Research Article

MICROBIOLOGICAL EVALUATION OF TAKRA BHEDA (BUTTERMILK) MENTIONED IN BHAVA PRAKASHA

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ABSTRACT

The aim and objectives of this study is the emerging concepts of probiotics proved buttermilk and curd as good probiotics. Today Scientists are developing genetically engineered probiotics which is of high market value. Takra which is explained as byproduct of ksheera (milk) is having Deepana (digestive) Pachana (metabolic capacities) and Shroto shodhana (cleansing properties). Many researches on buttermilk, its probiotic effect have been dealt with but the ayurvedic aspect of buttermilk is not taken for any research yet. Classification of takra is based on dilution factor which includes Ghola, Mathita, Takra, Udasvith and Cacchika is taken for the study. The study was planned to validate, microbiological aspect of takra bheda. Total 5 samples of five different variety of Takra were taken for the study, which was prepared under standard condition. Microbial analysis includes total *Lactobacillus* Count of Takra bheda explained in Bhāvaprakāśa, Total Microbial Load and Total *Lactobacillus* Count in Intestinal swab taken from the Wistar albino rats fed with different Takra bheda. The microbiological evaluation was done by standard Plate Count Method. One way ANOVA post hoc Tukey Kramer is in stat graph pad software. It is observed that Takra showed increased *lactobacillus* count followed by Cacchika. Total microbial load in the intestinal swab was for rat fed with Takra variety followed by Mathita. Total *lactobacillus* count in the intestinal swab was more for rat fed with Mathita variety followed by takra variety.

Keywords: Takra bhedās, Ghola, Mathita, Takra, Cacchika, *Lactobaccilus* count, Total microbial load, microbiological study.

INTRODUCTION

The digestive system of human beings is having trillions of microorganisms which colonizes the gut and forms the gut micro flora.[1] There are approximately two kilograms of microorganisms in the gut of a healthy human being which is existed in a balanced and organized manner. They play an important role in the healthy survival of the human being. Human body contains beneficial flora, opportunistic flora and transitional flora all together constitute the total microbial load. The microorganisms which protects out gut are called beneficial bacteria which include members of *Bifidobacteria* and *Lactobacteria* (*L. acidophilus*, *L. rhamnosus*)[2]

In our classics there is no specification of the term probiotics, but the functional aspect of probiotics is explained in the context of Takra varga. Ayurveda has a unique concept of Buttermilk, where it classified Takra into different types. One such classification is based on dilution factor. No researches have been conducted yet in this area. This is a preliminary research which aims to find the difference in total *Lactobacillus* count and Total microbial load in Takra bheda mentioned in Bhavaprakasha[3].

MATERIAL AND METHODS

Study settings were college of Dairy science and technology Mannuthy, SDM Centre for Research in Ayurveda and Allied Science, Udupi, V.P.S.V Ayurveda College Kottakkal.

Animal species for study: Wistar albino rats (150-250) of either sex from animal house attached to Pharmacology laboratory, SDM Centre for Research in Ayurveda and Allied Sciences, Udupi, Karnataka. Females were nulliparous and non-pregnant. All the animal experiments were carried out in Pharmacology Laboratory, SDM Centre for Research in Ayurveda in Allied Science after the prior permission of Institutional Ethics Committee. The experimental protocol was approved by the Institutional Ethics Committee of SDM Centre for Research in Ayurveda and Allied Sciences, SDM College of Ayurveda Campus, Kuthpady, Udupi on 14/03/2017. Approval No: SDMCRA/IAEC/CPCSEA/KT-05, after exemption of research proposal by IEC, V.P.S.V. Ayurveda College, Kottakkal, as per proceedings of NO: IEC/CI/05/16; dated 28-04-2016.

Animals were acclimated to laboratory environment for seven days prior to the onset of experiment. Total 3 animals of either sex with an average weight between 150 and 250 mg were selected and grouped as one and they were kept in separate metabolic cages after proper labelling. Like these 18 rats were randomly selected and divided into 5 groups. Dose calculation was based on standard reference Paget's and Burn's table (1964). Wistar Albino rats: Human Dose x 0.0118 x 5 = 'X' g/kg x Animal body weight. And was fixed as 30 ml. Fixed doze of sample was administered orally through libitum in polypropylene bottles with stainless steel sipper tube. Drug was administered for 7 days for the study.

