

Research Article

Effect of the Steroidal Saponins of *Fructus tribuli* on Growth of Three *Lactobacillus acidophilus*

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Abstract: Effect of the steroidal saponins of *Fructus tribuli* on growth of *Lactobacillus acidophilus* LA04, LA05 and LA06 was studied by measuring optical density at 600 nm (OD₆₀₀) and pH using MRS media as the control. The addition of steroidal saponins (w/v) was 0.05, 0.10, 0.15, 0.20 and 0.25%, respectively. Results were as follows: addition of steroidal saponins of *Fructus tribuli* could promote the growth of *Lactobacillus acidophilus* LA04, LA05 and LA06 and the optimum concentration of steroidal saponins was all 0.25% for the three strains.

Keywords: *Fructus tribuli*, *Lactobacillus acidophilus*, probiotics, steroidal saponins

INTRODUCTION

Lactobacillus acidophilus, which is Gram-positive, non-spore-forming rods with rounded ends that occur singly, in pairs and in short chains, is one of the most commonly recognized species of the genus *Lactobacillus* and was first isolated from the intestinal tract and vagina of humans and animals (Gopal, 2011). *Lactobacillus acidophilus* has been shown to play an important role in promoting and maintaining human health and is accepted as probiotics (Salminen *et al.*, 1998; Guarner and Schaafsma, 1998). The health benefits include reduction of gastrointestinal symptoms in lactose-intolerant individuals, relief from symptoms of constipation, treatment of infantile diarrhea, prevention of travellers' diarrhea and activity against *Helicobacter pylori* (Choi *et al.*, 2011; Tabbers *et al.*, 2011; Rerksuppaphol and Rerksuppaphol, 2010; Jain *et al.*, 2010).

Tribulus terrestris is an annual creeping herb of the *Zygophyllaceae* family, native to warm temperature and tropical regions of the old world in Southern Europe, Southern Asia, Africa and Northern Australia (Al-Bayati and Al-Mola, 2008). In traditional Chinese medicine, *Fructus tribuli*, the fruit of *Tribulus terrestris*, which is called "Ji Li" in China, have long been used for the treatment of eye trouble, cutaneous pruritus, edema and abdominal distention, emission and morbid leucorrhea, inflammation and tracheitis as well as vitiligo (Zhang *et al.*, 2005; Yan *et al.*, 1996). Nowadays, the anticancer and nutrition providing properties of this plant also have been reported (Neychev *et al.*, 2007; Christophe *et al.*, 2008). The

steroidal saponins of *Tribulus terrestris* are considered to be one of the factors responsible for biological activity of products derived from this plant and many pharmaceutical preparations and food supplements based on the saponin fraction from this plant are on sale worldwide (Dragomir *et al.*, 2008).

In previous research, we found that the hot water extract of *Fructus tribuli* can promote the growth of *Lactobacillus acidophilus* LA09 (Shu *et al.*, 2011). The purpose of the present work was to study effect of steroidal saponins from *Fructus tribuli* on growth of *Lactobacillus acidophilus* LA04, LA05 and LA06.

MATERIALS AND METHODS

Materials: The probiotic lactic acid bacterial strains, *Lactobacillus acidophilus* LA04, LA05 and LA06 were obtained from College of Life Science and Engineering, Shaanxi University of Science and Technology. All *Lactobacillus acidophilus* strains were transferred three times in MRS medium (Hopebio, Qingdao, China) before use. The inoculum concentration was 2% (v/v) and the growth temperature was kept at 37°C.

The steroidal saponins of *Fructus tribuli* were purchased from Xi'an Tianyuan Biotech Co., Ltd., (Xi'an, China).

Growth conditions: Steroidal saponins (*Fructus tribuli* P.E., 90% purity) was supplemented to the anaerobic tubes containing MRS media at 0.05, 0.10, 0.15, 0.20 and 0.25% (v/v), respectively. MRS media without steroidal saponins was allowed to grow under the same conditions as a control.

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All media were disinfected at 118°C for 15 min. Active strains transferred three times were inoculate to each anaerobic tube which was cooled to room temperature and cultured at 37°C, then measured biomass and pH at incubation 12, 18 and 24 h, respectively.

The experiment was completed in triplicate and means were calculated for each experimental group at each data collection point.

pH measurement: The pH of organism was measured through a pH-meter (pHS-3C Shanghai Precision Scientific Instrument Co., Ltd., Shanghai, China).

Growth determination: In order to monitor the growth of each strain, the optical density at 600 nm (OD₆₀₀) of the cultures was measured through a spectrophotometer (SP-756PC, Shanghai Spectrum Instruments Co., Ltd., Shanghai, China).

RESULTS AND DISCUSSION

Effect of steroidal saponins on growth of *Lactobacillus acidophilus* LA04: Effect of different concentrations of *Fructus tribuli* steroidal saponins on the growth of *Lactobacillus acidophilus* LA04 showed in Fig. 1 to 3.

