

## Effects of some common spices and herbs (aqueous extract) on the growth of probiotic *Lactobacillus Furfuricola* under gastrointestinal simulations

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### ABSTRACT

Spices and herbs are considered as very beneficial in traditional medicines. We compared the GIT non-simulated conditions with GIT simulated conditions and the effects of aqueous extracts (10%) of some common oriental spices and herbs on the growth of *Lactobacillus furfuricola* a gut bacterium. In pure culture studies, *L. furfuricola* was found to enhance the growth in the presence of Carom Seeds (*Trachyspermum ammi*), Turmeric (*Curcuma longa*), Cinnamon (*Cinnamomum verum*), Garlic (*Allium sativum*) and a rapid decrease in growth in the presence of Onion (*Allium sepa*), Blackseed (*Nigella sativa*) and Ginger (*Zingiber officinale*), whereas no effect in the presence of Mint (*Mentha*), Coriander Seeds (*Coriandrum sativum*) and Fenugreek Seeds (*Trigonella foenum*) was observed after 48 hours by taking optical density at 600nm by using Spectrophotometer. Human gut simulatory conditions were given artificially under controlled environment and *Lactobacillus furfuricola* was found to be shown more enhanced growth, indicating that their consumption may favour the growth of probiotics species in the gut. Spices and herbs have potential to enhance the growth of gut microflora.

### Key words

Spices and Herbs, *Lactobacillus furfuricola*, Microbiota, Gastrointestinal tract, GIT-Simulated conditions, Millipore filter

## 1. Introduction

The people of Asia especially South Asians have used spices for different purposes like give colour to their food and for adding aroma to their cooked food. Spices are also being used in traditional medicines to improve gut digestion. The change in diet can modified the composition of gut microbiota. (Macfarlane and Macfarlane, 2003). An adequate amount of food is very beneficial for host gut microflora including humans (Metwaly *et al.*, 2018). Specific spices can have enhanced the growth of beneficial bacteria (Ravindran *et al.*, 2017).

A lot of research has been done on the relationship between gut microbiota and their effects on the digestive system as a result of growing awareness of how gut microbes might affect human health. (Gibson *et al.*, 1995).

There has been much research on the interaction between the gut microbiota and micronutrients and their effects on the digestive system as a result of growing recognition of the ability of gut microbes to affect human health. (Gibson *et al.*, 1995).

Spices and herbs derived from seeds, leaves of a plant, bark, rhizome of a plant and fruit often contain phytochemicals and spices has been used for medicinal purposes from early times (Kocaadam *et al.*, 2017)

There is a little amount of information is present about the effect of aqueous extract of spices on the growth of probiotic *Lactobacillus furfuricola*. The present study focused on the effect of 10 common oriental spices on the growth enhancement of probiotic under the effect of non-GIT simulations and GIT simulations.

## 2. Material and Methods.

### 2.1. Preparation of aqueous extract under non-GIT Simulations

Garlic rhizome, mint leaves, onion rhizome, fenugreek seeds, ginger rhizome, carom seeds, black seeds, coriander seeds, turmeric rhizome and cinnamon bark are all brought from local food market (Lahore, Pakistan) in raw condition than grinded and an extremely fine powder is obtained by using fine test sieves by Sigma-Aldrich® Solutions .10g of each spice in 100ml or (10g/100ml) was placed in a dark place and after three days filtered by using Whatman filter paper no. 1 and substrate was oven dried at 50°C temperature and then aqueous extract was prepared of 10% concentration (w/v) by passing through Millipore filter and stored the aqueous extract at 4°C temperature.

## 2.2. Preparation of aqueous extract under GIT Simulations

10g of each spice powder form was treated with simulatory fluids by using (Minekus *et al.*, (2014) protocol with little modification and total volume was raised up to 100ml and placed in a dark place for three days filter the substrate and oven dried at 50°C and raised volume by adding distilled water to make 10% solution (w/v) the substrate is passed through Millipore and stored at 4°C temperature.

## 2.3. Pure culture bacterial strains.

The bacterial culture used in the studies was as follows: Pure culture of *Lactobacillus furfuricola* slants provided by Prof. Dr. Javed Iqbal Qazi, Institute of Zoology, University of the Punjab, Lahore Pakistan was taken and grown statistically in MRS Broth for revival. Culture was incubated anaerobically at 37°C for 48 to 72 hours.

