

## Research Article



## SEPARATION OF PROTEOLYTIC COMPONENTS OF IRAN *FICUS CARICA* LATEX BY COLUMN CHROMATOGRAPHY AND ELECTROPHORESIS AND LATEX ANTI- CORN ACTIVITY

F. Pourmorad<sup>\*</sup>, S. Honary, M. Azadbakht, H. Asgarirad, G. Golmohammadzadeh

Department of Medicinal Chemistry, Faculty of Pharmacy, Mazandaran University of Medical Sciences, Sari, Iran.

\*Corresponding author's E-mail: [pourmoradf@yahoo.com](mailto:pourmoradf@yahoo.com)

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### ABSTRACT

There are different species of *Ficus* (fig tree) which its various parts are used in traditional medicine in Iran. Environmental conditions cause significant impact on *Ficus* latex activity. Using crude latex with proteolytic activity by people for corn and wart led us to study the proteolytic activity of *Ficus carica* L. var. *genuina* latex. Ficin specific activity in latex measured by casein method and obtained 833.4 unites per mg of crystalline ficin. Electrophoresis and column chromatography of the latex showed the existence of proteolytic components. Plasters containing crude latex were administered to volunteers affected by corn. Iran *Ficus* latex showed considerable proteolytic activity in corn management.

**Keywords:** *Ficus*, column chromatography, electrophoresis, Ficin.

### INTRODUCTION

Fig tree, *Ficus carica* L. [Fam. Moraceae], has more than 800 varieties with medicinal properties. Figs have documented as anti-ulcer, antidiabetic, antibacterial and antiparasitic and antioxidant<sup>1, 2-8</sup>. Compounds from the latex of *Ficus carica*, as well as synthetic analogs, have been shown to suppress abnormal cell proliferation<sup>2</sup>. Also some angiotension converting enzyme inhibitors were separated from the latex<sup>9</sup>. Recently, an unusual thermostable aspartic protease isolated from the latex of *Ficus racemosa*<sup>10</sup>. The proteolytic activity of latex is believed that is from an enzyme named ficin. This enzyme is similar to papain in structure and it contains sulfhydryl group as the main involving part for the proteolytic activity. The proteolytic activity of the latex can be determined by hydrolysis of casein or synthetic compounds in vivo and in vitro. The casein method for proteolytic activity determination is based upon the estimation of the amount of the small molecular weight digestion products (trichloroacetic acid- soluble material) formed from proteins in presence of the enzyme. The method is involved by measurement of the absorbance of the trichloroacetic acid filtrate at 280 nm<sup>11</sup>. Warts and corns are non-cancerous skin growths in the top layer of the skin. There are some treatment for these symptoms like applying salicylic acid gel, solution or plaster, "painting" with cantharidin, cryotherapy (freezing), electrosurgery (burning), laser treatment, inject each wart with an anti-cancer drug called bleomycin and interferon and Immunotherapy<sup>12</sup>. The proteolytic activity of latex in different species of the genus *Ficus* is quite different. It is even different in different varieties of the same species of *Ficus*. In folk medicine it has been used as a remedy for treatment of warts and corns with significant response. It is necessary to determine the activity of the latex to use it as a pharmaceutical remedy. Therefore, we decided to extract the active compound,

determine the activity and prepare a pharmaceutical form in order to perform a preliminary clinical study.

### MATERIALS AND METHODS

#### Chemicals

Crude latex was taken from *Ficus Carica* in Mazandaran province of Iran in September. All the compounds and reagents were purchased from Merck or Fluka Company. Crystalline ficin was prepared according to Walti<sup>13</sup>.

#### Determination of activity by casein method

A stock solution of casein made by suspending of 1 g. of casein in 100 ml of sodium phosphate buffer (0.1 M, pH 7.0) containing EDTA (0.001 M) and mercaptoethanol (0.007 M). The suspension was heated for 15 minutes in boiling water and it was stored in the refrigerator for one week. Prior to use, the suspension was placed in a water bath at 35° C for at least 5 minutes.

A solution of crystalline ficin was poured into the centrifuge tubes and the volume in each tube was brought to 1 ml with buffer and the tubes were placed in the water bath. 1 ml of casein solution was added into the tubes at 30 seconds intervals. 3 ml of 5% trichloroacetic acid (TCA) was added to each tube exactly 20 minutes after the addition of casein. The content of the tubes were mixed well. The tubes were removed from the bath, allowed to stand for an hour, and centrifuged for 20 minutes. The absorbance of the supernatants was read at 280 nm. The reading was corrected for the values of blanks. One unite of enzyme activity was determined as the activity which gives rise, under the proper conditions, to an increase of one unite of absorbency at 280 nm per minute digestion.



