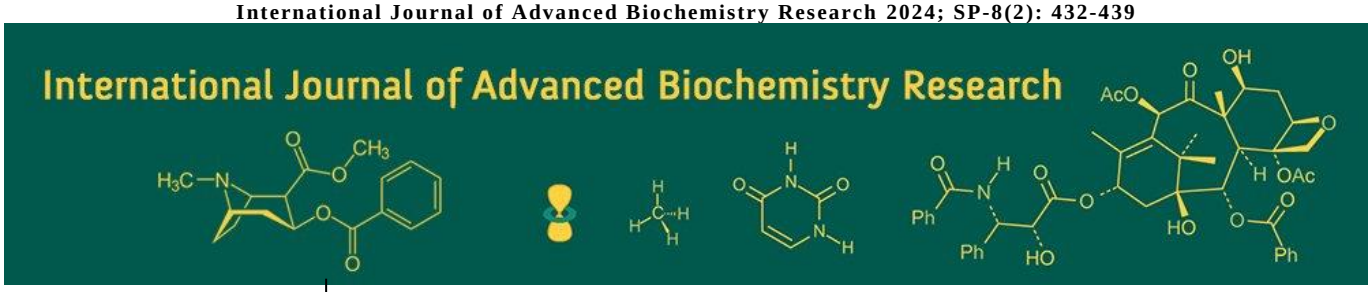

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Effects on antimicrobial and antioxidant properties of milk by addition of fruits and herbs in preparation of flavoured milk

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Abstract
The confluence of taste and nutrition in herbal-flavoured milk has emerged as a hot topic in this age of increased health awareness. Examining the possible effects on health and wellbeing, this introduction explores the symbiotic interaction between antioxidant and antibacterial capabilities within some fruits. The importance of comprehending the dynamic interaction of these bioactive substances is growing as Functional foods are sought after by consumers. In order to help people make better food choices and experience better health outcomes, this research intends to decipher the complex impacts of herbal-flavoured milk's antioxidant and antibacterial qualities.

Keywords: Flavour, antimicrobial, antioxidant, milk, herbal, fruits

Introduction
There have been new lines of investigation opened up as a result of the current drive toward healthier eating habits. One example of this is the combination of herbal-flavored milk with certain fruits, which is at the intersection of the abundance of nature and the development of nutritional study [1]. Creating a beverage that is not only tasty but also functionally useful to one's health is a promising endeavour, and the combination of the antibacterial and antioxidant properties that are present in fruits has tremendous potential [2]. Research is being conducted on herbal-flavoured milk that is loaded with fruits because consumers are searching for alternatives to food and drink that are more beneficial to their cardiovascular health [3]. The incorporation of herbal extracts into milk, which is a traditional component of the diet, has resulted in the expansion of the nutritional landscape to include a new dimension. This combination contains the potential to bring about a fusion of aroma, taste, and maybe even favourable effects on one's health [4].
The purpose of this introductory part is to provide readers with an understanding of the antibacterial and antioxidant properties of some fruits that are combined with milk that has a distinctive herbal taste [5]. In order to better understand the significance of these chemical components to human health, this inquiry is being carried out today [6, 7]. With the help of a mix of contemporary nutritional science and herbal remedies that have been used for centuries, researchers are hoping to unravel the intricate web of connections that is responsible for the health advantages that these innovative dairy beverages provide [8, 9].
Throughout the history of traditional medicine, substances have been held in high esteem for their antimicrobial properties [10, 11]. These properties indicate that they have the potential to prevent or delay the spread of germs [12]. The ancient use of herbs and fruits in medicine may be attributed to the inherent antibacterial and food-preserving characteristics that herbal and fruit products possess [13, 14]. By acquiring a greater knowledge of how these characteristics manifest themselves in flavoured milk, it may be possible to facilitate the manufacture of functional meals that not only satiate the taste receptors but also offer a natural defense mechanism against potentially harmful illnesses [15, 16].
At the same time, we are interested in examining the antioxidant properties of these fruits in order to determine how they may be able to assist in the elimination of free radicals inside

the body [17]. There is a correlation between oxidative stress and a variety of chronic diseases as well as the aging process; antioxidants are vital in the process of lowering this stress [18, 19]. It is possible that including these fruits, which are rich in antioxidants, into milk compositions might be a novel method to enhancing the nutritional value of milk, which is a fundamental food, and adding to the lifespan of health in terms of health [20, 21].

