min in the heating mantle. The obtained guava leaf extract was then filtered using filter paper, transferred to a centrifuge tube, and stored in a refrigerator for further use.

2.5 | Preparation of the chocolate

The flowchart for the process of development of the chocolates is given below (Figure 1). The chocolate was prepared by *C. asiatica*, *A. esculentus*, and *P. guajava* in different proportions are represented in Table 2. The concentration variation of the *P. guajava* is doubled because of its higher antimicrobial activity against the tooth decaying organisms *S. mutans* and *L. acidophilus* (Besra & Kumar, 2018). The trial no.12 was chosen to be the optimized concentration of the final product based on the desired organoleptic properties and in which the further analysis was carried out.

2.6 | Nutritional, moisture and ash content analysis

The nutritional labeling for the samples, namely, with and without the addition of *C. asiatica*, *A. esculentus*, and *P. guajava* extracts to the compound chocolate (100 g), were analyzed by standard procedures (IS 5949:1990) (RA.2003) (FAO Method) (AOAC, 2016). The amount of moisture content and ash content in the samples was estimated using the analytical procedure (FSSAI manual, 2015).

2.7 | Stability testing

The stability of the formulated chocolate was analyzed by placing the sample in a refrigerator temperature and regular monitoring of the sample regarding color, taste, and overall acceptability.

2.8 | Antioxidant assay

The antioxidant activity of the treated and untreated chocolate was tested according to DPPH assay analysis with slight modification

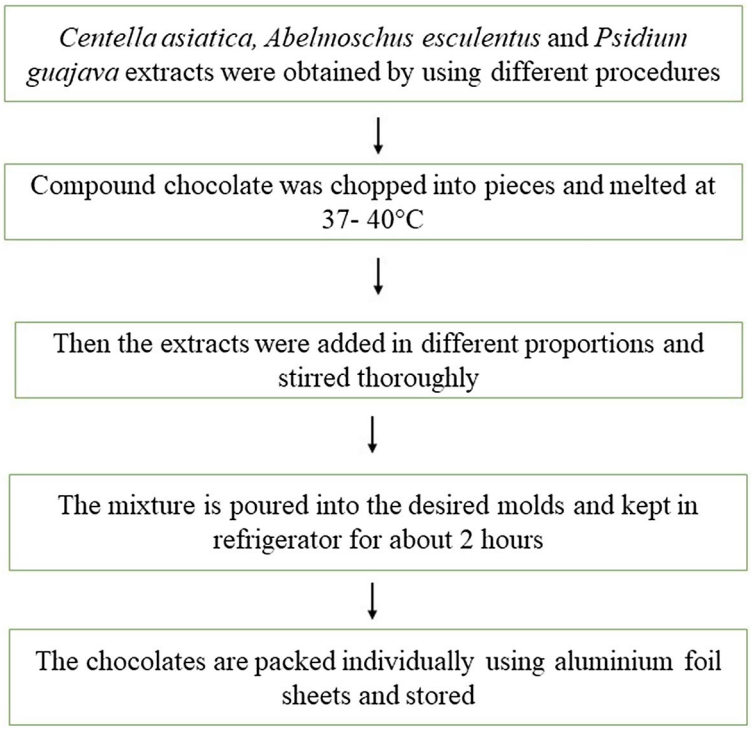

FIGURE 1 Process flow of chocolate formulation

TABLE 2 Formulation of chocolate with various concentrations of *Centella asiatica*, *Abelmoschus esculentus*, and *Psidium guajava* extracts

Trial No.	Compound chocolate (g)	*Centella asiatica* (ml)	*Abelmoschus esculentus* (ml)	*Psidium guajava* (ml)
1	100	1	1	2
2	100	3	3	4
3	100	4	4	6
4	100	6	6	8
5	100	7	7	11
6	100	8	8	14
7	100	9	9	15
8	100	11	11	18
9	100	11.5	11.5	20
10	100	12	12	21
11	100	12.5	12.5	22
12	**100**	**13**	**13**	**24**
13	100	13.5	13.5	25
14	100	14	14	27

Bold value indicates the optimized concentration of the chocolate.

