

## Nutritional and Functional Potential of Developed Low-Cost Leaf-Based Spreads: A Sustainable Approach for Enhancing Nutrition and Health

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### Abstract

Green edible leaves are a sustainable and economical solution for promoting health and nutrition. Nonetheless, their consumption remains limited due to insufficient awareness and appealing recipes. This study explores the nutritional and functional properties of leaf-based spreads made from lesser-known edible leaves (*Capsicum frutescens* and *Typhonium trilobatum*), highlighting their potential as low-cost solutions for alleviating hidden hunger. Spreads containing *C. frutescens* (CS), *T. trilobatum* (TS) and both the leaves (CTS) prepared in 3 variations each were analyzed for their sensory attributes. Variation 3 of each spread (CS3, TS3, and CTS3) demonstrated highest sensory score along with moderate macronutrients and a significant presence of micronutrients, fibre and functional properties compared to the control. Notably, higher amounts of iron, calcium, magnesium, phosphorous, vitamin C (11.28, 110.11, 68.78, 79.01, 56.30 mg/100 g),  $\beta$  carotene (495.03  $\mu$ g/100 g), phytochemicals namely, flavonoids, alkaloids, and total phenols (66.34, 93.43, and 346.15  $\mu$ g/100g), as well as antioxidant, antibacterial, and prebiotic potential were noted in CS3 than TS3 versus control. Interestingly, the spreads prepared by combining both leaves (CTS3) showed the highest nutritional and functional profile, indicating a synergistic combination. Hence, consumption of these spreads, especially CST3 followed by CS3 and TS3, may mitigate nutritional insecurity and advance health.

**Keywords:** Edible leaves; Functional; Health; Low-cost; Nutritional; Sustainable.

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### 1. Introduction

Green leafy vegetables have long been a staple of everyday diets with magnificent nutritional content and several health benefits. Despite the existence of a wide variety of plant species with morphological and physiological variations, many have not been fully utilized. This is chiefly because of a lack of awareness and promotion for their use, insufficient facilities for processing and value addition, bottlenecks in research and supply chain as well as limited options of appealing recipes for their consumption [1]. Most of them are adaptive, resilient to adverse climatic conditions as well as pests and can be grown on poor marginal soil [2]. These leaves serve as excellent sources of micronutrients

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including vitamins, and minerals and have been highlighted as crucial nutrient sources, especially in vegetarian diets [3, 4]. Besides, a number of these leafy greens are abundant in phytonutrients including bi-flavonoids and alkaloids that have been related to various therapeutic effects, particularly mediated through their antioxidant, antimicrobial, and prebiotic action [5].

According to the Food and Agriculture Organization (FAO), approximately 800 million people, primarily in developing countries, experience food and nutrition insecurity. This has further aggravated due to an inclination towards ready-to-use convenience foods that are often high in calories with a sub-optimal nutrient composition [6]. This in turn has negatively impacted the health of the population due to a loss of dietary diversification evidenced by heightened instances of non-communicable diseases along with malnutrition [7]. The agriculture, food and pharmacy sectors are investigating innovative approaches to not only satisfy the food and nutrition needs but also improve wellbeing. Besides, the diversity and importance of traditional green leafy vegetables have been highlighted because of shifting social values, the depopulation of rural areas, and the erosion of traditional knowledge [8]. Therefore, the present research focused on the development and analysis of convenient green leaf-containing spreads to address the issues of nutritional and food insecurity. This study also focussed on promoting health advancement through disease palliation through economical, ready-to-consume, and renewable options. The developed green leaf-based products may serve as viable options for encouraging sustainable diet and food security to adhere to the 2020 sustainable development goals [9]. These spreads were created through the infusion of lesser-known leafy greens of green chili (*Capsicum frutescens*) and karkol (*Typhonium trilobatum*) either alone or in conjunction. These samples were selected owing to their ease of availability and cultivation, affordable cost as well as the potential to promote good health. Even though used as food and medicine by certain rural tribes, the above leaves have not been fully exploited amongst the masses via easy-to-consume food [10,11]. The prepared products were evaluated for their nutritional and functional potential to demonstrate their potency in minimizing nutrient deprivation as well as communicable and non-communicable health issues upon consumption. Notably, though previous studies have developed and analyzed leaf-based products, the synergistic effect of suitably combining leaf samples in products had been minimally explored. Notwithstanding, the nutrient profile and benefits of the leaves has been indicated to be elevated when used in combinations versus individual use [12]. Therefore, this research has additionally estimated the effect of leaf coalescence on the studied attributes. The study aims to add to the exploration of lesser-known edible leaves and promote their use in food as convenient and sustainable alternatives to solve the issues related to nutritional and food insecurity. This approach also aims to support health augmentation, diversification and economic growth.