The growth of control group was rapid in incubation 12-18 h and then slowed down in 18-24, for the OD value was 1.625, 1.662 and 1.669 at incubation 12, 18 and 24 h, respectively.

With the increasing concentration of *Fructus tribuli* steroidal saponins, the OD values were rapidly increased and reached maximum 1.639, 1.789 and 1.781 at 0.25% steroidal saponins at incubation 12, 18 and 24 h, respectively. It must be note that, at incubation 12 h, OD value of the culture mediums added steroidal saponins were 1.598, 1.604, 1.605 and 1.618 at 0.05, 0.10, 0.15 and 0.20% density of steroidal saponins, respectively, which were lower than the control and they then increased significantly and surpassed the control group in 12-18 h, reached 1.713, 1.725, 1.745 and 1.741, respectively.

The pH of different culture mediums in contrast with the OD₆₀₀ has significant difference ranging from 4.03 to 4.38 (incubation time 12 h), 3.73 to 4.08 (incubation time 18 h), 3.73 to 4.06 (incubation time 24 h), which indicated that steroidal saponins has a significant promotion on growth of *Lactobacillus acidophilus* LA04 and the optimum concentration of steroidal saponins in MRS media was 0.25% at incubation 18 h.

Effect of steroidal saponins on growth of *Lactobacillus acidophilus* LA05: Effect of different concentrations of *Fructus tribuli* steroidal saponins on the growth of *Lactobacillus acidophilus* LA05 showed in Fig. 4 to 6.

The control group was rapidly growing from incubation 12 to 24 h, for the OD value was 1.476,

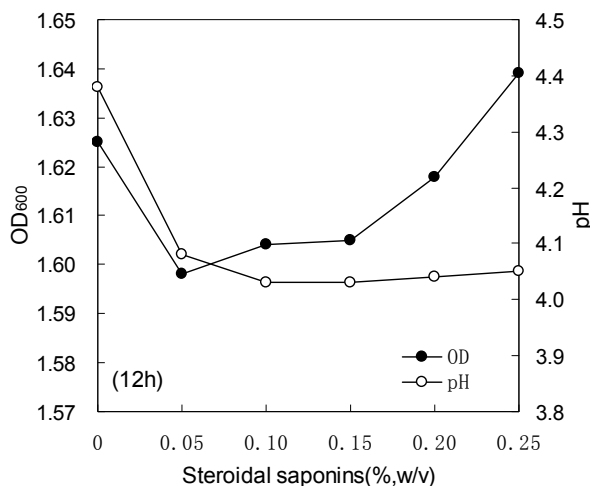


Fig. 1: Effect of steroidal saponins on growth of *L. acidophilus* LA04 (12 h)

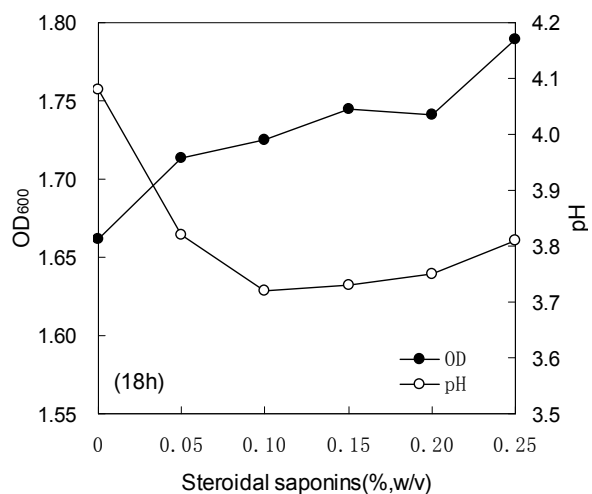


Fig. 2: Effect of steroidal saponins on growth of *L. acidophilus* LA04 (18 h)

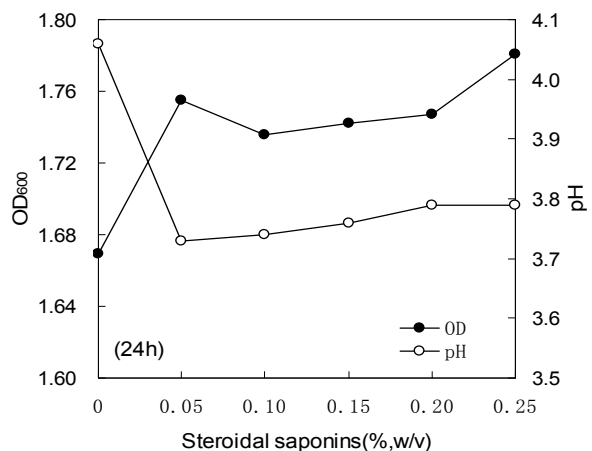


Fig. 3: Effect of steroidal saponins on growth of *L. acidophilus* LA04 (24 h)

