ISOLATION FUNGAL ENDOPHYTES OF *CHELIDONIUM MAJUS L.* AND IDENTIFY THEIR ANTI-INFLAMMATORY ACTIVITIES.

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Abstract. *Chelidonium majus* L. is rich in many isoquinoline alkaloids which are responsible for the anti-inflammatory, antitumor and cytotoxic bioactivities known for this plant. In the literature, these alkaloids can be found in *Chelidonium majus L.* endophytes has been shown to produce that live in its host plant. In this work, *Chelidonium majus L.* the anti-inflammatory activity of plant endophytes has been studied through Inhibition of Albumin Denaturation and Heat-induced hemolysis methods. Results showed that the anti-inflammatory activity of which is identified through method of Inhibition of Albumin Denaturation the endophyte FK23 fungus demonstrated 79.24% a pointer almost too close to diclofenac sodium. Heat-induced hemolysis method has shown that, aspirin's anti-inflammatory activity at 500 mcg/ml concentrations of the samples was 57.97%, FK14 ethanol fraction was 60.94%, FK23 ethanol fraction was 59.59%. Significantly, the anti-inflammatory activity of endophyte fungi showed activity even higher than aspirin.

Key words*:* *Chelidonium majus L*, endophytes, anti-inflammatory activity, Albumin Denaturation, hemolysis.

Introduction

Endophytes are microorganisms living inside plant tissues that synthesize various bioactive compounds, including alkaloids. These substances have significant pharmacological potential, including anti-inflammatory, antibacterial and antitumor properties, which makes endophytes promising objects for the development of new drugs[13].

Celandine (*Chelidonium majus L.*) due to the long presence of such chelidonin and sanguinarine alkaloids, its medicinal properties have been reported in traditional medicine [11]. Research shows that the endophytes of this plant can synthesize similar compounds, which opens up new opportunities for the use of these microorganisms in biotechnology.

The purpose of this study is to isolate celandine endophytes, screen their alkaloid composition and evaluate their anti—inflammatory activity. This will allow us to identify strains with high pharmacological potential, which can become the basis for the creation of new drugs based on natural compounds.

2.MATERIALS AND METHODS

2.1Isolation of endophytic fungi.

The collected plants were briefly washed under running tap water and the stems were cut into 2–3 cm long pieces. These pieces were rinsed in sterile water and then surface disinfected by soaking in 70% ethanol for 30 sec and 10% mercuric chloride (NaOCl) solution for 1 min. The disinfected stem pieces were then rinsed extensively in sterile water and drained. Next, they were cut longitudinally with a sterile scalpel and laid, with the exposed inner surface facing downwards,

on plates potato dextrose agar (PDA) (Hung and Annapurna, 2004). As controls, uncut, surface-disinfected stem pieces and non-disinfected pieces were also placed on the same agar. All plates were incubated for several days at 28°C.

2.2Preparation of Endophytic Fungi Extracts

The endophytic fungi isolates were inoculated, respectively, into 500 mL Erlenmeyer flasks containing 100 mL of potato dextrose liquid medium and cultured at 28 °C with 140recycles/minute for 7 days in a rotary shaker. Then biomass and cultured liquid were dried together in lyophilise. The resulting mass was dried 3days at 40^0C. Dried mycelia were crushed and extracted with 70% ethanol, the extracts were filtered and the filtrates were concentrated with a rotary evaporator.

2.3. Qualitative analysis of alkaloids of *Chelidonium majus L* endophytes. Through the Dragandorf and Wagner's tests, a qualitative analysis of the fungal isolates from which the plant was isolated was carried out[12].

Dragendorf test. 1 mL Dragendorff"reagent is added to 2 mL of the extract to form a purple red precipitate indicating the presence of alkaloids.

Wagner's test: Potassium iodide (2 g) and iodine (1.27 g) were dissolved in distilled water (5 mL) and the solution was diluted to 100 mL with distilled water. Few drops of this solution were added to the filtrate; a brown colored precipitate indicates the presence of alkaloids.

2.4. In vitro anti-inflammatory activity

2.4.1Inhibition of Albumin Denaturation

The 5ml of reaction mixture was comprised of 0.2ml of eggs albumin (from hens egg), 2.8ml of phosphate buffered saline (PBS, pH 6.4) and 2ml of varying concentration of extracts. Similar volume of double distilled water served a control. Then the mixture was incubated at 37^0C in BOD incubator for about 15 mins and then heated at 70^0 C for 5 mins. After cooling, their absorbance was measured at 660nm by using pure blank. Diclofenac sodium (standard drug) was used as reference drug and treated as such for determination of absorbance [3]. The percentage inhibition of protein denaturation was calculated as mentioned in membrane stabilization assay

$$\text{Percent inhibition} = \frac{\text{Abs}_{\text{Control}} - \text{Abs}_{\text{treated}}}{\text{Abs}_{\text{Control}}} \times 100$$

2.4.2Membrane Stabilization Property

Prepauration of Red Blood Cells (RBCs) Sspension [9].