## **2. Materials and Methods**

### **2.1. Materials**

All the assay reagents used for the study including Anthrone, Biuret, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Bovine serum albumin (BSA), Ascorbic acid, Ethylenediamine tetraacetic acid, Quercetin and Nutrient Agar were of analytical grade with purity index > 98 % and procured from Loba Chemie Private Limited. Kits for quantitative analysis of iron, and phosphorus was obtained from Micro Xpress, India, while those for calcium and magnesium were from Clini Quant-FSR, India and Coral Clinical System, India. Data was analyzed using colorimeter (S-9121, Systonic, India), spectrophotometer (U2910, Hitachi, Japan) and refractometer (RHB-55, Erma, Japan) and recommended titrimetric experiments.

## 2.2. Sample and strain selection

*Capsicum frutescens* and *Typhonium trilobatum* leaf samples (n = 3) from rural farms in West Bengal, India, were used to make value-added spreads. These spreads were prepared as sustainable, ready-to-consume, and cost-effective healthy and nutritious alternatives. The plant and leaves used have been displayed in Fig. 1.

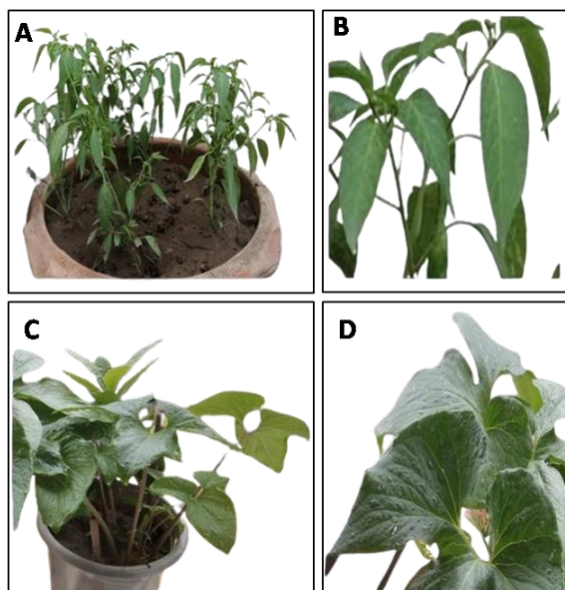


Fig. 1. Sample plant and leaves. (A) *Capsicum frutescens* plant, (B) *Capsicum frutescens* leaves, (C) *Typhonium trilobatum* plant, and (D) *Typhonium trilobatum* leaves.

## 2.3. Development of leaf-based spread and organoleptic property estimation

The leaves were used to develop spreads because of their ease of preparation and versatile use. Chickpeas were soaked overnight and then boiled in salted water. It was then integrated with ingredients mentioned in Table 1 and blended to a smooth paste to develop the control

or basic spread (BS). Fresh leaves of both *Capsicum frutescens* and *Typhonium trilobatum* were thereafter amalgamated with BS to create *Capsicum frutescens*-based spread (CS), *Typhonium trilobatum*-based spread (TS) as well as *C. frutescens* and *T. trilobatum*-based spread (CTS) (Table 1). The spreads were subsequently transferred into clean, pre-sterilized (110 °C for 15 min) jars and stored at  $\leq 4$  °C. Each leaf-based spread (prepared in three variations) was analyzed for its organoleptic properties with respect to the control spread BS (without leaves) through a 9-point hedonic scale by respondents (n = 150; age: 20-40 years) [13]. The best variations of each type were further analyzed for biochemical and functional properties.

Table 1. Variants of the leaf-based spreads.

