The Pharma Innovation Journal 2019; 8(10): 36-39

www.ThePharmaJournal.com

The Pharma Innovation

**ISSN (E): 2277- 7695**
**ISSN (P): 2349-8242**
**NAAS Rating: 5.03**
TPI 2019; 8(10): 36-39
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www.thepharmajournal.com
Received: 23-08-2019
Accepted: 24-09-2019

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# Effect of wheatgrass (*Triticum aestivum*) on bacterial count of Lassi

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**Abstract**
In this study, the attempts have been made to prepared lassi by utilizing wheat grass in different proportion and study the bacterial count of *lassi*. The level of wheatgrass extract was optimized on the microbial test such as total aerobic bacterial count, *E. coli*, *Salmonella* Spp. And *staphylococcus aureus*, were evaluated based on bacterial culture and wheatgrass was not contaminated with micro-organism. The data indicated that wheatgrass had proved to indicate the better quality for consumer health.

**Keywords:** Wheatgrass, Lassi, Microbial count

## 1. Introduction
Present era is the time of excellence in every sector of life from simple to complex and vice-versa. Every field changing tremendously due to the increasing scientific attitudes, modernization with industrialization and its impact on lifestyle could not be ignore in each field. The eating and selecting habit of food show so much variation due to the diversification in tradition and culture, purchasing power of consumers, need of lifestyle and specialty of food. The expectation from food are not only to furnish the essential nutrients required for the normal body growth but to impart the curative properties against different disorders or diseases observed normally in human beings. Such type of innovative foods claiming functional, health beneficial and somewhat medicinal are coming in market by different food companies example pro-biotic yoghurt, pro-biotic dahi/lassi, Arjuna ghee, low cholesterol ghee etc. Milk is one such carrier that has been effectively used to deliver claiming agent for targeted health benefits in the traditional Indian system as well as medical science. There are evidences to suggest that addition of certain herbs into milk products increased antioxidative stability, heat stability, alcohol stability. The application of herb as medicine are practice science civilization and popular in Ayurveda as jadi butti but as the development of scientific attitude and increasing curiosity its, application in dairy may result in raising their nutritional and medicinal values and enable development of value-added dairy products. Likewise, wheatgrass also having medicinal and nutritional properties being helpful for different ways is trying here for development of herbal lassi.
Wheatgrass is a food prepared from the cotyledons of the common wheat plant (*Triticum astivum*) Belonging to a family Gramineae. *Triticum* is genus of annual and biennial grasses yielding various types of wheat and is cultivated almost all over the world shoot of *Triticum aestivum* is called wheat grass.

## 2. Material and Methods
### 2.1 Materials
The following materials were used for the present investigation.

### 2.2 Collection of buffalo milk and wheat
Fresh and standardized buffalo milk for fat 6 percent and SNF 9 percent was procured from Natural Milk Pvt. Ltd., Latur. The wheat seed was purchased from the local market of Latur city of verity (2189).

### 2.3 Microbial cultures
The standard mixed dahi culture i.e. Standard dahi, contained *streptococcus thermophilus* and *lactococcus lactis* NCDC-167 (BD4) in this study was procured from the National collection of Dairy culture (NCDC), NDRI, Karnal.

### 2.4 Chemicals
Analytical (AR) or guaranteed grade (GR) reagents were used in the chemical analysis.

### 2.5 Packaging material
Plastic glasses were used for serving the developed *lassi* for sensory study and packaging was done in plastic bottles.

### 2.6 Equipment and accessories
Stainless steel vessels of requisite capacity, Muslin cloth, and standard weight balance, thermometer, gas shegdi, Mixture (HERO Mixture, 550 WATTS) etc. were used for preparation of dahi. Before using this material it was properly cleaned and washed with detergent solution. All the precautionary measures were considered during the conduct of trials to avoid contamination.

## 3. Methods
The following methods were followed for the preparation of wheatgrass extract, lassi and for evaluation of physico-chemical properties, sensory evaluation and microbial analysis of developed lasso from buffalo milk and wheatgrass extract in the present investigation.