## 2.4. Effect of spices on *L. furfuricola* growth under non GIT simulations

MRS Broth media was prepared and autoclaved. 5ml of media was taken in each autoclaved vial. The first vial was taken and inoculated with 0.1ml of *L. furfuricola* by removing equal amount of MRS Broth solution

In second vial 50µl of MRS Broth was removed with the help of micropipette and 50 µl of aqueous spice extract was added while in third vial 0.1ml and 50µl of MRS Broth were removed simultaneously and 0.1ml of inoculum was added followed by 50µl of spice extract. The fourth vial was used as a blank which only contain MRS Broth sample. Same procedure was repeated for each spice separately the vials were incubated at 37°C incubator for 48 to 72 hours. The experiment was performed in triplicates. After the incubatory period samples were taken and optical density was taken at 600nm.

## 2.5. Effect of spices on *L. furfuricola* growth under GIT simulations

Effect of spices on *L. furfuricola* growth under GIT simulations same procedure was followed as mentioned above with the difference is spices aqueous extract which were used was treated with GIT simulatory conditions. by with little modification (Minekus *et al.*, 2014). The experiment was performed in triplicates After the incubatory period samples were taken and optical density was taken at 600nm.

## 2.6. Data Analysis

The data was analysed by using SPSS software and Two-way ANOVA was applied and the p value is  $\leq 0.05$

### 3. Results

The probiotic growth in MRS Broth was taken as control and was compared with 10 different spices under GIT simulated and GIT non-simulated conditions.

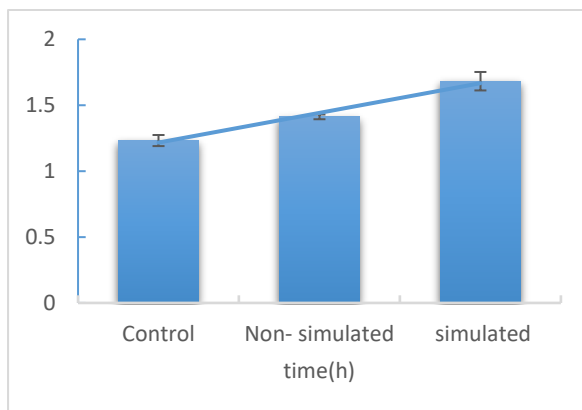
The growth of *Lactobacillus furfuricola* in aqueous extract of Turmeric (36%), Cinnamon (20%), Garlic (19%), Carom Seeds (56%) and Fenugreek Seeds was increased in both GIT simulated and GIT non-simulated conditions as compared to control as shown in Fig 1 (a-e)

In contrast, aqueous extract of Ginger, Onion (70%) significantly suppressed the growth of probiotic *Lactobacillus furfuricola* as shown in Fig 2 (a,b) after incubated period of 48 hours in both GIT simulated and GIT non-simulated conditions as compared to control.

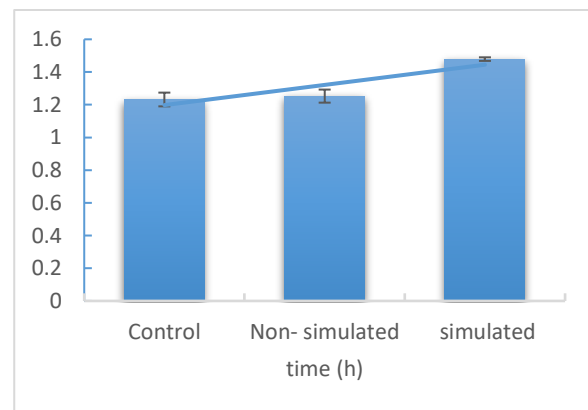
Mint aqueous extract has no effect on the growth of probiotic *L. furfuricola* it neither enhanced or suppressed the growth of probiotic under GIT simulated and GIT non simulated conditions as shown in Fig 3.

The aqueous extracts Black seeds and Coriander Seeds both didn't significantly enhanced the growth of probiotic rather suppressed the growth of the probiotic under GIT simulated conditions as seen in Fig 4 (a,b).