Ingredients (g/100 g)	Control (BS)	<i>Capsicum frutescens</i> based spread (CS)			<i>Typhonium trilobatum</i> based spread (TS)			<i>C. frutescens</i> and <i>T. trilobatum</i> based spread (CTS)		
		CS1	CS2	CS3	TS1	TS2	TS3	CTS1	CTS2	CTS3
Green chilly leaf	0	10	20	30	0	0	0	5	8	12
Kharkol leaf	0	0	0	0	10	20	30	5	12	18
Chickpea	25	25	25	25	25	25	25	25	25	25
Lemon juice	15	15	15	15	15	15	15	15	15	15
Sesame seeds	15	15	15	15	15	15	15	15	15	15
Garlic	6	6	6	6	6	6	6	6	6	6
Oil	12	12	12	12	12	12	12	12	12	12
Water	15	15	15	15	15	15	15	15	15	15
Black pepper powder	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Salt	10	10	10	10	10	10	10	10	10	10
Honey	0	2.5	5	7.5	2.5	5	7.5	2.5	5	7.5
Vinegar	0	2.5	5	7.5	2.5	5	7.5	2.5	5	7.5

#### 2.4. Proximate composition

The proximate components were analyzed based on methods recommended by the Association of Official Analytical Chemists (AOAC). The Anthrone method was used for the estimation of carbohydrates. Standard (1mg/mL D-glucose) or test solutions were mixed with Anthrone reagent (SRL Chemicals, India), incubated in a dark place for 20 minutes before measuring the absorbance at 620 nm in a colorimeter (Systonic, India) [14]. Proteins were estimated by combining standard (10 mg/mL Bovine serum albumin) or sample solutions (leaf-based spreads) with Biuret's reagent (Beacon Diagnostics, India). The mixture was incubated at 25 °C for 30 min followed by colorimetric assessment at 550 nm. The crude fat and fibre proportions were determined through Soxhlet semi-continuous extraction and acid-alkali digestion-based treatment (2/3N H<sub>2</sub>SO<sub>4</sub> and NaOH) [15]. The

moisture and ash percentage were estimated by oven drying thermo-gravimetric method and muffle furnace incineration at 600 °C [16].

### **2.5. Micronutrients**

The content of iron, calcium, magnesium, and phosphorus along with vitamin C and  $\beta$  carotene were determined in the leaves. The calcium and phosphorus concentrations were calculated using the o-cresolphthalein complexone (OCPC) and the Molybdate UV method, respectively. Iron and magnesium proportions were analyzed through the ferrozine and calmagnite method, individually. Reagents were combined as per the manufacturer's instructions followed by optical density (OD) measurement at the designated wavelengths. Concentrations of the above minerals were determined with respect to the corresponding standards. The vitamin C concentration was determined using the indophenol dye technique in comparison to standard ascorbic acid (5mg/mL). Briefly, 1 mL of 2,6-dichlorophenol indophenol dye was titrated with standard or sample solutions mixed with metaphosphoric acid (1:1). Lambert-Beer's law was used to compute the concentration of  $\beta$ -carotene. The ethanol extracts were subjected to centrifugation, separating funnel extraction followed by absorbance estimation at 452 nm in a spectrophotometer.

### **2.6. Phytochemicals**

Leaf-based spreads were analyzed for the presence of key phytochemicals. Flavonoids were determined by  $AlCl_3$ -based complex formation against quercetin standard followed by measurement of OD at 415 nm. The Folin–Ciocalteu assay with respect to standard Gallic acid (20 mg/100 mL) was employed for estimation of phenolics. This involved reduction of the Folin–Ciocalteu reagent by the phenolics resulting in the production of molybdenum–tungsten blue that was measured spectrophotometrically at 760 nm. Alkaloids were estimated by using  $NH_4OH$ . The samples were allowed to stand for 4-6 h with acetic acid in ethanol solution accompanied by filtration, evaporation of filtrate, and precipitation of alkaloids with  $NH_4OH$  [14].

### **2.7. Physicochemical parameters and anti-nutrients**

Total Soluble Solids (TSS) were calculated by a refractometer and expressed as degrees Brix. Titrable acidity (TA) was estimated by titration with 0.1 N NaOH with respect to standard oxalic acid [17]. Tannins and oxalates were estimated through quantitative titration with potassium permanganate. Phytates were measured by Ethylenediamine tetraacetic acid (EDTA)-dependent complexometric titration [18].

### **2.8. Free radical foraging capacity**

The free radical foraging ability representing the antioxidant capacity of the extracts was tested using a DPPH radical scavenging assay. 2.4 mL of 0.1 mM 2,2-diphenyl-1-

picrylhydrazyl (DPPH) solution was mixed with 1.6 mL of sample extracts at various concentrations (12.5-150 µg/mL) and incubated for 30 min away from light. The absorbance of the mixture was then measured by a spectrophotometer at 517 nm with ascorbic acid as a reference. The antioxidant potential of the samples was calculated by calculating the percentage of the difference in absorbance between the control and product extract with respect to the OD of the control [19].