### 3.1 Preparation of wheat grass
Wheatgrass was cultivated in laboratories from the local verity (2189) of wheat by sorting, cleaning and after overnight socking of wheat seed in tray, the sprouted seed was grown in soil as per shown in follow diagram. Wheatgrass was harvested after 7 to 10 days of old and used for extract preparation as per the method described by (Kumar, 2017 and Patel 2012) [9, 7].

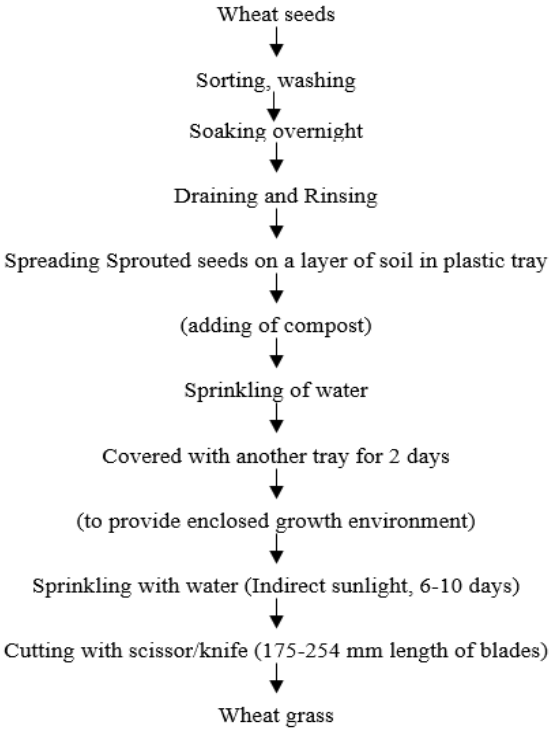


**Fig 1:** Flow Diagram 1: Method of growing wheat grass (*Triticum aestivum*) by (Kumar, 2017 and Patel 2012) [9, 7].

### 3.2 Preparation of wheat grass (*Triticum aestivum*) extract
Wheatgrass extract was prepared from the wheat grass cultivated in laboratory as per the method of Patel, 2012 [7] as shown in following flow diagram.

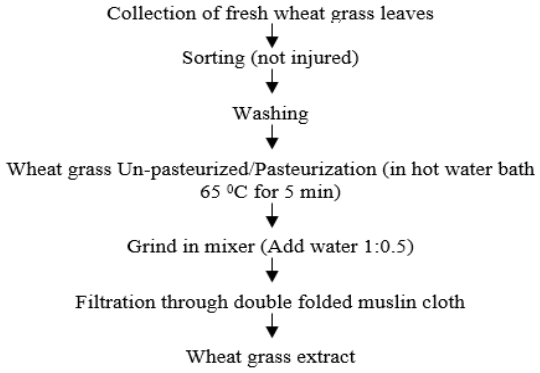


**Fig 2:** Flow Diagram 2: Preparation of wheat grass (*Triticum aestivum*) extract (Patel, 2012) [7]

### 3.3 Procedure
Wheatgrass extract was prepared from the wheat grass cultivated in laboratory as shown in flow chart. Wheatgrass extract was prepared by extracting the harvested grass in mixture (HERO Mixture, 550 WATTS) by using 0.5 percent water for easy and complete extraction of solid grass through following the steps sorting wheat grass, washing, grinding and filtration.

### 3.4 Treatment combinations
For preparation of herbal *lassi* by using *triticum aestivum* extract, the treatment combinations were finalized on weight basis as per follows:

$T_1$-100 Parts of curd
$T_2$-95 Parts of curd + 5 Parts of Wheat Grass extract
$T_3$-90 Parts of curd + 10 Parts of Wheat Grass extract
$T_4$-85 Parts of curd + 15 Parts of Wheat Grass extract

### 3.5 Preparation of herbal (*Triticum aestivum*) *lassi*
Wheat grass (*Triticum aestivum*) extract added herbal *lassi* was prepared as per the method of (Aneja and Mathur, 2002) [1] with slight modification and one stage of wheatgrass extract addition as shown in following flow diagram No.3.