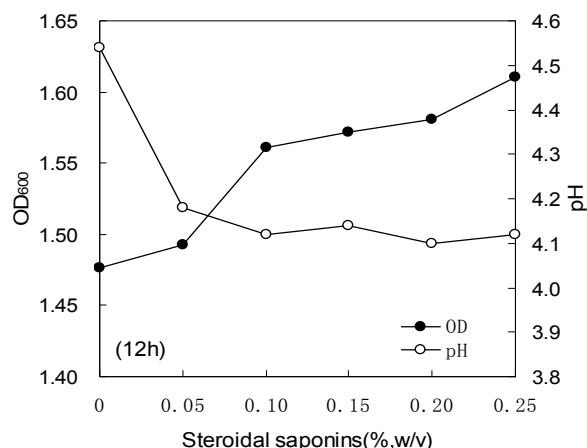


Fig. 4: Effect of steroidal saponins on growth of *L. acidophilus* LA05 (12 h)

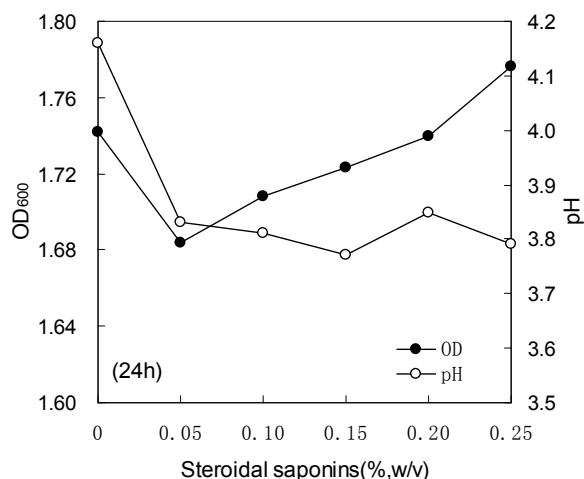


Fig. 6: Effect of steroidal saponins on growth of *L. acidophilus* LA05 (24 h)

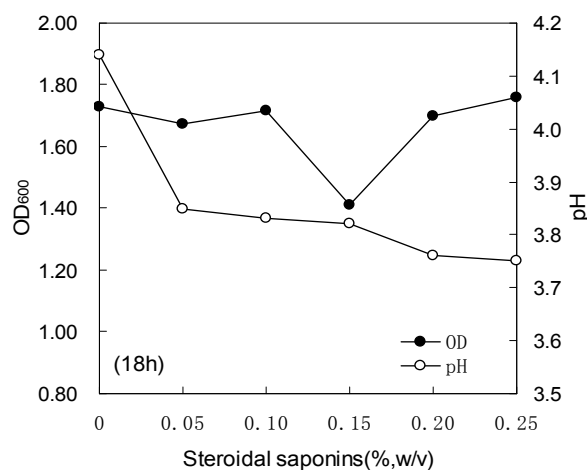


Fig. 5: Effect of steroidal saponins on growth of *L. acidophilus* LA05 (18 h)

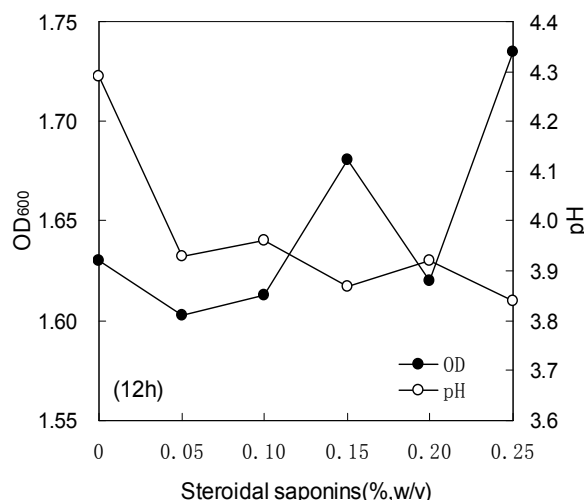


Fig. 7: Effect of steroidal saponins on growth of *L. acidophilus* LA06 (12 h)

1.728 and 1.742 at incubation 12, 18 and 24 h, respectively.

With the increasing concentration of steroidal saponins of *Fructus tribuli*, the OD values were rapidly increased and reached maximum 1.610, 1.757 and 1.776 at 0.25% steroidal saponins at incubation 12, 18 and 24 h, respectively. At incubation 12 h, the add of different concentrations of steroidal saponins significantly increased the OD values and reached 1.493, 1.561, 1.572 and 1.581 at 0.05, 0.10, 0.15 and 0.20% steroidal saponins, respectively, but the OD values were lower than the control group at incubation 18 and 24 h. Moreover, the decrease added 0.15% steroidal saponins at incubation 18 h was extremely significant which dropped to 1.412 and then increased to 1.732 at incubation 24 h, which was still lower than the control group.