### **2.9. Antimicrobial capacity**

The antimicrobial activity of the products was studied using the agar well diffusion method. Nutrient agar media (autoclaved at 121 °C and 15 lbs) inoculated with gram-positive bacteria (*Bacillus subtilis*), gram-negative bacteria (*Escherichia coli*), yeast (*Saccharomyces cerevisiae*), and mold (*Aspergillus niger*) were interacted with *C. frutescens* (CS), *T. trilobatum* (TS) as well as *C. frutescens* and *T. trilobatum* based spread (CTS) with reference to the control (BS). This assay measured the size of the growth inhibition zone (mm) against the test microbe with versus the control in agar wells post 24 hours of incubation (37 °C).

### **2.10. In vitro prebiotic potential**

The *in vitro* prebiotic potential of the products was estimated by inoculating them in de Man, Rogosa and Sharpe (MRS) media containing 1 % *Lactobacillus acidophilus* (ATCC 4356). The growth of the probiotic *L. acidophilus* was determined at regular intervals of 60 min up to 24 h at 560 nm. The prebiotic potential was estimated by determining the increase in growth upon the addition of the sample extracts compared to the control [20].

### **2.11. Cost analysis and product shelf life**

The shelf life on nutrient agar was tested using the microbiological spread plate method. Measured amounts of sample extracts were plated onto pre-solidified and pre-sterilized agar plates, incubated at 37 °C for 24–48 h, followed by growth determination. The mean of three replicate experiments was expressed as colony-forming units per milliliter (CFU/mL). The product's cost was estimated using the formula proposed by Rachman *et al.* [21].

### **2.12. Data analysis and statistics**

Microsoft Excel (version 2016) was used to calculate the mean, standard error, and standard deviation from the experimental data. Values were illustrated as mean± standard error of mean (s.e.m) of three or more individual investigations. Statistical analysis was performed through Analysis of variance (ANOVA-one-way). P value <0.05 at a 95 % confidence interval was considered statistically significant and represented as \*(p-value <0.05), \*\* (p-value <0.01), and \*\*\* (p-value <0.001).

### 3. Results and Discussion

Lesser-known and low-cost edible greens were incorporated to produce value-added nutritional and functional spreads as a sustainable and convenient alternative to combat food and nutritional insecurity along with health upliftment.

#### 3.1. Organoleptic characteristics

Table 2. Sensory analysis of the developed spreads.

Property	Control (BS)	<i>Capsicum frutescens</i> based spread (CS)			<i>Typhonium trilobatum</i> based spread (TS)			<i>C. frutescens</i> and <i>T. trilobatum</i> based spread (CTS)		
		CS1	CS2	CS3	TS1	TS2	TS3	CTS1	CTS2	CTS3
Appearance	6.06±0.5	6.78±0.2*	7.87±0.5*	8.24±0.09**	6.83±0.3*	7.68±0.2*	8.31±0.2*	6.34±0.8*	7.23±1.0	8.81±0.4*
Colour	5.68±0.7	5.77±0.4*	6.43±0.6*	7.41±0.1*	6.01±0.2*	7.33±0.3*	7.97±0.08**	5.8±0.5*	6.1±0.8*	7.62±0.1*
Taste	5.23±0.5	5.45±0.6*	6.32±0.3*	7.37±0.01**	5.77±0.4*	7.99±0.2*	8.16±0.1**	5.89±0.7*	6.76±0.9*	7.87±0.06**
Texture	5.97±0.1	6.11±0.5*	6.43±0.6*	7.56±0.03**	6.23±0.6*	6.29±0.3*	8.11±0.08**	6.45±0.9*	7.67±1.1	8.12±0.03**
Odour	5.66±0.2	5.97±0.3*	6.35±0.4*	7.89±0.05**	5.65±0.5*	7.11±0.3*	8.12±0.06**	5.71±0.8*	6.58±0.6*	7.93±0.1*
Overall ranking	5.78±0.2	6.13±0.1*	6.72±0.6*	7.74±0.03**	6.06±0.4*	7.16±0.4*	7.54±0.04**	6.31±0.9*	7.02±0.5*	8.16±0.05**

Organoleptic property analysis was done to check the palatability and acceptability of the developed products (Table 1). Leaf-containing products viz., *Capsicum frutescens*-based spread (CS), *Typhonium trilobatum*-based spread (TS), and *C. frutescens* and *T. trilobatum*-based spread (CTS) were found to display higher acceptance versus the control (without leaves) with respect to their appearance, taste, texture and overall acceptability. The third variant of all the spreads displayed the highest rating in all the sensory properties (7.74, 7.54, and 8.16 for CS3, TS3, and CTS3, respectively) which may be due to the addition of appropriate proportions of leaves along with other ingredients, especially honey and vinegar to balance the sensory characteristics (Table 2). The addition of leaves created a bright green appearance along with a lustrous finish due to the combination of honey, chickpea and vinegar, resulting in higher acceptability of the leaf-based products versus the control (BS) as also noted in previous studies [22]. Additionally, lemon juice, vinegar and honey helped in masking the bitterness of the leaves along with creating a desirable consistency and smoothness.

