

## Studies on Addition of Four o'Clock Flowers (*Mirabilis jalapa*) Extract on Physiochemical, Microbiological and Sensory Analysis Properties of Yoghurt

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### ABSTRACT

**Background:** Milk and other dairy products are the most stable which contains of minerals, vitamins and carbohydrates for all social groups and all age people. The additives of plants are multifunctional use in the dairy industry. Wild plants have the good property of vitamins, minerals, and some other biologically active substances. Because of these compounds, digestion has been improved.

**Materials and Methods:** This study was done in the food technology and nutrition department, School of Agriculture, Lovely Professional University, Phagwara, Punjab. *Mirabilis Jalapa* flowers have been gotten from Phagwara model town. The flowers collected at the evening, then were dried at the oven, and the second procedure is making powder of the flowers. Milk was collected from the local market then heat it and pasteurized for 5 minutes at 95 centigrade, cooling at 45 centigrade.

**Findings:** We observed that *Mirabilis Jalapa* flower extracts were not commonly integrated into the raw milk components. Various extract concentrations, ranging 0%, 2%, 4%, 6%, 8%, and 10%, respectively, were incorporated into raw milk, with a 5% lactobacillus culture, omitting the use of a control sample. After 24-hour fermentation period, the acidity levels of the samples ranged from 1.05% for the control to 1.45% for the 10% fortified yogurt. Over time, the pH values of the different extract concentrations exhibited a gradual decline. Moreover, with various concentrations of *Mirabilis Jalapa* extracts exhibited extended shelf lives, with durations of 24 days, 28 days, 32 days, and 34 days for extract concentrations of 2%, 4%, 6%, 8%, and 10%, respectively.

**Conclusion:** The experimental findings show that the fortified yogurt with *Mirabilis Jalapa*'s extract shelf life extended, adding more extracts means extending shelf life, whereas conventional yogurt exhibited a comparatively shorter shelf life of 23 days.

**Keywords:** Milk drinks, Plant extracts, *Mirabilis Jalapa*'s flowers, Yoghurt, Shelf life

### INTRODUCTION

*Mirabilis Jalapa* is one of these medicinal plants used for centuries for the treatment of various ailments; its leaves may be eaten cooked as well, but only as an emergency food. An edible crimson dye is obtained from the

flowers to color jellies and cakes. It is popularly known as four o'clock. It belongs to the family Nyctaginaceae. It is a large, herbaceous plant grown in gardens throughout India and Pakistan. The shape of the pollen grains of *Mirabilis Jalapa* is Spheroidal, oblate spheroidal, with a diameter ranging from 125-140  $\mu\text{m}$  and thickness of 10-15  $\mu\text{m}$ . Exine ornamentation is Spinulose; spinules 0.5-1  $\mu\text{m}$  high, randomly distributed, Aperture type Pantoporare with numbers ranging from 18-20 the diameter of aperture varies between 6.3-10  $\mu\text{m}$  while the membrane of the aperture is Margin ornate, membrane provided with spinulose and granules (Rozina, 2016).

The meaning of *mirabilis* in the Latin language is wonderful and the meaning of the *Xalapa* (*Jalapa*) is the capital of Veracruz which is in México. This is one of the crucial plant for multipurpose such as medicinal plant, used for the treatments of the wounds. This plant as well used for some other foods such as for the color of several cakes. This plant has been used for the dairy to get several results with shelf life. The posture will be prostrated many times. A big aspect of *mirabilis Jalapa* is that have flowers with different colors which grow in the same plant. The seed production is so high and rapid growth allows it to grow up from 30% to 50% in the plots of cane (Chetty et al., 2008). The aqueous as well ethanolic extract derived from the leaves of an indigenous medicinal plant *Mirabilis jalapa* (several flowered plants) were screened for antibacterial activities against *Staphylococcus aureus*.

An outstanding aspect of *Mirabilis jalapa* is that it has flowers with various colors and can be found simultaneously on the same plant. Moreover an individual flower can be splashed with different colors. Flower patterns are referred to as flakes (stripes of varying length), sectors (whole sections of flower) and spots. *Mirabilis Jalapa* introduces from tropical South America, but the tropical has naturalized, and also subtropical and temperate regions are included in it. *Mirabilis jalapa* usually grows 0.6-0.9m tall and just as wide. Leaves are pointed; flowers usually open from late afternoon onwards, hence the first of its common names. Flowers in group of three, flowers with five green bracelets; surround the perianth; usually yellow; crimson, white or variegated and opening in the evening (Rozina, 2016).