The pH of different culture mediums in contrast with the OD₆₀₀ has significant difference ranging from 4.10 to 4.54 (incubation time 12 h), 3.75 to 4.14

(incubation time 18 h) and 3.77 to 4.16 (incubation time 24 h), which indicated that steroidal saponins on growth of *Lactobacillus acidophilus* LA05 has an obvious effect and the optimum concentration of steroidal saponins in MRS media was 0.25% at incubation 12 h.

Effect of steroidal saponins on growth of *Lactobacillus acidophilus* LA06: Effect of different concentrations of *Fructus tribuli* steroidal saponins on the growth of *Lactobacillus acidophilus* LA06 showed in Fig. 7 to 9.

The control group kept tardily growing from incubation 12 to 24 h, for the OD value was 1.630, 1.641 and 1.665 at incubation 12, 18 and 24 h, respectively.

With the increasing concentration of *Fructus tribuli* steroidal saponins, the OD values were rapidly

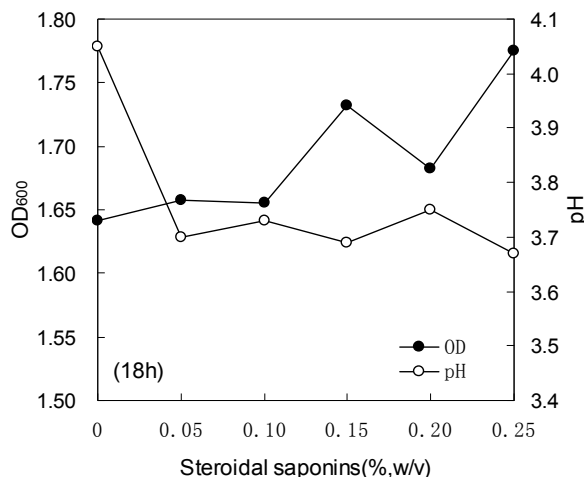


Fig. 8: Effect of steroidal saponins on growth of *L. acidophilus* LA06 (18 h)

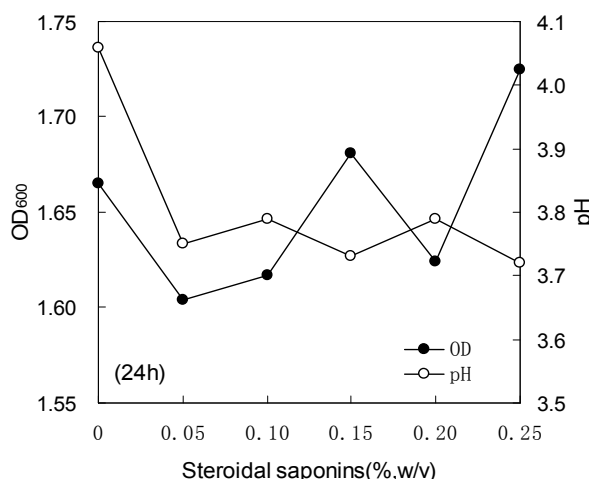


Fig. 9: Effect of steroidal saponins on growth of *L. acidophilus* LA06 (24 h)

increased and reached 1.735, 1.775 at 0.25% steroidal saponins and 1.681, 1.732 at 0.15% steroidal saponins at incubation 12 and 18 h, then decreased to 1.665 and 1.681 at incubation 24 h, respectively. The OD values of other groups also increased gradually from incubation 12 to 18 h and reached maximum 1.658, 1.655 and 1.682 at 0.05, 0.10 and 0.20% steroidal saponins, then decreased to 1.604, 1.617 and 1.624 at incubation 24 h, respectively, lower than the control. This may suggest that the add of *Fructus tribuli* steroidal saponins plays an active role on the growth of *Lactobacillus acidophilus* when incubate in a short time (18 h), but if the incubation time last long (24 h), this promotion effect would lose.

The pH of different culture mediums in contrast with the OD₆₀₀ has significant difference ranging from 3.84 to 4.29 (incubation time 12 h), 3.69 to 4.05 (incubation time 18 h) and 3.72 to 4.06 (incubation time 24 h), which indicated that steroidal saponins on growth

of *Lactobacillus acidophilus* LA06 has an obvious effect and the optimum concentration of steroidal saponins in MRS media was 0.25% at incubation 18 h.

CONCLUSION

Addition of steroidal saponins of *Fructus tribuli* in MRS broth has the significant promotion on growth of *Lactobacillus acidophilus* LA04, LA05 and LA06. The optimum concentration of steroidal saponins in MRS media was all 0.25% for the three strains.

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