#### 3.2. Proximate, bioactive, and anti-nutrient components in developed spreads

Variation 3 of the prepared spreads (CS3, TS3, and CTS3) certified with the highest organoleptic score as well as the raw leaves were estimated for proximate composition, including moisture, ash, carbohydrate, protein and crude fibre due to the importance of these

elements in supporting appropriate metabolism and homeostasis. The leaf-based spreads were observed to harbor moderate carbohydrate, protein and fat proportions with the amount higher in green chili leaf spread (13.08, 7.34, and 2.91 g/100 g) than kharkol (10.65, 6.83, 2.74 g/100 g). This may serve 10 %, of the recommended dietary allowance (RDA) for carbohydrates and 1 % for proteins and fats, respectively for an adult [23]. This was pursuant to a comparatively higher concentration of proximate components in raw green chili leaf versus kharkol leaves (Table 3). Owing to their modest calorific values fostered mainly by limited carbohydrates and fats, these may be used by people preferring hypocaloric options. The protein content, although mild, may be used to supplement other protein sources for improved health. Besides, originating from botanical sources, these may be preferred by the vegetarian population. All samples manifested a significant content of fibre versus the control. This may not only aid in the maintenance of digestive health but also foster the growth of favorable gut microbiota, hence upgrading health status upon consumption. Moreover, the leaf-based spreads displayed significant ash content indicating a notable mineral inhabitation with the amount being higher in CS3 compared to T3S (Table 3). Indeed, the selected samples demonstrated a notable presence of iron, calcium, magnesium, and phosphorus with their amounts being comparatively greater in CS3 versus TS3. This was in line with a higher content of the mentioned components in fresh *C. frutescens* than *T. trilobatum* leaves (Table 3). The existence of 11.28 mg/100 g and 9.01 mg/100 g of iron in CS3 and TS3 can remunerate a notable portion of the RDA requisites for this mineral. This qualifies them as suitable options for the development of iron-rich food and pharmaceuticals for combating the issues of anemia and other iron-deficiency conditions. Additionally, considerable presence of calcium, magnesium, and phosphorus in the *C. frutescens* (110.11, 68.78, and 79.01 mg/100 g) and *T. trilobatum* (96.22, 57.68, and 76.21 mg/100 g) containing spreads suggest the usefulness of the same in the maintenance of homeostasis of bones, circulatory as well the vascular systems [24]. Moreover, the spreads portrayed noteworthy vitamin C and  $\beta$ -carotene content, with the amounts being higher in CS3 spread than in TS3 (Table 3). Vitamin C may not only promote iron absorption but also bestow functional benefits including antioxidant and immunity-enhancing properties which may further be upgraded by the  $\beta$ -carotene content. The results of this study are in confirmation with earlier investigations that have also found edible leaves to be good micronutrient sources [25]. Phytochemical analysis revealed a considerable presence of key phytochemicals (alkaloid, phenols, flavonoids, and saponins) in leaf-containing spreads with CS3 displaying a moderately greater proportion than TS3. This was in accordance with a greater presence of these phytochemicals in fresh *C. frutescens* than *T. trilobatum* leaves. Interestingly, the nutritional and bioactive content in the spreads incorporated with both *C. frutescens* and *T. trilobatum* leaves (CTS3) was observed to be higher than those with individual leaf samples. This indicates them to be better options for combating nutritional security and health issues owing to the synergistic combinations of the leaves present in these products (Table 3). The spreads containing leaves were observed to harbor a lower moisture proportion due to the addition to leaves that concentrated the product as also noticed by a higher total soluble solid (TSS) content

in the same. The higher titrable acidity (TA) in these products (CS3, TS3, and CTS3) versus the control indicates better keeping quality and taste. Notwithstanding, the raw leaves and the leaf-based spreads were detected to manifest low anti-nutrients inclusive of tannins, phytates, and oxalates which may not curtail the absorption and bioavailability of the studied nutrients post-intake. Therefore, CTS3 followed by CS3, and TS3 may be attractive alternatives for the management of micronutrient deficiency disorders along with chronic and degenerative diseases owing to their nutrient and phytochemical content.

