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## Evaluation of antibacterial activity of silymarin against enteric bacterial pathogens

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### Abstract

Enteric bacterial pathogens cause various diseases and the major problem of these bacteria is antimicrobial resistance. Silymarin is a standardized extract from the milk thistle clinically has used as a hepatoprotective agent. The aim of this study was to investigate the antibacterial activity of silymarin against enteric bacterial pathogen strains. For evaluation of antibacterial activity, different concentrations of silymarin (ranging from 2-128 µg/mL) were tested against twelve enteric bacterial pathogens by microdilution broth method. The results of study showed that the minimal inhibitory concentration (MIC) of silymarin against all of tested strains was >128 µg/mL. It appears that silymarin is not beneficial drug for treatment of gram negative enteric bacterial pathogens.

**Keywords:** Silymarin, antibacterial activity, enteric bacterial pathogens

### 1. Introduction

Infections caused by enteric pathogens are an important cause of morbidity and mortality in developing countries that lead to the death of 3.3 to 6.0 million children and adults annually. Enteric bacterial pathogens are major cause of sporadic and epidemic diarrhea and gastroenteritis both in children and adults [1]. Antibiotics are mainly used for the treatment or prevention of infectious disease. Clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens. In addition, antibiotics are sometimes associated with adverse effects. So, there is increasing interest in medicinal plants as source of antimicrobial agents for the treatment of infectious diseases [2, 3].

Silymarin, an extract from the milk thistle (*Silybum marianum* L. Gaertn) plant, has been used from ancient times as herbal medicine. It has antioxidant, anti-inflammatory, anticarcinogenic, antimetastatic, antifibrotic, antiangiogenic and hepatoprotective effects [4]. Nowadays, it has been used as an alternative medicine for liver disorders. It is well-known that silymarin has antiviral effects against Hepatitis B and especially Hepatitis C viruses [5-8]. However, there are limited data about its antibacterial activity against enteric bacterial pathogens [9-11].

Because of wide usage of silymarin in medicine, the aim of this study is to determine its antibacterial effect against enteric bacterial pathogens.

### 2. Materials and Methods

#### 2.1. Silymarin preparation

The silymarin powder was purchased from Sigma (Seelze, Germany). Stock solution of silymarin was prepared at a concentration of 60 mg/mL in 1mL of dimethyl sulfoxide (DMSO).

#### 2.2. Bacterial strains

In the present study, 10 clinical bacterial strains including *Salmonella paratyphi* A, *Salmonella typhi*, *Salmonella paratyphi* B, *Salmonella paratyphi* C, *Salmonella typhimurium*, *Shigella flexneri*, *Shigella sonnei*, Enteropathogenic *E.coli*, *Vibrio cholerae* serotype Ogawa, *Vibrio cholerae* serotype Inaba and two ATCC strains: *E. coli* ATCC25922, *Shigella* ATCC12022 were tested.

#### 2.3. Determination of Antibacterial activity

The minimum inhibitory concentration (MIC) of silymarin was determined by microdilution broth method according to the CLSI recommendations. Bacterial strains were cultured on blood agar for 24 hours.

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Then several colonies inoculated in normal saline and its turbidity adjusted to 0.5 McFarland standard. Twofold serial dilution of different concentrations of silymarin (ranging from 4-256µg/ml) was prepared in Muller-Hinton broth and was added to each well. A standardized suspension of test bacteria was added to sterile U-bottom 96-well polystyrene tissue culture microtiter plates to obtain a final concentration of approximately  $1.5 \times 10^5$  CFU/ml. Plate contained an uninoculated control well and a positive growth control well. For all bacterial strains, the antibacterial effect of DMSO was checked. After 24 hours of incubation at 37 °C, the MIC was determined as the lowest concentration of the agent that completely inhibits visible growth as evaluated by naked eye.

### 3. Results & Discussion

Inhibitory effects of silymarin against different selected gram negative strains were performed at various concentrations from 2, 4, 8, 16, 32, 64, 128 µg/ml.

The minimal inhibitory concentration of silymarin against all of the 12 gram negative bacteria was >128 µg/mL. Turbidity of *Shigella* strains were lower than other tested strains at 64 and 128 µg/mL concentrations, demonstrating decrease of growth and the number of bacteria.

Among the herbal medicine, silymarin is widely used because of its hepatoprotective effects and minimal adverse effects [4, 12, 13].

Since, there were a few studies about effect silymarin against bacteria causing gastrointestinal infections. Current research is the first report on evaluating the antibacterial activity of silymarin against various important species of gram negative enteric pathogen bacteria specially *Vibrio* and *Salmonella* serotypes.

Results showed not significantly antibacterial effect against all tested organisms. Present findings are in agreement with observation of other researchers [9, 11, 14, 15]. According to our results there was a low growth of shigella strains that is consistent with Ahmad study which obtained 50% inhibition [16].

Although most of studies revealed inhibitory effect of silymarin on some gram positive bacteria [9, 11, 15], but a few ones showed this effect in some gram negative bacteria [10].

These conflicting results can be due to :1) different compounds and material preparation, purified silymarin fraction use in this study whereas other experiments use crude extract of plant, 2) different doses of silymarin were used in various studies, 3) different methods were applied for antimicrobial assay.

### 4. Conclusions

In conclusion, it seems that silymarin is not suitable candidate for treatment of gram negative enteric bacterial pathogens.

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