In cooler subtropical and temperate regions, when the weather becomes cool it will cool down it will die first frosts (especially after it fully matures stages and finished itself-seeding), and it will live or re-growing in the following spring from the tuberous roots. It is proved by several experiments that *Mirabilis Jalapa*'s extracts have moderate to potent antioxidant activity. *Mirabilis Jalapa* is a native plant of dry tropical regions of Central America and South America: Guatemala, Mexico, Chile and Peru are the places where *mirabilis Jalapa* has adapted to the environment.

It is now naturalized in many countries in Asia, some in Africa, some of the countries in the United States, the Middle East and Europe (Khurian, 2003). The extract of *Mirabilis Jalapa* flowers is not used in the yoghurt, but none of the previous studies have defined the extraction methods on yoghurt. The objectives of this study was to extract and efficiency of different extraction methods on plant extract and to standardize the concentration of plant extract in the cultured milk product after plant extract addition.

## MATERIALS AND METHODS

### *Preparation of Mirabilis Jalapa extract*

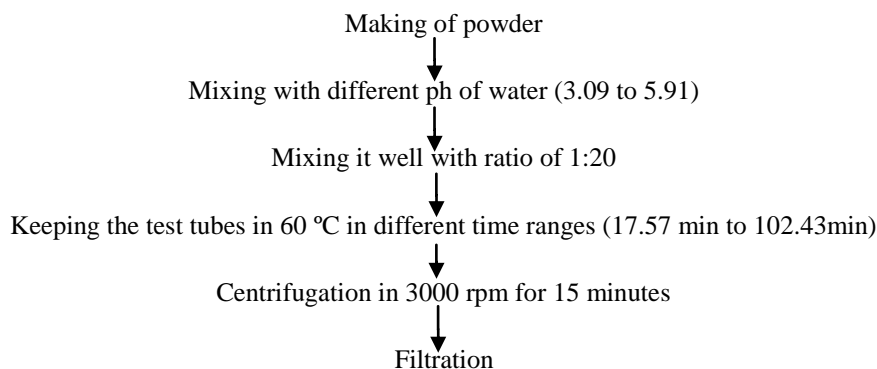
Flowers collected at the evening, the flowers were dried at the oven, and the second procedure is making powder of the flowers. Cleaning of extra pills from the powder. After the powder we made the different solutions and pH of water varies from 3 to 5. Mixing of the powder with different solution of 1:20, keeping all

solutions for different time ranges (17.57 h to 102 h). Centrifuged at 3000 ppm for 15 minutes. Centrifuged it adding of different solution vary from 0%, 2%, 4%, 6%, 8%, and 10%, respectively.

#### **Preparation of the yoghurt**

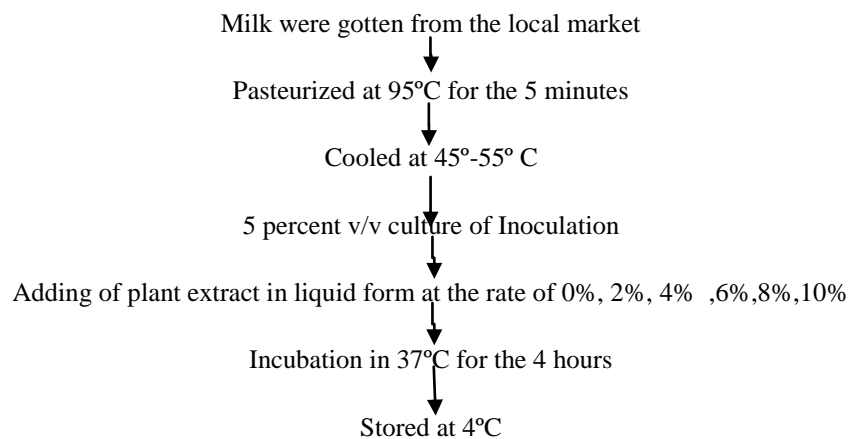
This study was done in the Food Technology and Nutrition department, School of Agriculture, Lovely Professional University, Phagwara, Punjab in the years of 2018-2020. *Mirabilis Jalapa* flowers have been gotten from Phagwara model town. Milk was collected from the local market than heat it and pasteurized for 5 minutes at 95 centigrade, cooling at 45 centigrade. An antioxidant comparative study, DPPH, ABTS, water activity, pH, CFU, Phenol, Flavonoid, Protein, FRAP, Lactic acid, Beta-carotene and shelf life. Flowers of the *Mirabilis Jalapa* were collected and separated from another part then kept in a tray dryer for 6 hours at 55 centigrade. The flower crashed in the grinder till the physical parts change to the powder form. The extraction was done by centrifuge with different pH of water.