Table 3. Proximate, bioactive, and anti-nutrient components in the raw leaves and the developed spreads.

Component	Raw Leaves (per 100 g leaves)		Developed Spreads (per 100 g product)			
	<i>Capsicum frutescens</i>	<i>Typhonium trilobatum</i>	Control (BS)	<i>Capsicum frutescens</i> based spread (CS3)	<i>Typhonium trilobatum</i> based spread (TS3)	<i>C. frutescens</i> and <i>T. trilobatum</i> based spread (CTS3)
Total Carbohydrate (g/100 g)	3.21±0.007	2.10±0.001	9.46±0.05	13.08±0.07*	10.65±0.01*	14.05±0.03*
Total Proteins (g/100 g)	2.70±0.007	1.97±0.007**	4.55±0.04	7.34±0.05*	6.83±0.07*	7.88±0.07*
Crude Fat (g/100 g)	0.71±0.01	0.46±0.01	2.10±0.02	2.74±0.04*	2.91±0.05*	3.17±0.04*
Crude fibre (%)	10.4±0.05	6.8±0.05	10.81±0.05	18.04±0.01*	20.15±0.05*	22.21±0.05*
Iron (mg/100 g)	15.61±1.05	13.62±1.15*	5.34±1.05	11.28±1.15*	9.01±1.07*	12.46±1.07*
Calcium (mg/100 g)	131.12±0.03	104.16±0.4	64.11±0.05	110.11±0.03	96.22±0.04	120.31±0.03
Magnesium (mg/100 g)	48.2±0.003	41.25±0.02	15.25±0.04	68.78±0.03**	57.68±0.02**	92.34±0.03**
Phosphorus (mg/100 g)	40.85±0.01	32.99±0.05	57.51±0.02	79.01±0.01**	76.21±0.02**	87.52±0.01**
Vitamin C (mg/100 g)	50.53±0.24	40.14±0.34	20.11±0.18	56.30±0.24*	40.24±0.34*	60.13±0.20*
β carotene (µg/100 g)	420.12±0.021	200.34±0.039	206.20±0.03	495.03±0.02***	368.05±0.04***	504.08±0.03***
Total phenols (µg/100 g)	164±1.32	145±1.25*	212.13±0.5	346.15±0.65**	331.04±0.46**	357.07±0.34**
Flavanoids (µg/100 g)	27±0.65**	17±0.11**	33.56±0.11	66.34±0.34*	43.31±0.30*	77.24±0.27*
Alkaloids (µg/100 g)	125±0.05	99±0.05	68.22±0.04	93.43±0.06**	89.10±0.12*	98.06±0.09**
Saponins (mg/100 g)	33.87±1.23	26.32±1.02	0.01±0.7	17.03±0.5*	14.06±0.3*	19.09±0.5*
Tannins (N)	0.33	0.30	0.34	0.65	0.68	0.71
Oxalates (N)	0.28	0.96	0.35	0.60	0.66	0.75
Phytates (%)	0.59	0.76	0.40	0.55	0.58	0.60
Moisture (%)	10.09±0.03	10.13±0.05	8.51±0.02	7.07±0.03*	7.28±0.05*	7.05±0.02*
Ash (%)	13.1±0.02	9.2±0.04	5.21±0.03	13.13±0.02*	10.22±0.04*	14.24±0.04*

Titration acidity	-	-	4.8	6.4	6.45	6.5
Total soluble solid (°Brix)	-	-	25	32.5	32.6	33.9