TABLE 3 Parameters and the rating scale of a 5-point Hedonic scale

Parameters	Rating scale
Appearance	5-Like Extremely
Color	4-Like Slightly
Flavor	3-Neither like nor Dislike
Texture	2-Dislike Slightly
Taste	1-Dislike Extremely

(Nilima & Hande, 2011). In this test, a stock solution of DPPH (1, 1-Diphenyl-2-picrylhydrazyl) was prepared by adding 4.3 mg of DPPH dissolved in 3.3 µl of methanol and the test tube containing stock solution was protected from sunlight by covering it with aluminium foil sheets. Three milliters of methanol in 150 µl of DPPH solution were taken as a control. Various concentrations of the samples from 100 mg/ml to 500 mg/ml were prepared and to each sample 3 µl of methanol and 150 µl of DPPH was added. Absorbance was taken after 15 min of incubation at 517 nm on a UV-visible spectrometer. The antioxidant activity was calculated using the formula.

$$\% \text{ scavenging} = \left[\text{Absorbance of (control} - \text{test sample)} / \text{control}\right] \times 100$$

2.9 | Sensory analysis

The sensory test was carried out by a 5-point hedonic scale method. The values were tabulated and the results were interpreted accordingly. The sensory form is created with 5-point hedonic scale with the following parameters and the rating scale as represented in Table 3.

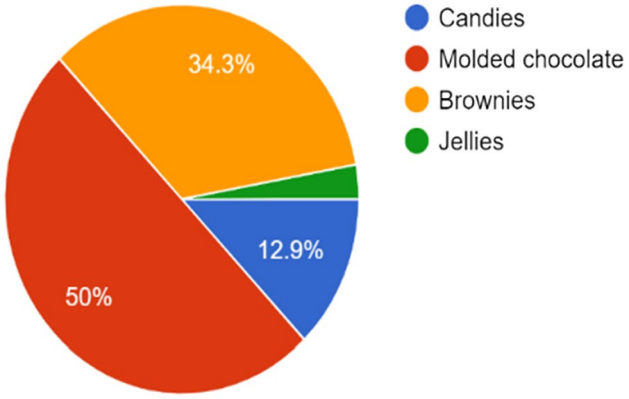

FIGURE 2 Consumer choice of preference over candies, molded chocolates, brownies, and jellies

2.10 | Antimicrobial analysis

The antimicrobial activity of the aqueous extracts was performed by agar well diffusion method with slight modification (Besra & Kumar, 2018). In this technique, the culture plates seeded with the tooth decaying organisms *S. mutans* and *L. acidophilus* by the agar incorporation method and the plates were allowed to solidify and punched with a sterile well puncher to make open wells. The open wells were filled with 100 µl of the extract. The plates were then incubated at 37°C for 24 hr.

2.11 | Shelf life analysis

The shelf life of the formulated chocolate was examined by the Total Plate Count (TPC) estimation procedure (Giménez et al., 2012). The microbial load was analyzed every 10 days over a period of 35 days. The results were tabulated and interpreted accordingly.