There is a growing awareness among consumers about the relationship between food and health, which has led to an increase in demand for products that are not only delicious but also functional [22]. This contemporary movement toward holistic health is in line with the introduction of herbal-flavoured milk, which has antibacterial and antioxidant properties similar to those of the milk itself [23, 24]. The agro-industrial potential of exotic fruit byproducts have been explored by Ayala-Zavala *et al*. (2011) and Gabbi, Bajwa, and Goraya (2018), respectively [25]. The physicochemical features of ice cream with processed ginger have also been researched. We have been motivated to look for methods to apply these notions to herbal-flavoured milk as a result of the insights that have been gained from these experiments [26].

Review of literature

Ayala-Zavala *et al*. (2021) [27] Fruits contain phenolic compounds in various parts of the fruit. For example, phenolic compounds in the peel have antioxidant, antimicrobial, and colorant properties; pulp has a lower concentration of phenolic compounds but is the primary source of antioxidants in human beings diet; and seed can also contain phenolics, primarily tannins, which have antimicrobial and antioxidant properties and protect this tissue so that the species can regenerate.

Gabbi *et al*., (2018) [28] The ice cream's overflow and acidity remained unaffected. During the freezing process, ginger rhizomes were added to the ice cream mixture in a variety of forms, including pulp, juice, candy, and powder. While ginger candy and powder raised total solids, ginger juice and paste reduced them. By using ginger in various forms, the fat and protein content (excluding powder) decreased, while the ash and fiber content (excluding juice) were enhanced in the ice cream that resulted. Adding various types of ginger greatly boosted the overall phenolic content and antioxidant activity. The inclusion of ginger preparations also boosted melting resistance and lowered ice cream overflow. The combination of 10% candy, 6% juice, 4% pulp, and 1% powder resulted in the greatest overall approval ratings.

Objectives

- To study the antimicrobial properties of the grape or herbal flavoured milk.
- To study the antioxidant properties of the grape or herbal flavoured milk.

Statement of the problem

The influence of the antibacterial and antioxidant capabilities inherent in some fruits or herbal-flavored milk is the urgent topic that this research seeks to address. Their possible health advantages are of utmost relevance given the increasing popularity of these items. Antimicrobial drugs and antioxidants both play important roles in fighting infections, and understanding these functions is crucial to solving the situation at hand. We hope that by filling in some of the gaps in our current understanding of the synergistic effects of these bioactive substances, our study may help people make better choices when it comes to making and drinking flavored milk variations that promote health.

Research methodology

Merlot (V1), Cabernet Sauvignon (V2), Busuioaca de Bohotin (V3), Muscat Ottonel (V4), ˇ Feteasca Neagr ˇ a (V5), Traminer (V6), and Sauvignon Blanc ˇ (V7) grape types, harvested in the fall of 2022 at full maturity, comprised the essential plant material for the experiment. Before pressing, the grapes were crushed and destemed. The maceration phase lasted for 24 hours for white types and 5 days for reds at 10 ◦C. Prior to examination, the marc that emerged from the pressing process was dried and put in the freezer. The marc samples were defrosted, dried in an air stream until they reached a consistent weight, ground into a powder, and then extracted using several solvents.

2.2. The Stage of Extraction: We examined two distinct approaches to the extraction. An extraction was accomplished by combining 50% water and ethanol. A second extraction was carried out using a 50% methanol and 50% water combination. Two separate sets of extracts were made by combining 2 grams of solid materials with 5 milliliters of water, stirring the mixture for 24 hours at room temperature, and then centrifuging it for 20 minutes at 4 degrees Celsius. We utilized the recovered supernatants for our study.