### 3.3. Functional properties

The developed spreads and the raw leaves were further tested for key functional properties including free radical inhibition, prebiotic potential and anti-microbial capacity. This was owing to the detection of significant amounts of bioactive components as well as fibre in the leaves and leaf-containing products (Table 3) that have previously been associated with these benefits [26]. As noted, CS3 (73.2 %), TS3 (65.3 %) and CTS3 (81.6 %) manifested a greater free radical inhibition compared to BS with the potential greater in CS3 than TS3 which is in concert with a greater free radical inhibition power of *C. frutescens* than *T. trilobatum* spreads. This agreed with a higher free radical inhibition in fresh *C. frutescens* than *T. trilobatum* (Table 4). Both the leaves and all the leaf-based spreads were also observed to demonstrate anti-microbial activity displayed by their ability to inhibit gram-negative *Escherichia coli*, gram-positive *Bacillus subtilis* along with fungi *Saccharomyces cerevisiae* and *Aspergillus niger* (Table 4). Results showed this property to be more pronounced in *Capsicum frutescens* and *C. frutescens* spreads (25.22, 35.56, 17.35, and 18.51 mm against *E. coli*, *B. subtilis*, *S. cerevisiae*, and *A. niger*) than *Typhonium trilobatum* and *T. trilobatum* spreads (18.65, 24.40, 12.18, and 14.30 mm against *E. coli*, *B. subtilis*, *S. cerevisiae*, and *A. niger*). Additionally, the leaves and the leaf-based spreads showed a significant prebiotic potential compared to the control as portrayed by the ability to hasten the proliferation of probiotic *Lactobacillus acidophilus*. This was evident by the heightened values of optical density (OD<sub>600nm</sub>) in the logarithmic phase mainly between 6-18 hours, compared to the BS (control). This was in accordance with a higher prebiotic potential of raw *C. frutescens* than *T. trilobatum* leaves versus negative control (without leaf extract) (Figure 1A). CS3, TS3, and CTS3 displayed a constant increase in the growth kinetics of *L. acidophilus* between 3 h to 18 h, with the effect being highest in CTS3 (0.63 at 18 h) followed by CS3 (0.55 at 18 h) and TS3 (0.47 at 18 h) (Fig. 2A). Similarly, raw *C. frutescens* fostered higher growth kinetics to *L. acidophilus* versus *T. trilobatum* leaves with respect to negative control (without leaves). After 18 h, all samples entered the stationary phase (Fig. 2). Noteworthy, the free radical scavenging, microbial inhibition, as well as probiotic acceleration, was found to the highest in spreads prepared with both *C. frutescens* and *T. trilobatum* (CTS3), suggesting the presence of a synergistic combination of leaves that may be complementing the functional properties derived from each of the individual leaf samples (Table 4). Consequently, the leaf-based spreads may be suitable for maintaining a healthy lifestyle and preventing the prevalence of infectious and lifestyle diseases. This may be owing to their potential to mitigate microbes, and free radicals as well as promote gut health demonstrated by their observed functional effects.

Table 4. Free radical inhibition and anti-microbial activity in developed spreads.

Sample	Component	Free radical inhibition (% inhibition)					Antimicrobial activity (Zone of inhibition in mm)	
		<i>Capsicum frutescens</i>	<i>Typhonium trilobatum</i>	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Saccharomyces cerevisiae</i>	<i>Aspergillus niger</i>	
Raw leaves	<i>Capsicum frutescens</i>	71.5±1.2	23.92±1.8	32.05±1.4	10.51±0.7	16.45±0.9		
	<i>Typhonium trilobatum</i>	62.3±0.8	17.71±1.01	27.12±1.1	7.30±0.4	10.64±0.7		
Leaf-based spreads	Control (BS)	52.3±0.01	10.04±1.02	12.03±0.98	8.02±0.86	8.86±1.01		
	<i>C. frutescens</i> based spread (CS3)	73.2±0.004***	25.22±2.13*	35.56±1.68*	17.35±1.12*	18.51±1.05*		
	<i>T. trilobatum</i> based spread (TS3)	65.3±0.005***	18.65±1.41*	24.40±1.95*	12.18±1.07*	14.30±0.53**		
	<i>C. frutescens</i> and <i>T. trilobatum</i> based spread (CTS3)	81.6±0.002***	32.12±0.04**	40.34±0.11**	19.21±1.88*	22.34±0.05**		

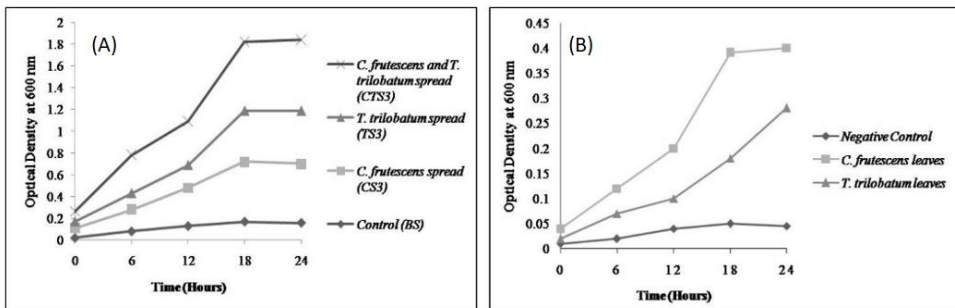


Fig. 2. Prebiotic potential of the leaves and the developed spreads. (A). Prebiotic potential of *Capsicum frutescens* based spread (CS3), *Typhonium trilobatum* based spread (TS3), and *C. frutescens* and *T. trilobatum* based spread (CTS3). (B). Prebiotic potential of *C. frutescens* and *T. trilobatum* leaves.

