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Research Article

Effect of Polysaccharides and Water Extract from *Fructus tribuli* on Growth of *Lactobacillus brevis*

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Abstract: Effect of polysaccharides and water extract from *Fructus tribuli* on growth of *Lactobacillus brevis* was studied by measuring the optical density at 600 nm and pH using commercial MRS broth as the control. The addition (mg/mL) of steroidal saponins was 0.10, 0.20, 0.30, 0.40 and 0.50 and the addition (%, v/v) of water extract was 1, 2, 3, 4 and 5. Results were as follows: The additions of polysaccharides and water extract had a significant promotion on the growth of *Lactobacillus brevis*. The optimum concentrations of steroidal saponins and water extract were 0.40 mg/mL and 5%, respectively.

Keywords: *Fructus tribuli*, *Lactobacillus brevis*, polysaccharides, probiotics

INTRODUCTION

Tribulus terrestris L. is an annual herb that grows worldwide, especially in the subtropical area of Asia, Africa, Europe, America and Australia (Topia *et al*., 1994; Kostova *et al*., 2002; Combarieu *et al*., 2003). The fruits of *T. terrestris* known to the Chinese as "Jili" are allowed to use in functional food by the Ministry of Health of China and used in the folk medicine in India, China, Bulgaria and other countries against various diseases, including impotence tonic, cardiovascular diseases, anti-hypertensive urinary anti-infective, anti-inammatory and immunosuppressive activities (Sangeeta *et al*., 1994; Somanandhan *et al*., 1999; Wu *et al*., 1999; Ali *et al*., 2001; Deepak *et al*., 2002; Wu *et al*., 2003; Ali *et al*., 2003a, b; Joshia *et al*., 2005). The occurrence of saponins, polysaccharides, alkaloids, amides, flavonoids and cinammic acid has been reported in Jili (Saleh *et al*., 1982; Wu *et al*., 1996; Yan *et al*., 1996; Wang *et al*., 1997; Li *et al*., 1998; Fang *et al*., 1999; Wu *et al*., 1999; Kostova *et al*., 2002).

In previous research, the hot water extract from *Fructus tribuli* was found to promote the growth of *L. acidophilus acidophilus*, *Lactobacillus bulgaricus* and *Bifidobacterium bifidum* (Shu *et al*., 2007, 2011a, b). In our previous research, we found the additions of steroidal saponins and flavonoid had a significant promotion on the growth of *Lactobacillus brevis*. The optimum concentrations of steroidal saponins and flavonoid were 0.20 and 0.15 mg/mL, respectively (Miao *et al*., 2012). The aim of our research was to study the effect of polysaccharides and water extract from *Fructus tribuli* on growth of *Lactobacillus brevis*.

MATERIALS AND METHODS

Microorganism: *Lactobacillus brevis* was obtained from Xijing University. The strain was grown three successive times in MRS broth (Hopebio, Qingdao, China), the inoculums was 2% (v/v), 37°C for 24 h (Shu *et al*., 2007).

Preparation of the extract from *Fructus tribuli*: *Fructus tribuli* was ground in a high speed disintegrator (Model SF-2000, Shanghai, China), then was extracted by distilled water (liquid: solid ratio (mL/g) 14:1, While the temperature (95°C) of the water bath was kept steady, The extraction in a 1.0 l stainless steel boiler in the water bath was stirred with an electric mixing paddle for 1.5 h, then obtained the water extract of *Fructus tribuli* by filtration and centrifugation.

Culture conditions: The MRS media were autoclaved at 118°C for 15 min and then added filter sterilized polysaccharides or water extract from *Fructus tribuli*. The concentration of polysaccharides added to MRS media in anaerobic tube was 0.10, 0.20, 0.30, 0.40 and 0.50 mg/mL, respectively. The concentration of water extract added to MRS media in anaerobic tube was 1, 2, 3, 4 and 5%, respectively. The sterilized MRS broth containing steroidal polysaccharides or water extract was inoculated with 2% (v/v) inoculum. The temperature was kept at 37°C and then determined growth of *Lactobacillus brevis* and pH at incubation 2, 5, 20, 24 and 27 h, respectively. MRS media without polysaccharides or water extract was included in this experiment as the control.

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The measurement of growth and pH: The growth of *Lactobacillus brevis* was evaluate by measuring the optical density at 600 nm (OD_{600}) by a spectrophotometer (SP-756 PC, Shanghai Spectrum Instruments Co., Ltd., Shanghai, China). The pH of MRS broth was measured by a pH-meter (pHS-3C Shanghai Precision Scientific Instrument Co., Ltd, Shanghai, China).