### Separation of proteolytic fractions from latex by column chromatography and electrophoresis

The latex of ficus fractionated by column chromatography (3cm× 70 cm) over silica gel eluting with solvents (table 1). First elution was started with hexane then polarity increased with chloroform and methanol. The fractions were evaporated in vacuo. Fractions were collected and combined according to TLC results. Electrophoresis of crude latex carried out to observe the proteolytic components in this variety of *Ficus*.

**Table 1:** Separation of *Ficus* latex fractions by column chromatography

Fraction	Eluent
1-25	CHCl <sub>3</sub> - CH <sub>3</sub> OH 95: 5
26- 34	CHCl <sub>3</sub> - CH <sub>3</sub> OH 90: 10
35- 50	CHCl <sub>3</sub> - CH <sub>3</sub> OH 80: 20
51- 55	CHCl <sub>3</sub> - CH <sub>3</sub> OH 70: 30
55- 70	CHCl <sub>3</sub> - CH <sub>3</sub> OH 50: 50
71- 101	CHCl <sub>3</sub> - CH <sub>3</sub> OH 40: 60
102- 200	CH <sub>3</sub> OH

### Preparation of plasters

Plasters contained 0.8 ml of latex were prepared and administered to 20 volunteers suffering from corn with an instruction in order to proper use.

### Statistical Analyses

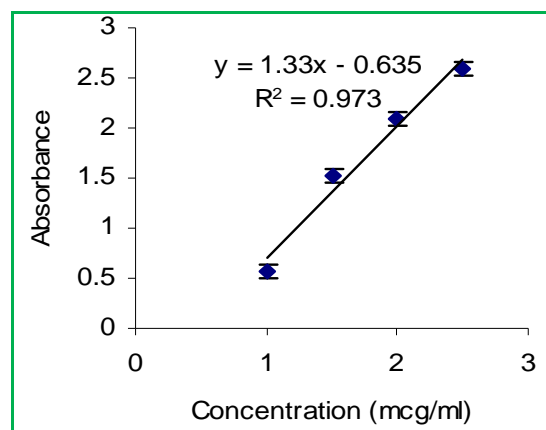
All values are expressed as mean ± S.E. Statistical analyses were performed by Student's *t*-test. The values of *p* lower than 0.05 were considered statistically significant (*p* is probability).

## RESULTS AND DISCUSSION

There are more than 1800 species of *Ficus* and at least 800 varieties of *Ficus carica* that each variety has its specific proteolytic activity. Studies of the ficin content of different species of the genus *Ficus* have revealed significant differences in proteolytic activity. Of 46 species of *Ficus* examined, only 13 exhibited appreciable proteolytic activity; the latex of *F. stenocarpa* had the highest specific activity, followed closely by latices of *F. carica* and *F. glabrata*. A total of as many as 26 chromatographically distinct active components were detected in 6 species of *Ficus*.

Variations in activity have also been observed in different varieties of the same species of *Ficus*. For example, in the latices of 9 varieties of *F. carica*, a total of at least 16 chromatographically distinct components were detected, the *Kadota* variety alone showing 10 distinct active components (6). So each variety has a proper activity and should be detect separately. In this study 4 proteolytic components were found by electrophoresis and 9 were detected by the column chromatography of latex. The crystalline ficin was prepared from crude latex in 20% yield by adjusting pH. The crystalline ficin was identified

by casein digesting method, inactivation by I<sub>2</sub> and hydrogen peroxide and IR spectroscopy. In casein method the activity of ficin was determined as 1.2 µg of ficin in each unites of proteolytic enzyme (Figure 1). The specific activity was 833.4 unites per mg of the crystalline ficin. Each plaster contained 0.83 g ficin. *Ficus* latex plaster was given to 20 volunteers who were suffering from corn in their feet. It was administered through 5-10 days depends on the corn size, twice a day. More than 70% of the patients had satisfaction regarding to the plaster effectiveness. In this study latex of *Ficus* showed a specific chromatographic profile and electrophoresis in compare with other species mentioned above. The proteolytic activity of latex due to proteolytic enzyme such as ficin caused a considerable anti-corn activity in the volunteers.



**Figure 1:** Standard curve of ficin in casein digest method at different times

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