Spices		Control	Simulated	<u>Non Simulated</u>
<i>Curcuma longa</i> (Turmeric)		1.23 ± 0.042	1.681 ± 0.018	1.42±0.07
<i>Zingiber officinale</i> (Ginger)		1.23 ± 0.042	0.798 ± 0.014	0.800 ± 0.013
<i>Cinnamomum verum</i> (Cinnamon)		1.23 ± 0.042	1.479 ± 0.011	1.252 ± 0.040
<i>Allium cepa</i> (Onion)		1.23 ± 0.042	0.364 ± 0.006	0.265 ± 0.049
<i>Allium sativum</i> (Garlic)		1.23 ± 0.042	1.472 ± 0.007	1.417 ± 0.044
<i>Trachyspermum ammi</i> (Carom Seeds)		1.23 ± 0.042	1.926 ± 0.011	1.466 ± 0.048
<i>Mentha</i> (Mint)		1.23 ± 0.042	1.262 ± 0.013	1.127 ± 0.016
<i>Nigella sativa</i> (Black seed)		1.23 ± 0.042	0.905 ± 0.027	0.903 ± 0.027
<i>Coriandrum sativum</i> (Coriander Seeds)		1.23 ± 0.042	1.183 ± 0.024	1.148 ± 0.014
<i>Trigonella foenum-graecum</i> (Fenugreek Seeds)		1.23 ± 0.042	1.546 ± 0.006	1.417± 0.005

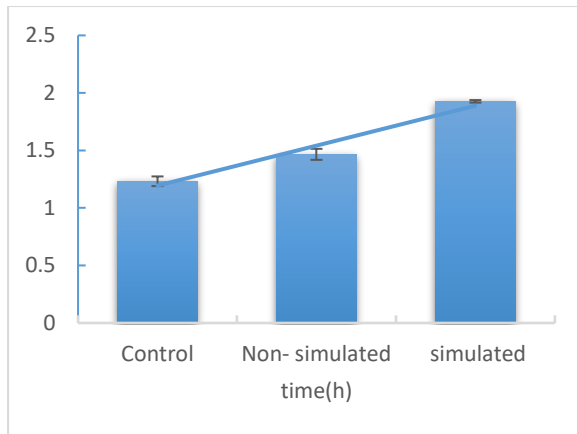


(a)

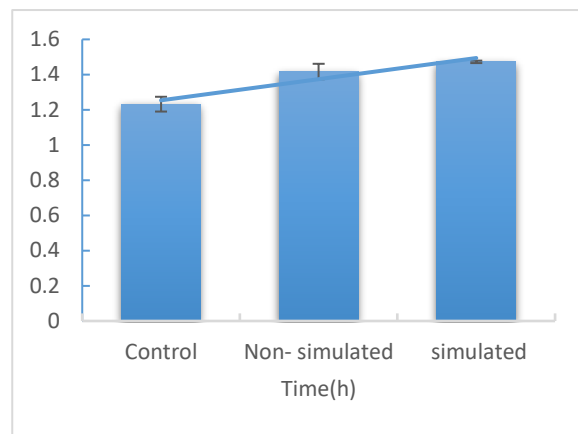


(b)

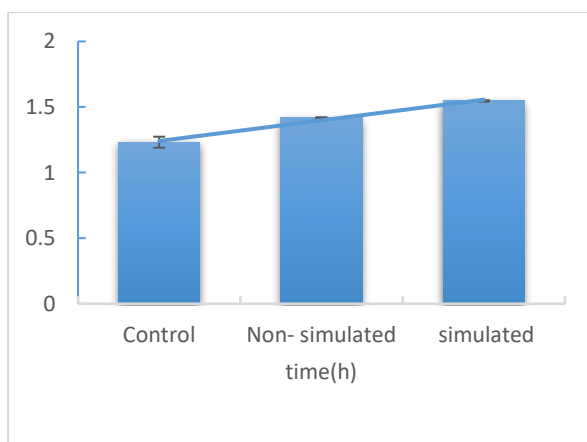
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(c)