RESULTS AND DISCUSSION

Effect of polysaccharides on growth of *Lactobacillus brevis*: The effect of polysaccharides on growth of *Lactobacillus brevis* and pH in the MRS broth containing polysaccharides from *Fructus tribuli* showed in Fig. 1 and 2.

With the concentrations of polysaccharides from *Fructus tribuli* increasing, The OD600 values of *Lactobacillus brevis* decreased gradually from 0.323 and 0.346 at the control to 0.249 and 0.254 at 0.50 mg/mL at 2 and 5 h, respectively, but the pH of the medium has not significant difference. The first few h were lag phase, so *Lactobacillus brevis* grew slowly and the addition of polysaccharides from *Fructus tribuli* had a slight inhibition on growth of *Lactobacillus brevis* at first few hours. At 20, 24 and 27 the OD 600 values of *Lactobacillus brevis* first increased gradually from 1.671, 1.822 and 1.952 at the control to 1.896, 1.991 and 3.244 at 0.40 mg at 20, 24 and 27 h and then decreased to 1.838, 1.945 and 2.972 at 0.50 mg/mL, respectively. Therefore, addition of polysaccharides from *Fructus tribuli* could promote the growth of *Lactobacillus brevis*. The optimum concentration of polysaccharides in MRS broth for *Lactobacillus brevis* was 0.40 mg/mL at incubation 24 h.

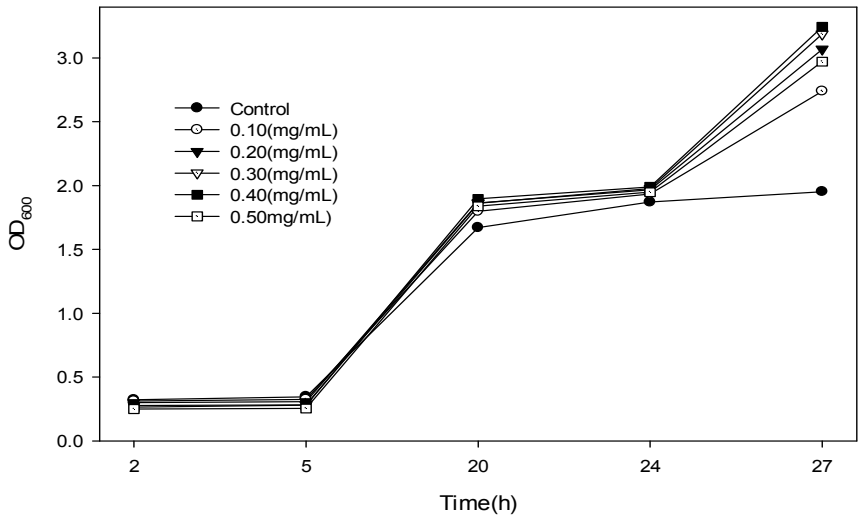

Fig. 1: The effect of polysaccharides from *Fructus tribuli* on the growth of *Lactobacillus brevis*

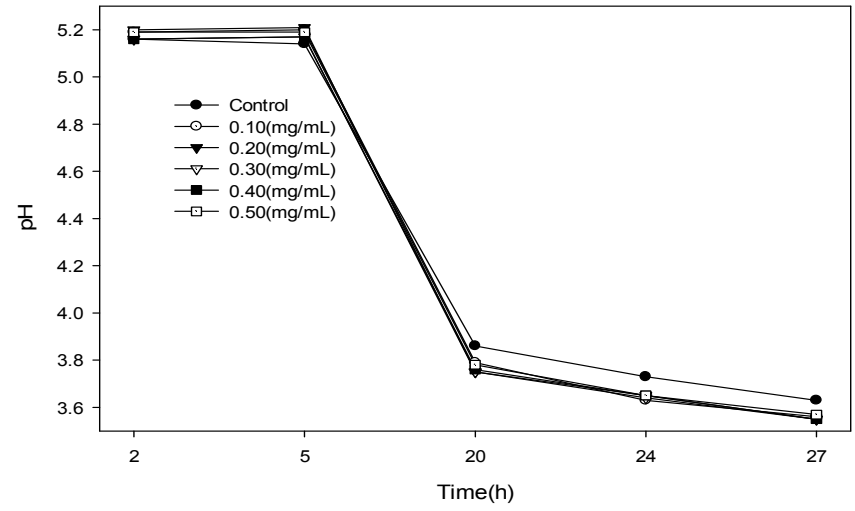

Fig. 2: The effect of polysaccharides from *Fructus tribuli* on the pH in the MRS broth