Methods of analyses

DPPH enzyme test: To measure the radical scavenging activity, the stable radical was bleached in a purple methanolic solution using the DPPH (2,2-diphenyl-1-picrylhydrazyl) technique. The typical reagents used were quercetin and butylated hydroxytoluene (BHT), two synthetic antioxidants that are hydrophilic and lipophilic, respectively. The elimination of DPPH absorption due to antioxidant activity is the metric for antioxidant effect. A DPPH solution (100 µM) was mixed with 980 µl of diluted extracts, yielding 20 µl. A UV-VIS JASCO V-530 spectrophotometer was used to assess the decrease in absorbance at 517 nm after a 30-minute incubation period. I = 100 (Ac-As)/Ac, where Ac is the absorbance of the control sample and As is the absorbance of the tested sample, was used to determine the percentage inhibition of the DPPH radical after the various samples were added. The inhibitory concentration IC50, which is the sample concentration needed to produce a 50% drop in initial DPPH radical absorbance, was also used to visually represent antioxidant activity. Triplicate runs of each experiment were performed.

Microbiological assays for antibiotic resistance of fruits

A variety of microorganisms were used to study the antibacterial activity, including *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853), and *Candida albicans* (ATCC 90028), which are pathogenic yeasts. We used a disc-diffusion technique (CLSI) to investigate the antimicrobial potential of certain bacteria. It was necessary to dilute each microbial culture in sterile 0.9% NaCl until the turbidity reached the level required by McFarland standard no. 0.5 (106 CFU/mL). The examined

microorganism suspensions were inoculated onto Mueller-Hinton agar Fungi (Biolab) and Mueller Hinton agar Oxoid. The agar surface in the Petri dishes was covered with sterile stainless steel cylinders that were 5 mm in internal diameter and 10 mm in height. Next, cylinders were filled with 100 µL of each of the examined substances (1–7), at a concentration of 10 mg/mL. The plates were allowed to sit at room temperature for 10 minutes to guarantee that the substance was distributed evenly.

Herbal material

1. **Spirulina (*Spirulina platensis*):** Manjul Spirulina Sanwardhan Sansthan in Jaipur, Rajasthan, India, is where the spirulina powder was bought in August 2018. An electronic balance was used to precisely measure three grams of spirulina. The mixture was strained through a muslin cloth after being added to one liter of homogenized, pasteurized milk. Various combinations of spirulina amounts were tested in milk, and samples were taken. Afterwards, a survey was conducted to assess organoleptic sensory qualities. Three grams of spirulina was determined to be the final concentration to be added based on flavor.
2. **Fennel (*Foeniculum vulgare Mill.*):** Nature land Organics Food Pvt. Ltd. of Rajasthan, India, supplied the organic fennel in September 2018. Using a mortar and pestle, it was reduced to a fine powder. After precisely measuring 15 grams of powdered fennel, it was blended with 1 liter of pasteurized and homogenized milk, stirred well, and strained through a cotton cloth. The process began with experimenting with various combinations of fennel to milk amounts, followed by sample preparation and organoleptic/sensory assessment. Fennel was chosen as the final concentration to be added after tasting fifteen grams.
3. **Tulsi (*Ocimum sanctum* L.):** The Department of Botany at Dayalbagh Educational Institute (Deemed to be University), Agra, has an organic herbal garden where the Tulsi was produced. The plants were harvested in September 2018 and their leaves were cleaned extensively with running water. One liter of pasteurized and homogenized milk was combined with five grams of tulsi leaves, ground in an electric mixer until the mixture was uniform, and then strained through a muslin cloth. First, we explored several combinations of Tulsi to milk amounts, prepared samples, and then evaluated them organoleptically and sensoryly. The final concentration to be added was five grams of Tulsi, based on taste.
4. **Lemongrass (*Cymbopogon flexuosus* (Nees ex Steud.) W. Watson):** Dayalbagh Educational Institute (Deemed to be University), Agra's herbal garden was the organic source for the lemongrass. In August, we collected the plants' fresh leaves, rinsed them under running water, and minced them. An electric mixer (Glen, Model No. 4011) was used to grind five grams of chopped lemongrass leaves into a homogeneous mass with one liter of pasteurized and homogenized milk. The mixture was then filtered through a muslin cloth. Organoleptic and sensory assessment followed the trial and error of various combinations of lemongrass leaf to milk concentrations.

Raw milk

We got our raw cow milk from a DEI in Gaushala, Agra. The raw milk's composition was 3.5% fat and 9.0% solid non-fat. Next, the raw milk was brought to a temperature of 35–40 ºC, strained through a muslin cloth, heated to 63 ºC for 30 minutes, and last, homogenized under pressure of 2500 psi. The fat content of the processed milk was homogenized to 1.5%.