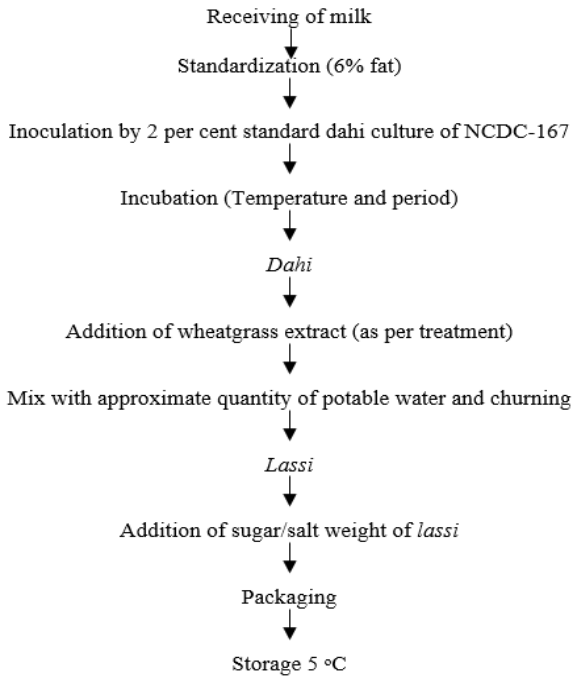


**Fig 3:** Flow Diagram 3: Preparation of wheat grass (*Triticum aestivum*) extract added herbal *lassi*. (Aneja and Mathur, 2002) [1]

### 3.6 Procedure
For preparation of dahi from buffalo milk using wheatgrass extract, standardized buffalo milk containing 6 percent fat and 9 percent SNF milk was pasteurized 65 °C for 5minute. After pasteurization cooling of milk 37 °C. After cooling the standard culture NCDC-167 was added in milk @ 2 percent and incubated at 37 °C for 10 hrs. The wheat grass extract was added after formation of *dahi* as per treatment. After that equal quantity of potable water was added and churned it by using churner. Then 15 percent sugar was mixed in it. The prepared *lassi* was packed in plastic bottles and stored at 5 °C until further study.

## 4. Results and Discussion
### 4.1 Bacterial count of selected treatments of fresh lassi
The fresh product prepared was subjected to microbiological analysis with respect to *lactobacillus* count, *lactococcus* count, yeast and mould count and coliform count. The microbial load of fresh product were estimated and expressed in $\log_{10}$ (cfu/gm) in forthcoming tables.

### 4.2 *Lactobacillus* count
The *lactobacillus* count of lassi prepared from *wheatgrass* extract mixed with buffalo milk was estimated at $10^{-4}$ dilution as per the method of pour plate, by Tharmaraj and Shah (2003) is tabulated in table 1.

**Table 1:** Lactobacillus count for wheatgrass lassi [$\log_{10}$ (cfu/gm)]

| Treatment \ Replication | R-I | R-II | R-III | R-IV | Mean |
|---|---|---|---|---|---|
| $T_1$ | 2.30 | 2.28 | 2.28 | 2.31 | 2.29[a] |
| $T_2$ | 2.25 | 2.34 | 2.36 | 2.28 | 2.31[b] |
| $T_3$ | 2.20 | 2.18 | 2.18 | 2.19 | 2.19[b] |
| $T_4$ | 2.32 | 2.30 | 2.29 | 2.28 | 2.30[bc] |
| S.E. ± 0.014 | | | C.D. at 5% 0.043 | | |

The values with different small letters superscripts row wise differ significantly at 5 percent level of significance.

From the table 1, it was observed that the average *lactobacillus* count of fresh sample of *wheatgrass lassi* were found to be 2.29, 2.31, 2.19 and 2.30 cfu per g for treatments $T_1$, $T_2$, $T_3$ and $T_4$, respectively. All treatments were found *lactobacillus* count as sufficient proportion and not so much variation observed between them. The inhibitory action of (*Triticum aestivum*) for the growth of lactobacilli was not observed in extract added treatments might be due to the less quantity of extract and supportive role of milk solid particularly milk lacose. But all samples ensured claim of health benefits by fulfilled the criteria of suggested minimum number of more than $10^4$cfu/g at the time of consumption.

### 4.3 *Lactococcus* count
For the preparation of dahi, standard dahi culture was used which contains lactobacilli and *lactococcus* spp. Therefore, the *lactococcus* count in developed lassi had been checked and is tabulated in table 2.