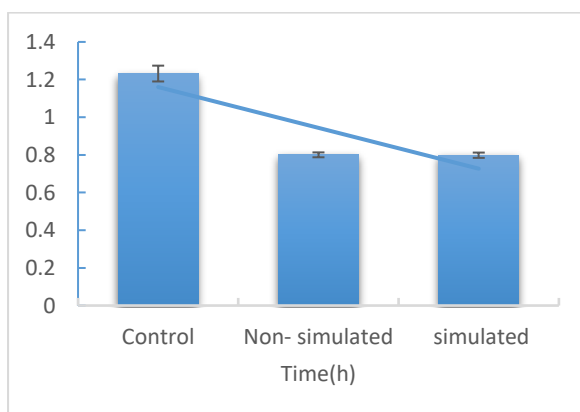


(d)

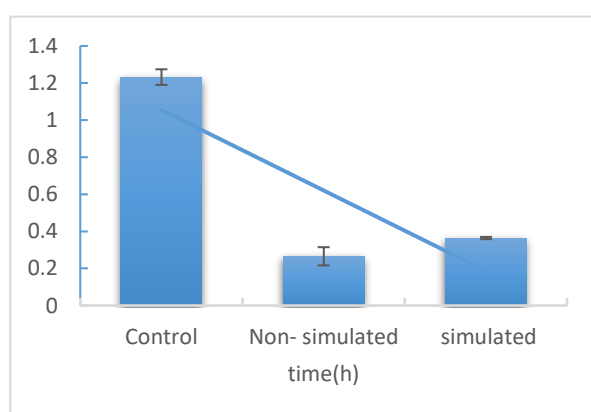


(e)

**Fig.1.** Effect of aqueous extract of (a) Turmeric (b) Cinnamon (c) Garlic (d) Carom Seeds (e)Fenugreek Seeds on the growth of *L. Furfuricola* in Control, GIT Non simulated and GIT Simulated conditions.

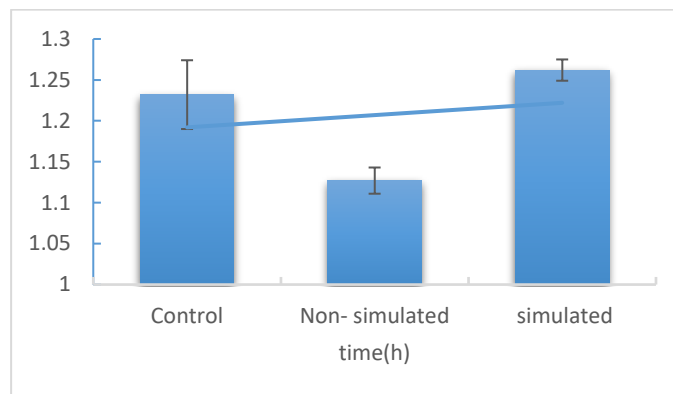


(a)

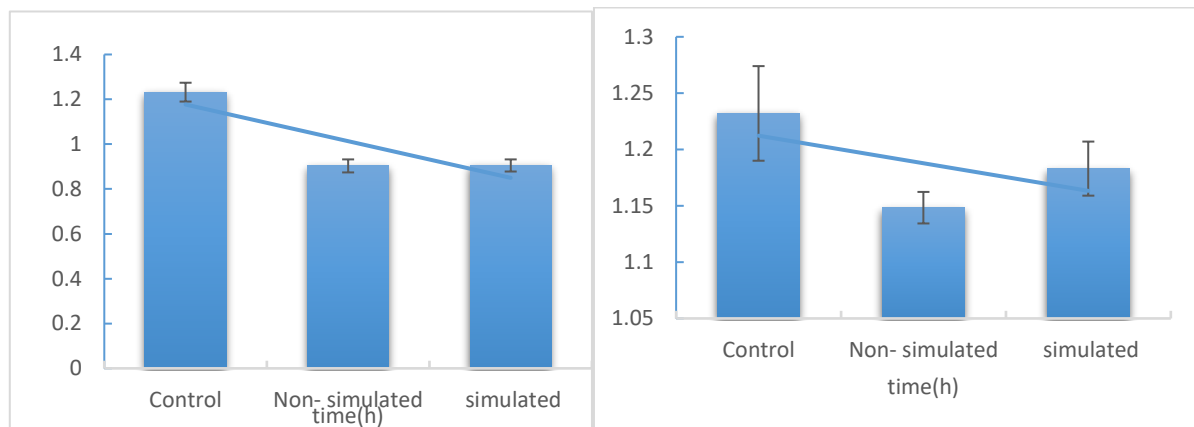


(b)

**Fig.2.** Effect of aqueous extract of (a) Ginger (b) Onion on the growth of *L. Furfuricola* in Control, GIT Non simulated and GIT Simulated conditions.