#### **Flow chart for extraction**



Powder of *Mirabilis Jalapa's* flowers was mixed in the ratio of 1:20 or 1 gram of powder mixed with 20 ml of water different ph were used for the mixed water (3.09, 3.5, 4.5, 5.5, 5.91) respectively, there were 13 treatments for choosing the best result of extract for using it in cultured milk product. Test tubes were kept in different time ranges at 60 degrees of Celsius (17.57 min, 30 min, 60 min, 90 min, and 102.43 min), respectively.

#### **Preparation of *Mirabilis Jalapa* enriched yogurt**



**Microbiological counts and physically examination**

The microbiological counts were done for colony forming unit different culture was made for 0 to 10 % and varied to different days. The shelf life was extended to 34 days. The 10% extract used were the most influence for the shelf life. However, physical examination was done base on texture, color, sensory analysis.

**Chemical examination and sensory evaluation**

Different chemical tests were such as, (Acidic range) with PH, beta- carotene, ABTS, DPPT, FRAP, Total polyphenol, flavonoids, water activity, colony forming units. Acidic test was done by different pH meters, beta carotene tests and all others test were done by the standards tests. But the sensory evaluation was done by the different senses. All of the concentration was looked by the seven professors, and the result was evaluated great for all of them.

**Statistical analysis**

The data generated from this experiment were entered, organized and processed for further analysis into Microsoft Excel Worksheet. Means and percentages were calculated through SPSS software 16 version.

**RESULTS**

The acidity range of Yoghurt starts from the first hour, because of fermentation which is occurring by lactic acid bacteria. Acidity is increasing as time passes more acidity will occur. Yoghurt acidity was 0.14 % in the initial step because there is low activity of bacteria and the acidity of 10% fortified Yoghurt was 0.13% at the initial step. After 24 hours the acidity range of the sample was 1.05% and 10 % fortified Yoghurt has an acidity range of 1.45% (Table 1).

**Table.1:** Acidic range of fortified Yoghurt with Mirabilis Jalapa extract in different time ranges

Concentrations	0 hr	After 1 hr	After 2 hrs	After 3 hrs	After 24 hrs
0%	0.14%	0.15%	0.19%	0.27%	1.05%
2%	0.13%	0.15%	0.18%	0.28%	1.23%
4%	0.14%	0.15%	0.18%	0.24%	1.42%
6%	0.13%	0.14%	0.17%	0.23%	1.42%
8%	0.13%	0.15%	0.18%	0.23%	1.43%
10%	0.13%	0.14%	0.17%	0.24%	1.45%

The pH of normal yoghurt at the first hour without adding any extract was found to be  $6.46 \pm 0.56$  because of less acidity and near neutrality. The pH range of different concentrations was decreased in excess of time; the pH of 10% fortified Yoghurt was  $6.12 \pm 0.56$  in the first hour. After 24 hours the pH range changed for the sample, it became  $4.4 \pm 0.56$  and for 10 % it became  $4.09 \pm 0.56$  (Table 2).