**Table 2:** *Lactococcus* count for wheatgrass lassi [$\log_{10}$ (cfu/gm.)]

| Treatment \ Replication | R-I | R-II | R-III | R-IV | Mean |
|---|---|---|---|---|---|
| $T_1$ | 2.29 | 2.34 | 2.34 | 2.31 | 2.32[a] |
| $T_2$ | 2.28 | 2.32 | 2.32 | 2.33 | 2.31[ab] |
| $T_3$ | 2.25 | 2.30 | 2.29 | 2.25 | 2.27[b] |
| $T_4$ | 2.22 | 2.28 | 2.28 | 2.30 | 2.27[b] |
| S.E. ± 0.013 | | | C.D. at 5% 0.042 | | |

The values with different small letters superscripts row wise differ significantly at 5 percent level of significance.

From the table 2 it was observed that the average *lactococcus* count of fresh sample of wheatgrass *lass* were found to be 2.32, 2.31, 2.27 and 2.27cfu per g for treatments $T_1$, $T_2$, $T_3$and $T_4$, respectively. When the *lactococcus* count was compared with *lactobacillus* count, it was observed that both bacilli and cocci were found near about in equal proportion in lassi. The treatments $T_1$ and $T_2$ at par with each other. The results recorded in the present investigation are compared with the present findings.

### 4.4 Coliform count
Table 3 indicates the coliform count of lassi prepared from wheatgrass extract and buffalo milk.

**Table 3:** Coliform count of fresh wheatgrass lassi (cfu per gm)

| Treatment \ Replication | R-I | R-II | R-III | R-IV | Mean |
|---|---|---|---|---|---|
| $T_1$ | 0.00 | 1.00 | 0.00 | 0.00 | 0.25[a] |
| $T_2$ | 0.00 | 0.00 | 2.00 | 0.00 | 0.50[b] |
| $T_3$ | 0.00 | 1.00 | 0.00 | 0.00 | 0.25[a] |
| $T_4$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00[c] |
| S.E. ± 0.306 | | | C.D. at 5% 0.943 | | |

The values with different small letters superscripts row wise differ significantly at 5 percent level of significance.

The Table 3 shows the coliform count of *wheatgrass lassi*. The range for coliform found in between 1 to 5 cfu per g. The higher count for coliform was observed in treatment $T_2$ (0.50 and lower in treatment $T_4$ (0.00cfu per gm). All the treatment was significantly different from each other. It was seen that the coliform count remained within the limit (maximum 100 c.f.u. /gm) prescribed by IS: 9617 (1880) indicated that all treatments were prepared at hygienic condition and suitable for consumption.

### 4.5 Yeast and Mould count
Table 4 indicates the yeast and mould count of *lassi* prepared from wheat grass extract blended with buffalo milk.
The Table 4 shows the yeast and mould count of fresh *wheatgrass lassi*. The yeast and mould was found in control treatment only which had count 01 cfu/gm. in each. The mixed *lassi* was blank for yeast and mould. It was seen that the yeast and mould count remained within the limit (maximum 100 c.f.u. /gm.) prescribed by IS: 9617 (1880). All treatments were prepared at hygienic condition and at par to each other.

**Table 4:** Yeast and mould count for fresh wheatgrass lassi (cfu per gm.)

| Treatment \ Replication | R-I | R-II | R-III | R-IV | Mean |
|---|---|---|---|---|---|
| $T_1$ | 1.00 | 2.00 | 1.00 | 0.00 | 1.00[a] |
| $T_2$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00[b] |
| $T_3$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00[b] |
| $T_4$ | 0.00 | 0.00 | 0.00 | 0.00 | 0.00[b] |
| S.E. ± 0.20 | | | C.D. at 5% 0.62 | | |

The values with different small letters superscripts row wise differ significantly at 5 percent level of significance.

## 5. Conclusion

Consumers can easily acquire pathogenic microorganism by consuming contaminated products. The good handling must be carried out starting from raw materials to finished products. The results we found from our study were there was no abnormal growth of total bacterial count and yeast and mould count in wheatgrass. The resultant finding indicated that the wheatgrass were processed under hygienic observance.

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