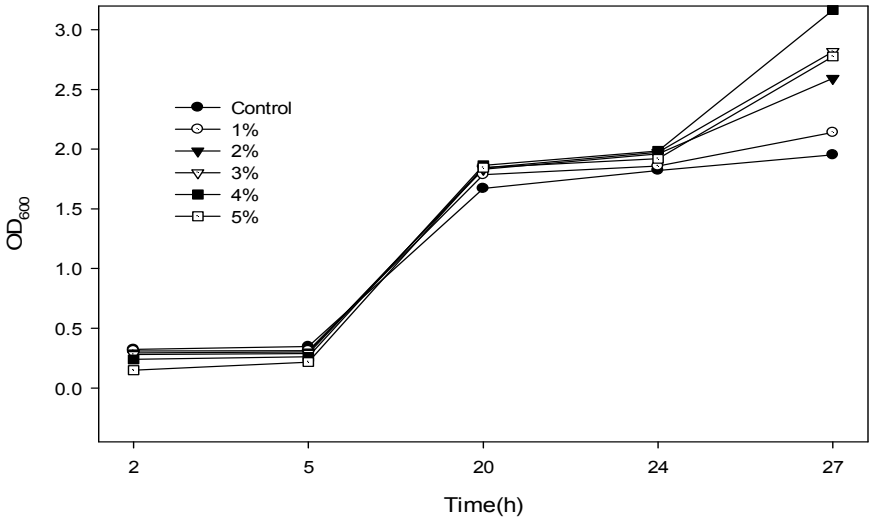

Fig. 3: The effect of water extract from *Fructus tribuli* on the pH in the MRS broth

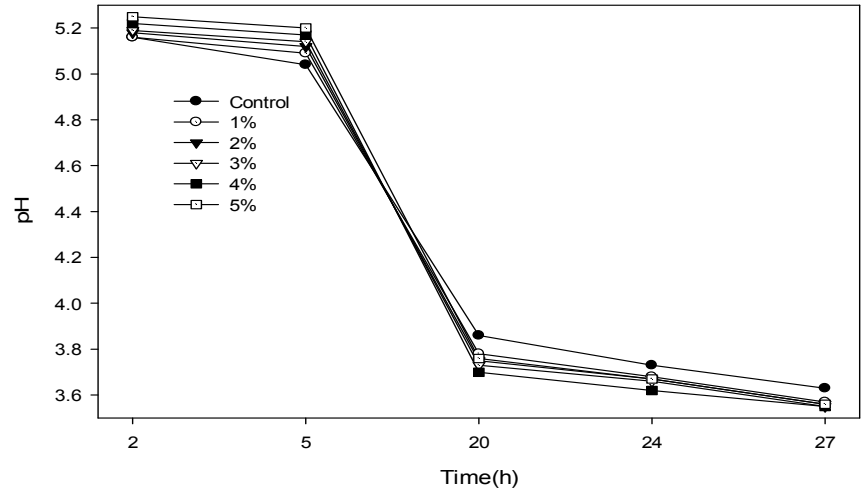

Fig. 4: The effect of water extract of *Fructus tribuli* on the pH in the MRS broth

Effect of extract of on growth of *Lactobacillus brevis*: The effect of water extract on growth of *Lactobacillus brevis* and pH in the MRS broth containing water extract from *Fructus tribuli* showed in Fig. 3 and 4.

With the concentrations of water extract from *Fructus tribuli* increasing, The OD 600 values of *Lactobacillus brevis* decreased gradually from 0.323 and 0.346 at the control to 0.148 and 0.217 at 5% at 2 and 5 h, respectively, but the pH of the medium has not significant difference. At 20, 24 and 27 h the OD600 values of *Lactobacillus brevis* first increased gradually from 1.671, 1.822 and 1.952 at the control to 1.863, 1.985 and 3.164 at 4% at 20, 24 and 27 h and then decreased to 1.845, 1.919 and 2.776 at 5%, respectively. Therefore, addition of water extract from *Fructus tribuli* could promote the growth of *Lactobacillus brevis*. The optimum concentration of water extract in MRS media for *Lactobacillus brevis* was 4% at incubation 24 h.

CONCLUSION

Addition of polysaccharides and water extract from *Fructus tribuli* has the significant promotion on growth of *Lactobacillus brevis* at incubation 24 h. The optimum concentrations of polysaccharides and water extract in MRS broth were 0.40 mg/mL and 5% for *Lactobacillus brevis* and the maximum OD_{600} values were 1.991 and 1.985 at 37°C for 24 h, respectively.

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