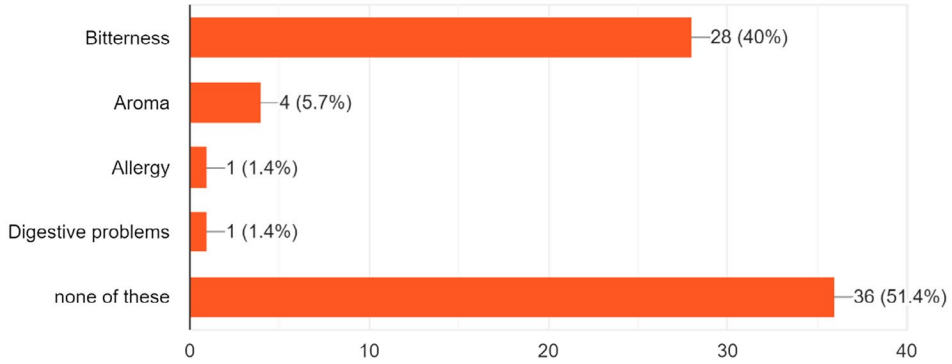

FIGURE 3 Reasons for the avoidance of *Centella asiatica* among consumers

FIGURE 4 (a) Final product, (b) Aluminium foil packed chocolates

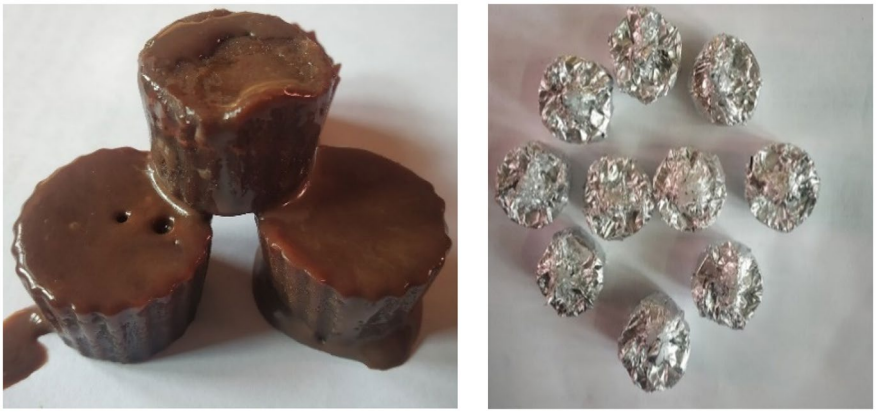

TABLE 4 Nutritional profile of the treated compound with the three phytomedicine extracts and untreated sample (control)

S. No.	Parameters	Method	Units	Results	
				Untreated	Treated
1	Energy (By Calculation)	FAO Method	kcal/100 g	518	483
2	Carbohydrates (By difference)	CTL/SOP/FOOD/262 - 2014	g/100 g	66.6	59.5
3	Total Fat	AOAC 20th Edn.2016, 920.39	g/100 g	26.3	25.7
4	Protein (Nx6.25)	AOAC 20th Edn.2016, 986.25	g/100 g	3.30	3.60
5	Total Sugars	FSSAI Manual 2015- Beverages, Sugars and Confectioneries	g/100 g	54.8	47.1
6	Dietary fibre	AOAC 20th Edn.2016, 985.29	g/100 g	4.06	4.26
7	**Sodium as Na**	**AOAC 20th Edn.2016, 969.23**	**mg/100 g**	**287**	**355**
8	**Calcium as Ca**	**IS 5949:1990 (RA.2003)**	**mg/100 g**	**41.5**	**67.8**
9	**Iron as Fe**	**AOAC 20th Edn.2016, 999.11**	**mg/100 g**	**1.72**	**2.34**
10	Moisture	FSSAI Manual 2015-Beverages, Sugars and Confectioneries	g/100 g	2.01	10.1
11	Ash		g/100 g	1.53	1.33

Bold value indicates increase in nutritional level than untreated.

3 | RESULTS AND DISCUSSION

3.1 | e-Survey

The total number of responses for the Google form that were circulated among various age groups of people is seventy (70) and results were recorded. The results interpreted from the statistical data stated that around 50% of the consumers prefer molded chocolates over candies, brownies, and jellies (Figure 2). Over 22.9% of people avoid the consumption of *C. asiatica* and the main reason is termed to be the bitterness (Figure 3). 54.3% consumers prefer the incorporation of the spinach in the form of baked items followed by 24.3% (chocolates) and 21.4% (smoothie).

3.2 | Preparation of the chocolate

The prepared herbal incorporated chocolate showed no significant changes in yielding when compared to the control chocolate prepared without herbs. Only marginal differences in hardness, color, and consistency were observed among the chocolates. The images (Figure 4) provided representing the shape of the chocolate formulated and the packaging. From the number of wrapping materials available in the market, aluminium foil sheets are selected due to their ability to maintain consistency, stability, and also provide a complete seal from the environment. These sheets also help toward maintaining the shelf life of the product.