In Uttar Pradesh, specifically Daurala Sugar Mills Sucrose was sourced. Afterwards, a blender was used to further grind it into a fine powder.

Honey was bought from Forever Living in Mumbai via their internet store. One liter of milk was mixed with twenty-seven grams of honey, which was measured precisely. Before conducting organoleptic/sensory assessment, several combinations and permutations of honey were first added to milk. In terms of flavor, the settled-upon concentration-27 grams of honey-was ideal.

Microbiological analysis of herbal flavoured milk

The presence of Coliform, Total plate count, yeast, and mould were studied by microbiological testing of herbal flavoured milk.

Coliform

As part of the experiment, distilled water was added to a 250 ml conical flask. Carefully added to this was 10.38 grams of Violet Red Bile Agar (VRBA). The flask was then coated with silver foil and a cotton plug was used to seal it. Boiling the agar for two minutes followed a thorough mixing. Keep in mind that autoclaving was purposefully omitted from this procedure.

Cooling the agar solution to 45ºC then boiling it. The next step was to conduct serial dilutions with a 9:1 ratio, starting at 10^-1 and going all the way up to 10^-6. A petri dish was filled with one millilitre of the diluted solution using a micropipette. Finally, the agar medium was prepared for further tests by pouring 10–12 ml of media onto the plate and letting it settle.

Total plate count

There were a number of methodical procedures involved in making Plate Count Agar (PCA) in the lab. First, a 250 ml conical flask was filled with measured amounts of distilled water. Plate Count Agar (PCA)-5.9 grams-was then added to make sure everything was well mixed. To keep things as clean as possible, a cotton plug and some silver foil were used to cover the flask.

To ensure the medium was completely sterile, it was autoclaved. After autoclaving, it was given enough time to cool down to the proper temperature. A 9:1 ratio was used for the serial dilutions, which ranged from 10^-1 to 10^-6. With extreme care, a micropipette was used to delicately distribute one milliliter of the diluted material onto a petri dish.

The 15 ml PCA medium was carefully added to the plate, mixed gently, and then rotated in both clockwise and anti-clockwise directions to make sure it was well distributed. It was then left to harden the agar medium. The finished plates were turned upside down and kept in an incubator at a temperature of 35 to 38 degrees Celsius for 24 hours to allow for the best possible growth and enumeration of microbes.

Yeast and Mould

In a 250 ml conical flask, 250 ml of distilled water was added. I next added 10.2 grams of Chloramphenicol Yeast Glucose Agar (CYGA) to the distilled water, stirring constantly to ensure a complete mixture. To provide a sterile environment, the flask was sealed with a cotton plug and wrapped with silver foil.

To make sure the medium was completely sterilized, it was autoclaved at 121 °C. It was necessary to let the agar medium cool to an appropriate temperature after autoclaving. From 10^-1 to 10^-6, serial dilutions were carried out in a 9:1 ratio. With precision, a micropipette was used to transfer a millilitre of the diluted material onto a petri dish.

The last step in preparing the plates was to pour the CYGA medium over them in the recommended quantity. To make sure the sample was evenly distributed, the mixture was gently swirled and spun in both clockwise and anti-clockwise directions. It was then left to harden the agar medium. For optimum microbial incubation and analysis, the finished plates were inverted and put in an incubator set between 35 ºC and 38 ºC for 24 hours.

A flavour-infused milk treatment using ultraviolet light

The herbal-flavoured milk that was made was treated with ultraviolet light. Three distinct varieties of ultraviolet light are known as UV-A, UV-B, and UV-C. A research on food and dairy products found that each of these rays had an average wavelength range. The most appropriate one is UV-C (Chawla *et al*., 2021). The milk was placed in a wooden box and treated with ultraviolet light. The measurements of the wooden crate were: Measurements: 53.5 cm in length, 31.5 cm in width, and 30.5 cm in height. An ultraviolet-light-emitting tube was installed inside the wooden container.