**Table 2:** PH range of fortified Yoghurt with Mirabilis Jalapa extract in different time ranges

Concentrations	Aft 1hr	Aft 2 hr	Aft 3 hr	Aft 4 hr	Aft 5 hr	Aft 24 hrs
0%	$6.46 \pm 0.56$	$6.3 \pm 0.65$	$6.28 \pm 0.55$	$6.09 \pm 0.62$	$5.2 \pm 0.56$	$4.4 \pm 0.56$
2%	$6.42 \pm 0.59$	$6.28 \pm 0.64$	$6.2 \pm 0.58$	$6.05 \pm 0.65$	$5.02 \pm 0.64$	$4.3 \pm 0.65$
4%	$6.31 \pm 0.62$	$6.18 \pm 0.56$	$6.19 \pm 0.65$	$5.98 \pm 0.55$	$5.01 \pm 0.56$	$4.27 \pm 0.62$
6%	$6.32 \pm 0.56$	$6.17 \pm 0.64$	$6.1 \pm 0.56$	$5.88 \pm 0.64$	$5.0 \pm 0.65$	$4.2 \pm 0.56$
8%	$6.3 \pm 0.61$	$6.1 \pm 0.64$	$6.1 \pm 0.58$	$5.72 \pm 0.56$	$4.92 \pm 0.62$	$4.15 \pm 0.64$
10%	$6.12 \pm 0.56$	$6.1 \pm 0.58$	$6.0 \pm 0.55$	$5.6 \pm 0.55$	$4.89 \pm 0.56$	$4.09 \pm 0.56$

The beta-carotene is present in very low amounts in Yoghurt and Mirabilis Jalapa. In the sample the range of beta carotene is 0.01 mg/gr it varies in different concentrations from 0.01 mg/gr (0 %) to 0.24 mg/gr (10%) (Table 3).

**Table 3:** Beta-carotene present in fortified Yoghurt with Mirabilis Jalapa extract

Concentrations	mg/100 gr
0%	0.01
2%	0.01
4%	0.1
6%	0.13
8%	0.21
10%	0.24

Table 4 shows the ABTS and DPPH contents in different concentrations such as 0, 2,4,6,8 and 10 per cent. These two content show the different properties in Yoghurt and several other foods, the ABTS started from  $75.74 \pm 0.51\%$  and reached  $74.6 \pm 0.59\%$ . The DPPH shows  $52.7 \pm 0.49\%$  to  $74.42 \pm 0.41\%$ . It was 6 samples and has shown different readings of different properties.

**Table 4:** ABTS and DPPH content of fortified Yoghurt with Mirabilis jalap extract

Concentration	ABTS assay %	DPPH %	Reducing Power %
0%	$75.74 \pm 0.51$	$52.70 \pm 0.49$	$28.36 \pm 0.65$
2%	$63.30 \pm 0.47$	$60.03 \pm 0.57$	$28.92 \pm 0.54$
4%	$64.78 \pm 0.54$	$60.92 \pm 0.42$	$29.25 \pm 0.52$
6%	$70.40 \pm 0.35$	$70.33 \pm 0.29$	$30.01 \pm 0.47$
8%	$70.40 \pm 0.42$	$73.00 \pm 0.35$	$30.36 \pm 0.39$
10%	$74.60 \pm 0.59$	$74.42 \pm 0.41$	$30.88 \pm 0.42$

Phenol and flavonoids are present in the above range; it varies from  $17.32 \pm 0.74$  GAE/g to  $58.23 \pm 0.69$  GAE/g. As more extract of Mirabilis Jalapa, the phenol content will be increased because Mirabilis has the potential capacity of phenol. The total flavonoids can be estimated by the estimation of CE/g it varies from  $119.34 \pm 0.61$  CE/g to  $160.30 \pm 0.41$  CE/g. The 10 % of Mirabilis Jalapa extract used in Yoghurt has a more amount of the total flavonoids (Table 5).

**Table 5:** Total polyphenol and flavonoids present in fortified Yoghurt with Mirabilis Jalapa

Concentrations	Total polyphenol (mg GAE/g)	Total flavonoids(mg CE/g)
0%	$17.32 \pm 0.74$	$119.34 \pm 0.61$
2%	$25.25 \pm 0.61$	$124.23 \pm 0.41$
4%	$38.34 \pm 0.51$	$136.51 \pm 0.47$
6%	$43.23 \pm 0.59$	$145.12 \pm 0.39$
8%	$48.05 \pm 0.64$	$152.21 \pm 0.54$
10%	$58.23 \pm 0.69$	$160.30 \pm 0.41$

The water activity of Yoghurt is one of the important activities to show how soon bacteria attack the Yoghurt and make it spoil. More concentration has different water activity. The water activity of the sample and whole fortified Yoghurt is near the range of 1. The temperature affects the water activity, if someone gets the reading of samples the entire temperature of the room should be 25 centigrade. In this experiment, the temperature was about  $11^{\circ}\text{C}$  and  $12^{\circ}\text{C}$  (Table 6).