3.3 | Nutritional analysis

By performing nutritional analysis, the protein, dietary fiber, and the iron contents were increased in the treated sample compared to the untreated (compound chocolate) sample, out of which the amount of calcium, sodium, and iron has increased significantly from 41.5 to 67.8 mg, 287 to 355 mg, and 1.72 to 2.34 mg, respectively, as shown in Table 4. The absorption of *C. asiatica*, *A. esculentus*, and *P. guajava* extracts has led to the increase in the moisture content from 2.01 g (Untreated sample) to 10.1 g (Treated sample). Thus, this product is calcium, sodium, and iron-enriched chocolate.

TABLE 5 The overall acceptability and the stability check of the chocolate over a period of 35 days

Day	Color	Taste	Overall acceptability
10	No change	No change	Good range of acceptability
25	No change	No change	Good range of acceptability
35	No change	No change	Average range of acceptability

3.4 | Stability testing

The stability analysis showed no change in the case of color, taste, and overall acceptability even after the 35th day of the production of the chocolate. The result (Table 5) states that the stability of the chocolate is well maintained in the case of refrigeration temperature.

3.5 | Antioxidant analysis

The anti-oxidant analysis was performed for both the treated and the untreated sample. From the results obtained, it is observed that the treated sample possesses 52.15% of antioxidant activity when compared to the untreated sample which possesses 47.85% of antioxidant activity (Figure 5). The antioxidant activity of the treated sample is represented in (Table 6). Hence, 4.3% increase in the antioxidant activity is observed and interpreted from the test performed.

3.6 | Sensory analysis

The sensory analysis of the formulated chocolate was performed using a 5-point hedonic scale. The sensory analysis form was circulated among various age groups of people and the results were recorded. The average score of the results recorded was then represented (Table 7) in the form of a table segregated among various age groups of people.

3.7 | Antimicrobial analysis

The aqueous extracts from *C. asiatica*, *A. esculentus*, and *P. guajava* were used as the test samples each of 35 µl in well no.1 of the plate and the rest well no.2, 3, and 4 is filled with diluted treated, untreated samples, and negative control (water). The zone of inhibition in the two plates seeded with *S. mutans* and *L. acidophilus*

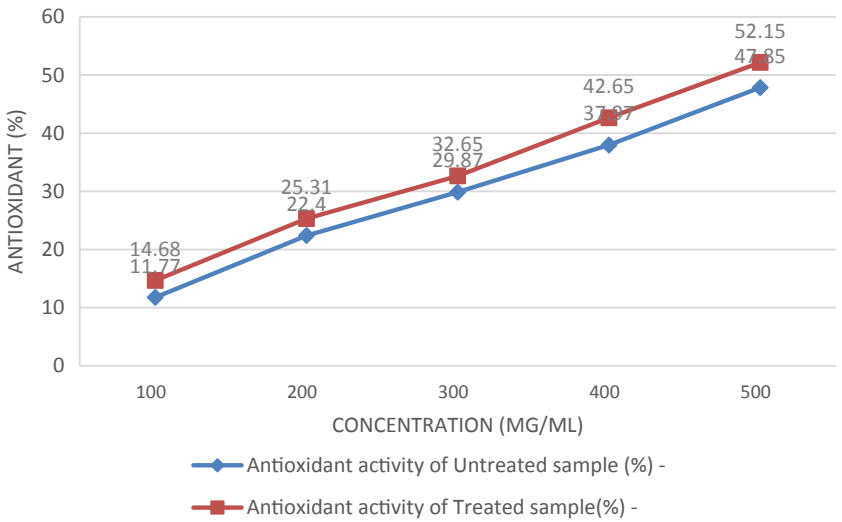

FIGURE 5 Comparative graphical representation of the antioxidant activity between the untreated and treated sample over various ranges of concentrations

was observed after 24 hr of the incubation period as shown in (Figure 6).