Statistical analysis

A 3x3x5 factorial arrangement was used in the experimental design to evaluate total phenolic, flavonoids, and antioxidant capacity. The three varieties were 'Haden', 'Ataulfo,' and 'Tommy Atkins.' The three types of tissues were peel, seed, and pulp. The five types of extracts were MPE, MNPE, EPE, ENPE, and water infusion. A 3x2x5 factorial design is used for the investigation of antibacterial activity: We employed three different varieties ('Haden', 'Ataulfo', and 'Tommy Atkins'), two different types of tissue (peel and seed), and five different extracts (MPE, MNPE, EPE, ENPE, and water infusion), in that order. Using the 2007 NCSS software, an analysis of variance (ANOVA) was conducted to see whether there were significant differences across treatments ($p < 0.05$), and a Tukey-Kramer test was used for comparison ($p \leq 0.05$).

Results and Discussion

A DPPH test for antioxidant activity

The capacity of quercetin and BHT, two antioxidants found in nature and in synthesized form, to donate hydrogen atoms was tested using the stable free radical DPPH. Table displays the findings of the DPPH bleaching test, which was used to evaluate the antioxidant activity. The DPPH radical was scavenged to varying degrees by all of the extracts.

Table 1: Extracts' DPPH-based antioxidant activity

Sample		V1	V2	V3	V4	V5	V6	V7	Quercetin	BHT
IC50 (µg/mL)	Extract with ethanol	24.21 ± 0.82	34.60 ± 1.02	33.42 ± 0.91	20.93 ± 0.79	20.59 ± 0.75	30.71 ± 0.96	44.04 ± 1.24	5.59 ± 0.13	15.88 ± 1.06
IC50 (µg/mL)	Extract with methanol	98.142 ± 4.31	134.48 ± 5.27	144.9 ± 6.41	132.17 ± 5.11	115.78 ± 4.29	119.86 ± 4.35	127.96 ± 4.74	5.59 ± 0.13	15.88 ± 1.06

Table 2: The Antimicrobial Effects of the Analyzed Chemicals

Compounds			1	2	3	4	5	6	7	Ciprofloxacin 5 µg/disc)	Fluconazol (25 µg/disc)	Voriconazol (1 µg/disc)
Diameter of inhibition zones (mm)	*S. aureus*	ATCC 25923	–	–	–	–	–	–	–	27.7 ± 0.06	NT*	NT*
	E. coli	ATCC 25922	–	–	–	–	–	–	–	30.0 ± 0.00	NT*	NT*
	Pseudomonas aeruginosa	ATCC 27853	–	–	–	–	–	–	–	30.0 ± 0.00	NT*	NT*

Better antioxidant activity is indicated by a lower IC50 value, which also indicates a larger bleaching impact. Depending on the extraction solvent, one might see differences. The DPPH scavenging activity of all the extracts that were tested was lower than that of the standards, quercetin and BHT. The extracts V5 and V4, which were ethanol-derived, had the greatest radical scavenging activity (20.59 µg/mL and 20.93 µg/mL respectively), and there was a significant association between the DPPH scavenging activity and total phenolic content. Using the DPPH technique, both V2 and V3 extracts demonstrated similar levels of antioxidant activity; this finding was also associated with their total polyphenol concentration. The reduced content of polyphenols (2.03 2.13 mg GAE/mL) may explain why extracts V3, V2, and V7 showed a weaker antioxidant effect (IC50 value = 33.42 µg/mL, 34.60 µg/mL, and 44.04 µg/mL, respectively). V7 < V2 < V3 < V6 < V1 < V4 ~ V5 < BHT < quercetin is the antioxidant activity order that might be determined based on the data obtained by the DPPH technique.

This approach indicated that all of the examined extracts had strong antioxidant properties, with an IC50 value below 50 µg/mL [12]. The V1 sample (with an IC50 of 98.142 µg/mL) and the V5 extract (with an IC50 of 115.78 µ g/mL) exhibited the highest antioxidant activity in the methanol-based second extract. There was a significant connection between the scavenging activity on DPPH and the total phenolic content.

After looking at the data, we can determine that V3 is the most antioxidant, followed by V2, V4, V7, V6, V5, V1, BHT, and finally quercetin. The results demonstrated that the examined extracts had modest antioxidant properties, according to this approach.