Mode of drug preparation: The different Takra bhedās where prepared at the Laboratory, SDM research Centre, Udupi and laboratory settings of V.P.S.V Ayurveda College, Kottakkal, daily for experimental and analytical study respectively (As per the standardized procedure).

Standardization of procedure for preparation of takra bhedas

The milk used for the preparation of the Takra bhedā was procured from the Safa Farm, Chudalapaara, and Kottakkal. A cow was selected, and milk was collected from that particular cow every day at same time, at 3 pm and it was boiled in vessel by keeping it over water in a sterilizer and was allowed to cool down to 37°C. At that particular temperature the milk was inoculated with two buttermilk starter cultures designated as *L. acidophilus* and yogurt culture (5 ml of *L. acidophilus* and 5 ml of yogurt culture for 1000 ml of milk). Inoculation was done at 9 pm and kept overnight kept for 15 hours and pH of the buttermilk was continuously observed with pH meter and when the pH became 4.5, the curd obtained was taken and churned using Mathana Yantra. The churning time was standardized as 30 minutes, (as after 30 minutes of churning when buttermilk samples were analyzed for fat content it was nearly 0.1-0.2%).[4]

Ghola: churned for 30 minutes without addition of water and butter was not removed.[5]

Mathita: churned for 30 minutes without the addition of water and removed the butter.[5]

Takra: churned for 30 minutes by adding 25 ml of water for 100 ml of curd and removed the butter.[5]

Udasvith: churned for 30 minutes by adding 50 ml of water for 100 ml of curd and removed the butter.[5]

Cacchika: churned for 30 minutes by adding 100 ml of water for 100 ml of curd and removed the butter.[5]

Five varieties were taken in five sterile bottles and were transported to College of Diary Science and technology Mannuthy in ice box filled with ice packs, for Analytical study.

Requirements

Laminar air flow, thermometer, Quebec colony counter, incubator, water bath, autoclave, samples in screw cap tube, Casein Soya bean Digest Agar Medium, MRH Agar, 99 ml sterile water blanks, 9 ml dilution blank, 1.0 ml pipette, sterile test tube, sterile petri dish.

Estimation of Total Microbial Load by standard Plate Count Method

Procedure

Sample to be counted was prepared and prepared serial solution (1:10) of the sample in a laminar air flow and transferred l ml of each dilution to a sterile labelled petri plates, added 10 ml of the melted cooled Casein Soya bean Digest Agar Medium to each plate. Rotated each plate gently, immediately after the addition of the medium for uniform distribution of organism and allowed the agar to solidify; incubated all the 6 plates at 37 C for 48 hours.

Observation

Observed the plates for bacterial colonies and count the number of colonies using a Quebec colony counter.

Estimation of *Lactobacillus* Count by standard Plate Count Method

Procedure

Sample to be counted was prepared and prepared serial solution (1:10) of the sample in a laminar air flow, transferred l ml of each dilution to a sterile labelled petri plates, add 10 ml of the melted cooled MRS agar medium to each plate. Rotated each plate gently, immediately after the addition of the medium, for uniform distribution of organism and allowed the agar to solidify. Incubate all the 6 plates at 37 C for 48 hours.

Observation

Observe the plates for bacterial colonies and count the number of colonies using a Quebec colony counter.

Statistical analysis

It was done by on way ANOVA followed by post hoc tukeykramer.

RESULT

***Lactobacillus* count in takra bheda**

Data showed that in the Takra bheda, *Lactobacillus* count was more in the Takra variety which is highly significant compared to Ghola, Mathita, Takra, Udasvith and only significant increase when compared to Cacchika. Cacchika also showed statistically significant increase when compared to Ghola. Mathita showed significant increase when compared to Ghola and non significant variation when compared to Udasvit and Cacchika.

Total microbial load in intestinal swab

Takra showed highly significant increase in total microbial load when compared to other four varieties. Mathita showed statistically highly significant decrease when compared to Takra and highly significant increase when compared to other three varieties. Ghola showed nonsignificant decrease when compared to Udasvith and Cacchika.

***Lactobacillus* count in intestinal swab**

Mathita variety showed statistically highly significant increase when compared to the Ghola, Udasvith and Cacchika. Takra also showed statistically high significant increase with Ghola Udasvith and Cacchika. Ghola showed non-significant increase with Udasvith and Cacchika.