**Fig.3.** Effect of aqueous extract of mint on the growth of *L. Furfuricola* in Control, GIT Non simulated and GIT Simulated conditions



(a)

(b)

**Fig.4.** Effect of aqueous extract of (a) Blackseed (b) Coriander seeds on the growth of *L. Furfuricola* in Control, GIT Non simulated and GIT Simulated conditions.

#### 4. Discussion

The effects of regularly used spices were examined to see if they had any beneficial effects on pathogenic bacterial growth as well as their potential to function as prebiotics for the growth assistance of intestinal microbiota under gastro- intestinal simulatory circumstances. *Shigella spp.* was used to study the growth of hazardous bacteria, while the *Lactobacillus furfuricola* strain was utilised to monitor the growth of good bacteria. At 600 nm, optical density measurements were made.

## Effects of some common spices and herbs (aqueous extract) on the growth of probiotic *Lactobacillus Furfuricola* under gastrointestinal simulations

According to Altuntas *et al.*, (2019) research's *Allium sativum*, a spice, has proved highly helpful for the growth of the good bacteria *Lactobacillus acidophilus* while also inhibiting the formation of food-borne harmful bacteria. Moreover, the current investigation has drawn similar conclusions.

People's risk of developing peptic ulcer disease has been found to be decreased by a variety of well-known spices and herbal supplements. The effectiveness of spices and herbal xenobiotic in treating stomach mucosal injury is still up for dispute. However, several common spices and herbal supplements might help in the fight against peptic ulcer disease in people. According to research, the various components of spices, such as phenolic compounds and the curcumin included in turmeric, are particularly useful for the Lactic Acid Bacteria (LAB) portion of the gut microbiota (Ganjre *et al.*, 2015)

The bacterium *Lactobacillus furfuricola* has demonstrated excellent results in the presence of the following spices as its growth has been seen by optical density method. The results have shown that with GIT simulated conditions under descending order, the spices have shown the following results. According to the data, Carom showed increased growth for the helpful bacterium *Lactobacillus furfuricola*, while Onion showed the least growth.

Many sulphur-containing chemicals have predominated the biochemistry of the *Allium* species, giving it a distinctive flavour, odour, and the majority of its intense biological activity. And same in the case of garlic and onion. Spices and herbs is defined as prebiotics because they promote the growth of beneficial bacteria. (Hutkins *et al.*, 2016).

Culinary spices enhanced the growth of lactic acid bacteria and acetic acid bacteria (Lu *et al.*, 2019). Phytochemical substances present in spices and herbs may favour the growth of beneficial bacteria in the intestine (Martinez *et al.*, 2020). Some foods can stimulate growth because these bacteria can utilise their constituents as substrates, or they can increase nutrient intake by altering bacterium metabolism. (Madurenia *et al.*, 2016).

Dietary spices and medicinal herbs contain phenolic extracts that might affect the growth of beneficial bacteria (Chan *et al.*, 2018). our results conclude that spices and herbs do exhibit prebiotic effect to increase the growth of *lactobacillus furfuricola*. Many species of lactic acid bacteria exhibit the potential to treat the effects of diarrhoea in gastrointestinal tract (Vanderhoof *et al.*, 1999). Seeds spices have diverse array of phytochemicals that supports the growth of gut microbiota (Rathore *et al.*, 2013). Spices used in non-random fashion, but they have many beneficial aspects on growth of microbiota (Sherman *et al.*, 1999). spices promoted the growth of beneficial bacteria (Sugasni *et al.*, 2018) and similar results were resulted in present study.

Herbs and spices like coriander, fenugreek, mint and rosemary etc., are all considered leafy spices. They not only improve the flavour, colour, and texture of meals while they are fresh and/or processed, but also the health and well-being of consumers thanks to the abundance of potent phytonutrients they contain. (Sharangi *et al.*, 2013.)



## 5. Conclusion.

Spices have a long history of usage as a traditional medicine and also for cooking purposes like adding colour and aroma to the cooked food. The present study has enlightened the beneficial role of some common spices for promoting the growth of *Lactobacillus furfuricola* a beneficial bacterium.

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