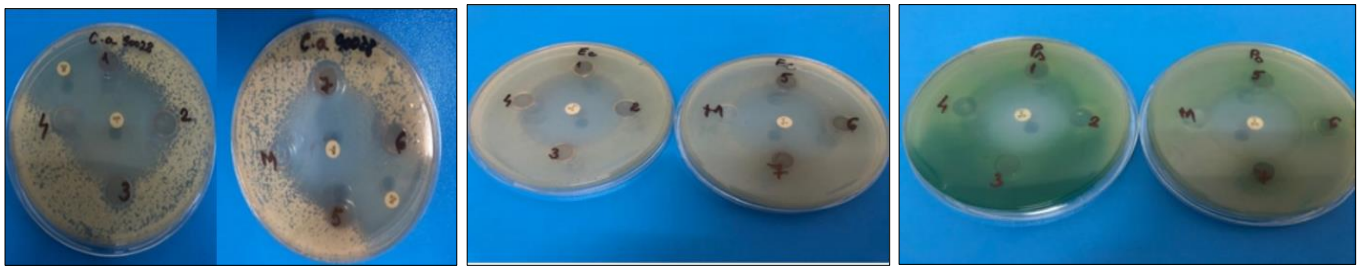
Possible correlation with increased concentrations of phenolic compounds, these findings show that various active principles may enhance the antioxidant capabilities of

natural goods. The varied antioxidant capabilities of these extracts might be attributed to the wide range of phytochemical compositions and contents as well as the specific extraction methods used. When getting ready to extract something, an ethanol solvent can be best.

Antimicrobial activity of fruit: Table shows the inhibitory zone sizes (in mm) for each of the substances that were tested. The experiments were repeated three times. Means + standard deviations are used to represent the results. All of the substances tested showed little effectiveness against both Gram-negative and Gram-negative bacteria. Only the V1 and V7 extracts showed any antifungal action at all. Results showing that grape pomace extract inhibits fungal and bacterial development include.

Table 3: Terpene concentration in the samples that were analyzed (±SD)

Sample		V1	V2	V3	V4	V5	V6	V7
TPC (mg GAE/mL) ±SD	Extract with ethanol	2.7075 ± 0.002	2.08 ± 0.002	2.1375 ± 0.001	2.76 ± 0.001	2.7775 ± 0.002	2.5725 ± 0.003	2.03 ± 0,003
TPC (mg GAE/mL) ±SD.	Extract with methanol	2.421 ± 0.09	0.642 ± 0.03	0.404 ± 0.02	0.771 ± 0.05	1.452 ± 0.07	1.242 ± 0.05	0.884 ± 0.06



A B C

Fig 1: Grape pomace extract's effect on the development of *Candida albicans* ATCC 90028 (A), *Escherichia coli* ATCC 25922 (B), and *Pseudomonas aeruginosa* ATCC 27853 (C)

Assessing the Antioxidant Capacity of Flavoured Milk

Table 4: Antioxidant activity as gallic acid milligrams per hundred millilitres for each of the four milk flavours

Flavour		Spirulina	Lemongrass	Tulsi	Fennel
Total phenolic content (as Gallic acid). mg/100ml	With 3% sugar Non- UV	19.1	7.7	14.9	12.2
	UV treated with 3% sugar	8.7	7.7	8.1	12.6
	With honey	-	21.1	-	-

This might be because the matrix and phenol types of these substrates are different. For instance, among the phenols in fennel, the most important ones were discovered to be trans-anethole, estragole, and fenchone; in lemongrass, there were considerable quantities of phytochemicals.

When compared to five servings of fruits and vegetables, three grams of spirulina had stronger anti-inflammatory and antioxidant activity. Researchers found that synaptic acid, salicylic acid, caffeic acid, and trans-cinnamic acid are among of the primary phenolic chemicals found in *Spirulina platentis*.

These findings have practical implications for the commercial sector, demonstrating that when UV therapy is included, the antioxidant properties of lemongrass and fennel are most advantageous.

Bacterial characteristics of herbal milk

Table 5: Characteristics of spirulina flavoured milk-treated with UV on microbes

Parameter		Total Plate Count (cfu/ml)	Yeast and Mould (cfu/ml)	Coliform (cfu/ml)
Results	Day 0	<10	<10	<10
	Day 7	<10	<10	<10
	Day 14	<10	<10	<10
	Day 21	<10	<10	<10
	Day 28	<10	<10	<10

The result was less than 10, which was within the specified limits, for all three of the aforementioned criteria for milk flavor spirulina that was treated with UV light. Since the milk's microbiological burden was reduced after UV treatment, the product had a longer shelf life. Hence, sun exposure was one way to prolong the freshness of milk.