The control used was the Ciprofloxacin disc. Effective result with a diameter of 7 mm and 8 mm has been obtained from the well no.1 which contained only the aqueous extracts of *C. asiatica*, *A. esculentus*, and *P. guajava*. The well no.2 with the treated sample shows antimicrobial activity against both the test organisms with a diameter of 5 mm and 4 mm. And no activity found in the untreated and the negative control.

3.8 | Shelf life analysis- standard total plate count estimation

The number of colonies grown in the formulated chocolate was found using total plates count estimations periodically on 1st, 10th, 20th, 30th, and 34th day. The colonies were counted using colony counters and the values were represented in (Table 8).

The aerobic CFU/gm of the sample in the case of chocolates is termed to be $<10^5$ (Satisfactory), 10^5-$<10^6$ (Acceptable), $>10^7$ (Unsatisfactory), as per the norms (Gilbert et al., 2000). In relation to the norms and the data obtained (Table 8), the chocolates were considered to be safe for consumption among customers until the 30th day after the production..

A significant change in the process of chocolate preparation by the incorporation of the nutritional rich ingredients would minimize the nutritional-based disorder in humans (David et al., 2011). The current study of the chocolate formulation in combination with herbal extracts and powder shows an increased level of micronutrients such as calcium, sodium, and iron. The sensory analysis that has been undertaken concludes that the formulated chocolate is accepted among the consumers irrespective of their age groups a side. The 52.15% of antioxidant analysis of the formulated chocolate (phytolate) helps toward attaining bone strength and the chocolate also possesses good antimicrobial activity against the tooth decaying organisms such as *S. mutans* and *L. acidophilus*. The product seemed to be consumable until the 30th day. Thus, from the above observations obtained from performing various analyses, it is concluded that the formulated chocolate phytolate was highly nutritive rich providing the human body with a sufficient amount of micronutrients. A similar type of research reports says that health and weight management benefits can be achieved by plant-centered plates (Hever, 2016; Orlich et al., 2013).

TABLE 6 The percentage of antioxidant activity of the treated sample over various concentrations at 517 nm

S. No.	Conc. (mg/ml)	Absorbance @ 517 nm	Antioxidant (%)
1.	Control	0.79	–
2.	100	0.674	14.68
3.	200	0.59	25.31
4.	300	0.532	32.65
5.	400	0.453	42.65
6.	500	0.378	**52.15**

Bold value represents the increase in antioxidant activity.

TABLE 7 The average data score attained from the overall responses recorded through the sensory analysis form

Sensory parameters	5–15 years	15–25 years	25–40 years	Above 40 years	Average
Appearance	4.2	4.31	4.25	3.75	4.12
Color	4.2	4.31	4	3.5	4
Flavor	4	4.31	4	4.75	4.26
Texture	4.2	4.29	4	3.5	3.99
Taste	4.08	4.05	5	5	4.53

FIGURE 6 (a) Agar plate seeded with *Streptococcus mutans*, (b) Agar plate seeded with *Lactobacillus acidophilus*

TABLE 8 The number of colonies formed in the plate over a period of 34 days (NG- no growth detected)

Day	CFU per ml
1	NG
10	1.2×10^2
20	2.8×10^2
30	9.4×10^6
34	7.8×10^8

4 | CONCLUSION

Chocolate fortified with *C. asiatica*, *A. esculentus*, and *P. guajava* contain an elevated level of calcium, sodium, and iron and thus making it nutritional rich. This herbal-based chocolate, delineate micronutrition, emphasizing specific youngish, and geriatric concerns and offer guidance to a nutritionist and other healthcare practitioners to support patients toward successful utilization of nutrition to improve their health. Chocolate aslo reported to contain natural antioxidants and this was further enhanced by making it an antiplaque property without affecting the desirable quality of original chocolate.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

DATA AVAILABILITY STATEMENT

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

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