**Table 6:** water activity of fortified Yoghurt with Mirabilis Jalapa extract with different temperatures

Concentrations	Water activity	Temperature °C
0%	1.004	11.2
2%	1.003	12.1
4%	0.996	11.3
6%	1.001	12
8%	1.002	11.7
10%	0.999	11.5

Shelf life is one of the important factors in all raw and processed foods. The shelf life of the Yoghurt and fortified Yoghurt is different each concentration has its own shelf life. The shelf life of the sample Yoghurt was 23 days in 4 centigrade, but the shelf life of other concentrations were found to be 24 days, 28 days, 32 days and 34days, respectively (Table 7).

**Table 7:** Shelf life of fortified Yoghurt with Mirabilis Jalapa extract stored in 4 °C

Concentrations	Shelf life
0%	23 days
2%	24 days
4%	24 days
6%	28 days
8%	32 days
10%	34 days

The colony-forming unit (CFU) in Yoghurt depends on the culture of the bacteria. Lactic acid bacteria (LAB) affected more on yoghurt and with time passing the range of lab increased. CFU had different effect at different times, in the first week created a few colonies but in the fourth week, the colonies raised up. The colonies in the first week were  $36 \times 10^6$  and 4th week were found to be  $59 \times 10^6$  but it is different in each concentration (Table 8).

**Table 8:** CFU/ml (colony forming units) for fortified Yoghurt with Mirabilis Jalapa extract by different times.

Concentration	First week	10th day	Second week	19th day	Third week	Fourth week
0%	$36 \times 10^6$	$39 \times 10^6$	$43 \times 10^6$	$46 \times 10^6$	$54 \times 10^6$	$59 \times 10^6$
2%	$34 \times 10^6$	$35 \times 10^6$	$41 \times 10^6$	$45 \times 10^6$	$48 \times 10^6$	$53 \times 10^6$
4%	$28 \times 10^6$	$32 \times 10^6$	$36 \times 10^6$	$39 \times 10^6$	$48 \times 10^6$	$49 \times 10^6$
6%	$24 \times 10^6$	$26 \times 10^6$	$34 \times 10^6$	$35 \times 10^6$	$42 \times 10^6$	$45 \times 10^6$
8%	$23 \times 10^6$	$25 \times 10^6$	$33 \times 10^6$	$35 \times 10^6$	$41 \times 10^6$	$43 \times 10^6$
10%	$22 \times 10^6$	$26 \times 10^6$	$35 \times 10^6$	$36 \times 10^6$	$40 \times 10^6$	$42 \times 10^6$

Table 9 shows the protein content present in the Yoghurt and Mirabilis jalapa extract. The protein of normal Yoghurt without any fortification is 3.62 mg/100 gr and the other 5 concentrations are  $3.66 \pm 0.67$ mg/100 gr,  $3.69 \pm 0.59$ Mg/100 gr,  $3.72 \pm 0.55$ mg/100 gr,  $3.88 \pm 0.51$ mg/100 gr,  $3.93 \pm 0.61$ mg/100 gr. The Mirabilis Jalapa extract doesn't have more content of protein.

**Table 9:** protein content in fortified Yoghurt with *Mirabilis Jalapa* extract

Concentrations	mg/100 gm
0%	3.62 ± 0.74
2%	3.66 ± 0.67
4%	3.69 ± 0.59
6%	3.72 ± 0.55
8%	3.88 ± 0.51
10%	3.93 ± 0.61

Foods have the most important factor which is known as sensory analysis it is been checked by 6 faculty of the food science and nutrition department it is shown in table 9 and marked as above for all points of color, taste, texture, appearance, aroma, mouth feel, overall acceptance. All marks are varying from 7.5 to 8.75 (Table 10).