Table 6: Microbiological characteristics of spirulina flavored milk that has not been treated with UV light

Parameter		Total Plate Count (cfu/ml)	Yeast and Mould (cfu/ml)	Coliform (cfu/ml)
Results	Day 0	79×106	<10	12
	Day 7	79×107	<10	14×102
	Day 14	52×108	<10	22×103
	Day 21	40×109	<10	29×103
	Day 28	49×109	<10	32×103

The Total Plate Count and Coliform count grew till day 28 for Non-UV treated milk taste spirulina, but the values for yeast and mold remained steady at 10. The non-UV treated milk, on the other hand, had a shorter shelf life due to its greater bacteria count.

Table 7: Bacterial characteristics of milk-flavored lemongrass – UV

S. No.	Parameter	Results				
		Day 0	Day 7	Day 14	Day 21	Day 28
1	Total Plate Count (cfu/ml)	<10	<10	<10	<10	<10
2	Yeast and Mould (cfu/ml)	<10	<10	<10	<10	<10
3	Coliform (cfu/ml)	<10	<10	<10	<10	<10

Within the specified limitations, it was noted that the values of the three parameters mentioned earlier for the milk flavor lemongrass-Ultraviolet were less than 10. Consequently, the milk had a longer shelf life after being treated with UV light, which reduced the microbial burden. It follows that UV therapy was one way to lengthen the time milk could be kept.

Table 8: Microbiological characteristics of non-UV treated lemongrass milk

S. No.	Parameter	Results				
		Day 0	Day 7	Day 14	Day 21	Day 28
1	Total Plate Count (cfu/ml)	98×101	80×102	102×102	40×103	52×104
2	Yeast and Mould (cfu/ml)	<10	<10	<10	<10	<10
3	Coliform (cfu/ml)	<10	13×101	13×102	15×104	16×104

Total Plate Count and Coliform count both rose up to day 28 for milk taste lemongrass, however Yeast and Mold remained stable at 10 each. The non-UV treated milk, on the other hand, had a shorter shelf life due to its greater bacteria count.

Table 9: Tests for microbes in tulsi flavored milk- UV treated

S. No.	Parameter	Results				
		Day 0	Day 7	Day 14	Day 21	Day 28
1.	Total Plate Count (cfu/ml)	<10	<10	<10	<10	<10
2.	Yeast and Mould (cfu/ml)	<10	<10	<10	<10	<10
3.	Coliform (cfu/ml)	<10	<10	<10	<10	<10

The values for all three of the aforementioned criteria for milk flavor tulsi - Ultraviolet were found to be less than 10, falling within the specified range. Consequently, the milk had a longer shelf life after being treated with UV light, which reduced the microbial burden. Hence, exposing milk to ultraviolet light was one way to prolong its storage life.

Table 10: Bacterial count of tulsi flavored milk - non UV-treated

S. No.	Parameter	Results				
		Day 0	Day 7	Day 14	Day 21	Day 28
1	Total Plate Count (cfu/ml)	61×105	89×106	199×107	49×108	56×109
2	Yeast and Mould (cfu/ml)	<10	<10	<10	<10	<10
3	Coliform (cfu/ml)	<10	15×101	25×102	24×103	30×103

For milk-flavored tulsi, the Total Plate Count and Coliform count rose up to day 28, however the Yeast and Mold counts remained steady at 10. The non-UV treated milk, on the other hand, had a shorter shelf life due to its greater bacteria count.

Table 11: Microbiological characteristics of fennel flavored milk - UV treated

S. No.	Parameter	Results				
		Day 0	Day 7	Day 14	Day 21	Day 28
1	Total Plate Count(cfu/ml)	<10	<10	<10	<10	<10
2	Yeast and Mould(cfu/ml)	<10	<10	<10	<10	<10
3	Coliform (cfu/ml)	<10	<10	<10	<10	<10

The value of milk flavor fennel - Ultraviolet for all three of the aforementioned characteristics was less than 10, which was within the specified range. Consequently, the milk had a longer shelf life after being treated with UV light, which reduced the microbial burden. Hence, exposing milk to ultraviolet light was one way to prolong its storage life.

Table 12: Microbiological characteristics of fennel flavored milk- non-UV treated.