### 3.4. Cost and self-life

Table 5 displays the shelf life of the developed spreads through the microbiological plating method. The products prepared without any chemical preservatives demonstrated an enhanced shelf life at refrigerated storage (4 °C) compared to storage at ambient temperature (30 °C) (Table 5). The total viable microbes were found within acceptable limits (< 100 CFU) up to 30 days. However, microbiological quality was found to diminish from the 20<sup>th</sup> day of storage as viewed by a high microbial load under both conditions. Notably, the leaf-containing products portrayed an improved shelf life than control (without leaves) which may be attributed to the anti-microbial action portrayed by the studied leaves. Therefore, CS3, TS3, and CTS3 can be consumed till one month of its preparation, especially when stored at low temperatures. Notwithstanding, it would be safe to consume

them within a limited duration after production (within 20 days) to maintain the desired quality and safety. Moreover, adding preservatives may extend the shelf life of the developed leaf-based spreads. These spreads were found to be low cost owing to the use of leaves along with other economical and readily available supporting ingredients with CS3, TS3, and CTS3 costing Rupees 30.00 for 100 g of the product versus the control that was found to cost Rupees 25.00. Hence, CS3, TS3, and CTS3 were found to be reasonably priced nutritious and functional ready-to-consume options that may be accessed by people across all socioeconomic backgrounds for health benefits.

Table 5. Cost and shelf life of the developed spreads.

Storage Time (Days)	At Room temperature (30 °C) CFU/mL				Under Refrigeration (4 °C) CFU/mL			
	Control (BS)	CS3	TS3	CTS3	Control (BS)	CS3	TS3	CTS3
1	2	1	1	1	0	0	0	0
10	10±1	8±1	9±1	5±1	6±1	<5	<5	<5
20	68±2	47±3*	52±2*	38±3*	38±4	25±2	27±1	18±1
30	100±3	88±2*	89±1*	76±2*	70±3	47±1*	52±2*	38±2*
Cost/100g								

#### 4. Conclusion

Green leafy vegetables are an attractive option for promoting nutrition and food security as well as the upgradation of health. Being climate resilient, easily cultivable, economical, and vegetarian sources, these may be sustainable alternatives that can be incorporated by major sections of society. To promote their utilization, ready-to-consume spreads were developed by incorporating two less popular edible leaves viz; leaves of green chili (*C. frutescens*) and kharkol (*T. trilobatum*) that were selected based on their minimal cost, ease of cultivation and availability. Nutritional and functional analysis demonstrated these leaves and the leaf-containing spreads to possess considerable amounts of important micronutrients including iron, calcium, magnesium, phosphorus, vitamin C and  $\beta$ - carotene along with fibre as well as moderate levels of macronutrients with the content being higher in spreads prepared with green chili leaves (CS3) than the kharkol leaves (TS3) as also observed in the raw leaf extracts. The products also displayed a significant proportion of important plant-based phytochemicals especially, phenolics, alkaloids, and flavonoids with their amounts being greater in CS3 than TS3 versus the control (without leaves). Noteworthy, spreads prepared with both leaves (CTS3) displayed a considerable increased presence of the studied nutrients and bioactive components signifying the leaves to be present in a synergistic concoction that may manifest greater benefits upon consumption. Additionally, the fresh leaves and the leaf-based spreads portrayed considerable free radical scavenging capacities, inhibitory action against *E. coli*, *B. subtilis*, *S. cerevisiae*, and *A. niger* as well as prebiotic abilities exhibited by their potential to expedite the growth kinetics of probiotic *Lactobacillus acidophilus* demonstrating notable functional properties with the intensity being higher in CTS3 followed by CS3 and TS3, which may be attributed to the

phytochemicals detected in them. The developed products were observed to possess an affordable cost with an enhanced shelf life at low-temperature storage. Therefore, utilization of these leaves through food product development into low-cost ready-to-consume spreads may aid in the amelioration of nutrition status and well as food security, mitigation of micronutrient deficiency along with alleviation of a spectrum of diseases.

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