DISCUSSION

No regular pattern in the microbial growth in Takra bhedā was observed. No direct correlation can be stated with the dilution factor, the *lactobacillus* count and total microbial load. Also, no mutual interrelation was found between the amount of fat in the Takra, microbial load and *lactobacillus* count.

However, Takra and Mathita variety have the ability to improve the probiotic bacteria in the intestine. Udasvith and Cacchika provide least support to the growth of bacteria in the intestine this might be because of the increased amount of water in these two forms when compared to other varieties. In addition to that in a study[4] conducted on physicochemical analysis of Takra bheda explained in Bhava Prakash it is observed that acidity decrease with increase in dilution. This might also have contributed to the decrease in *lactobacillus* count in these two varieties. The study also inferred that butter milk is better probiotic than curd as the Ghola form which is mentioned in the classics is curd only even though it is a form of Takra for Acharya Bhava Prakash.

Table 1: One-way ANOVA for *lactobacillus* count in takra bhedas

Group	Mean ± SEM
Ghola	7155555 ± 185592
Mathita	11560000 ± 480740
Takra	19400000 ± 133166
Udasvith	9800000 ± 264575
Cacchika	14900000 ± 838650

Table 2: Post hoc test for *lactobacillus* count in takra bhedas

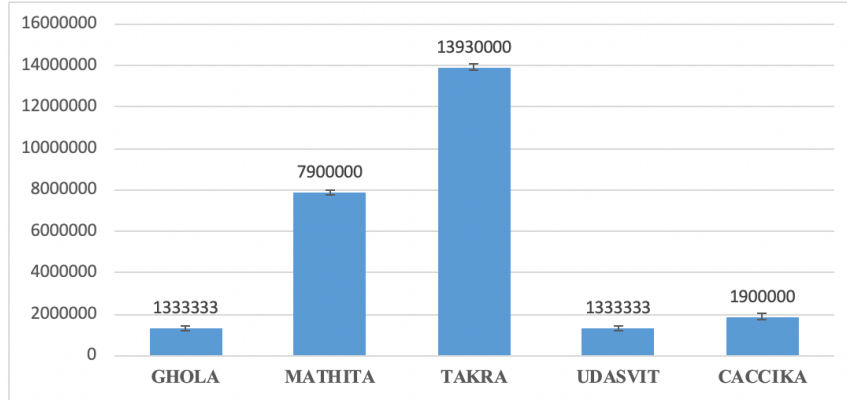| Group	Group v/s	Value of sig.	P value
Ghola	Mathita	*	P < 0.05
	Takra	***	P < 0.001
	Udasvith	NS	P > 0.05
	Cacchika	***	P < 0.001
Mathia	Takra	***	P < 0.001
	Udasvith	NS	P > 0.05
	Cacchika	NS	P > 0.05
Takra	Udasvith	***	P < 0.001
	Cacchika	*	P < 0.05
Udasvith	Cacchika	**	P > 0.05
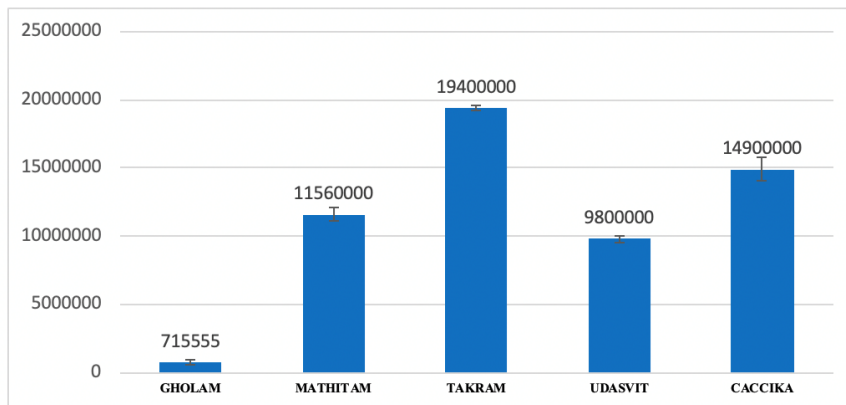
Graph 1: *Lactobacillus* count in Takra bheda

Table 3: One-way ANOVA for Total Microbial Load in intestinal swab

Group	Mean ± SEM
Ghola	1333333 ± 120185
Mathita	7900000 ± 513160
Takra	13930000 ± 417665
Udasvith	1333333 ± 88192
Cacchika	1900000 ± 378794