S. No.	Parameter	Test-Method	Results				
			Day 0	Day 7	Day 14	Day 21	Day 28
1	Total Plate Count (cfu/ml)	WAS 5402:2012 R.A.2018	66×105	95×106	72×107	97×108	91×109
2	Yeast and Mould (cfu/ml)	WAS 5403:1999 R.A.2018	<10	<10	<10	<10	<10
3	Coli-form (cfu/ml)	WAS: 5401 (P-1)2012 R.A.2018	16	20×101	27×102	<10	<10

Yeast and mold levels remained steady at a ten-value throughout the milk flavor fennel experiment; however, the total plate count rose until day 28 [29]. Up to the fourteenth day, the figure for Coliform continued to rise. Untreated milk showed a higher bacteria count than UV-treated milk. The non-UV treated milk, on the other hand, had a longer shelf life due to a greater microbiological count [30].

Conclusions

Findings from the research on the antioxidant and antibacterial effects of herbal-flavored milk shed light on the possible health advantages of adding certain herbs and fruits to dairy products. The results highlight the importance of these bioactive substances in enhancing antibacterial or antioxidant actions. To find the best formulations, further research on the synergistic effects in certain combinations is needed. There is hope for the future of functional or health-promoting foods with herbal flavors in herbal-flavored milk. To further understand these interactions and how they affect public health, future studies should dig farther. Only then will we be able to design dairy products that are both novel and nutrient dense.

Findings of the study

Normal sterilized milk had the greatest mean moisture and pH values when it came to milk flavour lemongrass, while the honey combined sample had the lowest. The samples of honey-mixed milk and conventional sterilized milk and UV-treated milk had the lowest titratable acidities, expressed as percentages of lactic acid. The honey-mixed sample had the greatest total solids, whereas the UV-treated sample had the lowest. When looking at milk-flavoured Tulsi, it was found that the sample including honey had the greatest mean moisture, whereas the one with regular sterilized milk had the lowest. Normal sterilized milk had the highest pH, while the honey blended sample had the lowest. The honey-mixed sample had the maximum titratable acidity, expressed as a percentage of lactic acid, whereas the UV-treated milk sample had the lowest.

The honey blended sample had the lowest total solids and regular sterilized milk the most. When it came to milk flavour fennel, the honey combined sample had the greatest mean moisture content, while the regular sterilized milk sample had the lowest. Normal sterilized milk had the highest pH, while the honey blended sample had the lowest.

The honey-mixed sample had the maximum titratable acidity, expressed as a percentage of lactic acid, whereas the UV-treated sample had the lowest. The honey blended sample had the lowest total solids, while conventional sterilized milk had the most. In terms of phenolic content (measured in milligrams per hundred millilitres), the milk-flavoured spirulina had the greatest value, while the non-UV treated samples of tulsi, fennel, and lemongrass had the lowest. For samples treated with UV light, fennel had the highest value, followed by spirulina, Tulsi, and lemongrass, which had the lowest value once again. All three parameters—total plate count, yeast and mould, and coliform-were found to be within limits when microbiological testing of the flavoured milk was conducted.

There is a favourable relationship between total phenolic content and scavenging activity. The measurement of phenolic components from grape pomace was improved by extracting it with ethanol. There was promising evidence that the grape varietals under consideration included bioactive substances with the potential to extend the shelf life of food and cosmetic items when used as natural antioxidants. No samples showed any discernible action against either Gram-negative or Gram-positive bacteria. Only the V1 and V7 extracts showed any antifungal action at all. There is a lot of promise for the use of grape marc in the food and pharmaceutical industries as a source of trans-resveratrol.

Scope for further research

The current investigation on the antioxidant and antibacterial effects of herbal-flavored milk provides a solid groundwork for further investigations in this area. Potentially fruitful avenues for future research include studying the synergistic effects of various herbs and fruits in order to determine the best combinations for maximum bioactivity. Research on the effects of herbal-flavoured milk on certain illnesses, including infections or those caused by oxidative stress, may also provide useful information. Investigating the bioavailability of these bioactive chemicals is an encouraging step toward understanding their mechanism of action in the body. To further guarantee the broad adoption of these health-enhancing drinks, it would be beneficial to examine customer perception and acceptability in order to shape product development plans.

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