**Table 10:** Sensory analysis of fortified Yoghurt with *Mirabilis Jalapa* extract

Concentration	Color	Taste	Texture	Appearance	Aroma	Mouth feel	Overall acceptance
0%	8.0 ± 0.75	8.08 ± 0.89	8 ± 0.91	8.75 ± 0.71	7.5 ± 0.75	8.25 ± 0.91	8.5 ± 0.82
2%	8.25 ± 0.71	8.16 ± 0.82	8 ± 0.84	8.66 ± 0.78	8 ± 0.47	8 ± 0.84	8.00 ± 0.74
4%	8.41 ± 0.85	8.33 ± 0.79	8.25 ± 0.79	8 ± 0.59	8.25 ± 0.74	7.5 ± 0.75	8.00 ± 0.75
6%	8.66 ± 0.74	8.25 ± 0.87	8.66 ± 0.74	8 ± 0.69	7.5 ± 0.69	7.5 ± 0.66	8.08 ± 0.73
8%	8.66 ± 0.67	8.25 ± 0.74	8.08 ± 0.69	8.33 ± 0.56	7.5 ± 0.57	8.5 ± 0.71	8.33 ± 0.66
10%	8.75 ± 0.61	8.41 ± 0.69	8 ± 0.70	8.08 ± 0.54	8 ± 0.49	8 ± 0.81	8.00 ± 0.64

## DISCUSSION

The experiment revealed that the Yoghurt acidity is 0.14 % in the initial step because there is low activity of bacteria and acidity of 10% fortified Yoghurt is 0.13% at the initial step. After 24 hours the acidity range of the sample is 1.05% and 10 % fortified Yoghurt has an acidity range of 1.45%. The findings correlate with a finding by (Gahrue et al., 2015). The presence of a high amount of tannins in the extract of *mirabilis Jalapa* showed the potential antibacterial and antiviral properties of the plant (Liya et al., 2020), who reported that the extracts of *Mirabilis Jalapa* reduced the multiplication of Tobacco mosaic virus (TMV) by 50% when added to the inoculums (Kumar et al., 2011).

Two phenolic compounds were found to show the antifungal activity against candida albicans (antioxidant and antimicrobial activities of various solvent extracts from *Mirabilis Jalapa* tubers by the findings of (Abdelkhalek et al., 2020). It is proved by several experiments that *Mirabilis Jalapa's* extracts have moderate to potent antioxidant activity reported by (Yang et al., 2001). *Mirabilis Jalapa's* flowers' methanolic extract contains several compounds showing spasmolytic activity such as some routines, sit sterol-d-glycosides and ursolic acid. Although, some additional studies are needed to explain the mode of action of *Mirabilis Jalapa's* extract (Akiyama et al., 2001). The root of *Mirabilis Jalapa's* is known as ethanol extracts and has anti-diabetic activity. Oral administration of the *Mirabilis Jalapa L.* (10mg/kg and 20mg/kg) is the ethanoic extract of root. Streptozotocin can induce diabetic rats for 12 days and mice for 28 days (Sunilson et al., 2008).

The yoghurt which is fortified by 10 % of extract has more antimicrobial activity then sample or 2 %. The antioxidant activity is increasing by the addition of more extracts of *Mirabilis Jalapa*. Shelf life is about 33 days

for 8 and 10 percent of fortified yoghurt but normal yoghurt has the shelf life of 23 days. As time get pass the acidity is increasing because of the activity of the bacteria. In first hours ph is high but after 24 hours it became less and it will have high acidity. CFU is not high in first time it is increasing after one week. it is observed that *Mirabilis Jalapa* has high antioxidant property and 10 % of fortified yoghurt has high antioxidant activity in comparing with 0 %, 2% , 4 %, respectively. I can state the *Mirabilis Jalapa* is one of the most crucial medicinal plants for the new research as I describe about its different properties, all researchers can use the different parts of this medicinal plant.

## CONCLUSION

The present study focuses on the addition of *Mirabilis Jalapa* extract on the physicochemical, microbiological and sensory analysis property of yoghurt. The experimental findings show that the fortified yogurt with *Mirabilis Jalapa's* extract shelf life extended, adding more extracts means extending shelf life, whereas conventional yogurt exhibited a comparatively shorter shelf life of 23 days.

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