Table 4: Post hoc test for Total Microbial Load in intestinal swab

| Group	Group V/S	Value of sig.	P value
Ghola	Mathita	***	P < 0.001
	Takra	***	P < 0.001
	Udasvith	NS	P > 0.05
	Cacchika	NS	P > 0.05
Mathita	Takra	***	P < 0.001
	Udasvith	***	P < 0.001
	Cacchika	***	P < 0.001
Takra	Udasvith	***	P < 0.001
	Cacchika	***	P < 0.001
Udasvith	Cacchika	NS	P > 0.05

Graph 2: Total Microbial Load in intestinal swab

Table 5: One-way ANOVA for *Lactobacillus* count in intestinal swab

Group	Mean ± SEM
Ghola	1666667 ± 88192
Mathita	5100000 ± 404145
Takra	4255555 ± 296273
Udasvith	133333 ± 88192
Cacchika	233333 ± 88192

Table 6: Post hoc test for *Lactobacillus* count in intestinal swab

Group	Group V/S	Value of sig.	P value
Ghola	Mathita	***	P < 0.001
	Takra	***	P < 0.001
	Udasvith	NS	P > 0.05
	Cacchika	*	P < 0.05
Mathita	Takra	NS	P > 0.05
	Udasvith	***	P < 0.001
	Cacchika	***	P < 0.001
Takra	Udasvith	***	P < 0.001
	Cacchika	***	P < 0.001
Udasvith	Cacchika	NS	P > 0.05
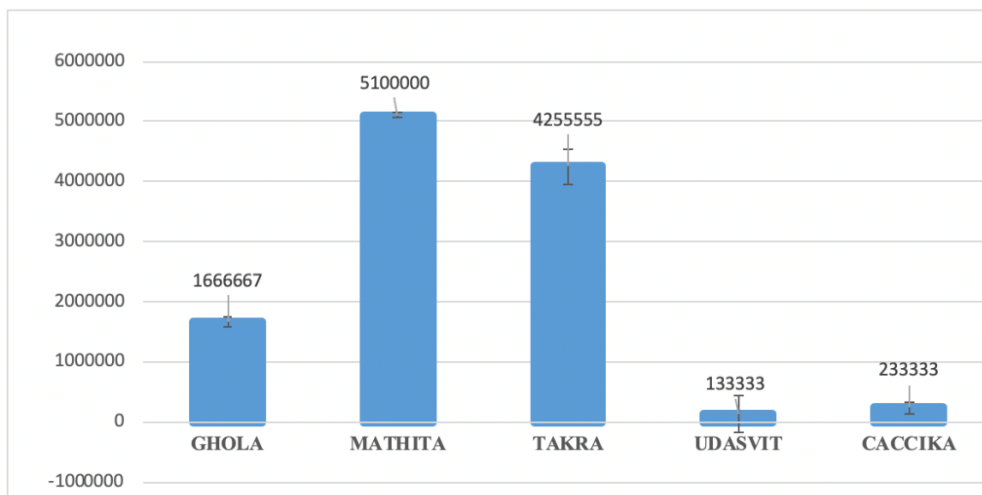
Graph 3: *Lactobacillus* count in intestinal swab

It is observed that *lactobacillus* count in the intestinal swab was not in accordance with the *lactobacillus* count in Takra bheda. Internal environment might have influenced the growth of *lactobacillus* flora rather than oral administration. The internal factors like pH, secretions like gastric acid, bile, gastric and jejunal mucosa, intestinal motility[6] might have varied to a different extent on rats fed with different varieties of Takra that can be given as probable explanation for non-compliance of *lactobacillus* count in Takra bheda and in intestinal swab after different varieties of Takra.

Some additional findings inferred from the study is that Ayurveda attributed Deepana property to Takra variety and Cacchika variety of Takra bheda[4]. Here in this study total *lactobacillus* in Takra bheda were significantly high for Takra and Cacchika variety. Researches shows that probiotic bacteria improve appetite, this study also concluded the same.[7]

CONCLUSION

Thus, it is concluded that, difference in the microbiological parameters i.e. Total Microbial Load and Total *Lactobacillus* Count in the different variety of Takra bheda explained in Bhava Prakash prepared in standard condition and also in intestinal swab after administering Takra bheda to Wistar albino rats.

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