The blood was obtained from a healthy human volunteer who had not taken any nonsteroidal anti-inflammatory drugs for 2 weeks before the experiment. The blood sample was centrifuged at 3000 rpm for 10 min and was washed 3 times with equal volume of normal saline. The amount of blood was measured and re-constituted as 10% v/v in normal saline suspension.

Heat-induced hemolysis.

The activity was done according to the protocol of Sakat et al. and Shinde et al. [9,10] with some modifications. The reaction mixture (2 ml) comprised 1 ml test sample at different concentrations (100 -500 µg/ml) and 10% RBCs suspension (1ml). For control, only saline was added to the test tube. Aspirin as a reference was used. The reaction mixture was incubated in water bath for 30 min, at 56°C. The reaction was stopped by cooling the reaction mixture under running tap water. The reaction mixture was then centrifuged at 2500 rpm for 5

min. The supernatant collected was used to take absorbance at 560 nm. The percentage inhibition of hemolysis was calculated as follows:

$$\text{Percent inhibition} = \frac{Abs_{Control} - Abs_{treated}}{Abs_{Control}} \times 100$$

3.RESULTS AND DISCUSSION

More than 20 alkaloids have been identified in the *Chelidonium majus* plant, of which the most well-known are isoquinoline alkaloids, which are included in the list of anticancer drugs [11]. Endophytic fungi have been isolated from various parts of *Chelidonium majus*, including the root, stem, leaves and flowers. As a result, 28 pure isolates of fungi were obtained (Fig.1).



Fig.1 Isolation fungal endophytes of *Chelidonium majus L.*

A qualitative analysis of endophytic extracts for the presence of alkaloids was carried out using Dragendorff and Wagner reagents [12]. According to the results of two methods, 2 fungal isolates - FK14 and FK23 showed the most intense positive reaction to the presence of alkaloids and were selected for further studies on anti-inflammatory activity.

The results of the conducted studies demonstrated that the maximum antiinflammatory activity was recorded for the fungal isolate FK23, amounting to 79.4%, and for the isolate FK14 - 70.8% at a concentration of 500 µg/ml. These indicators were comparable to the activity of diclofenac sodium, which reached 81.1%. It is especially noteworthy that the anti-inflammatory activity of the endophytic fungus FK23 turned out to be practically comparable to the action of diclofenac sodium(Fig.2).

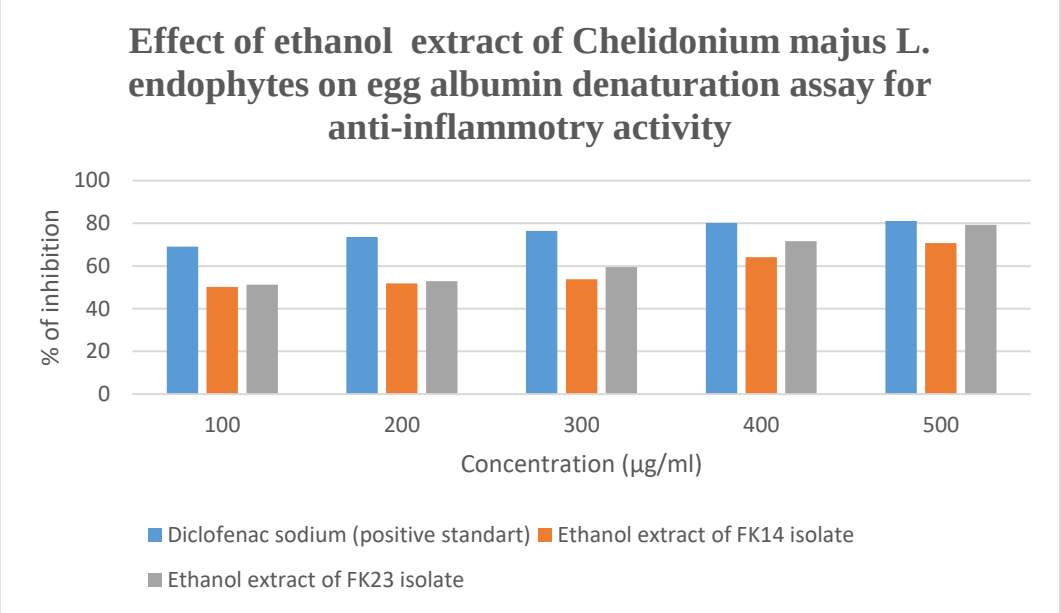


Fig.2 **Effect of ethanol extract of Chelidonium majus L. endophytes on egg albumin denaturation assay for anti-inflammotry activity**

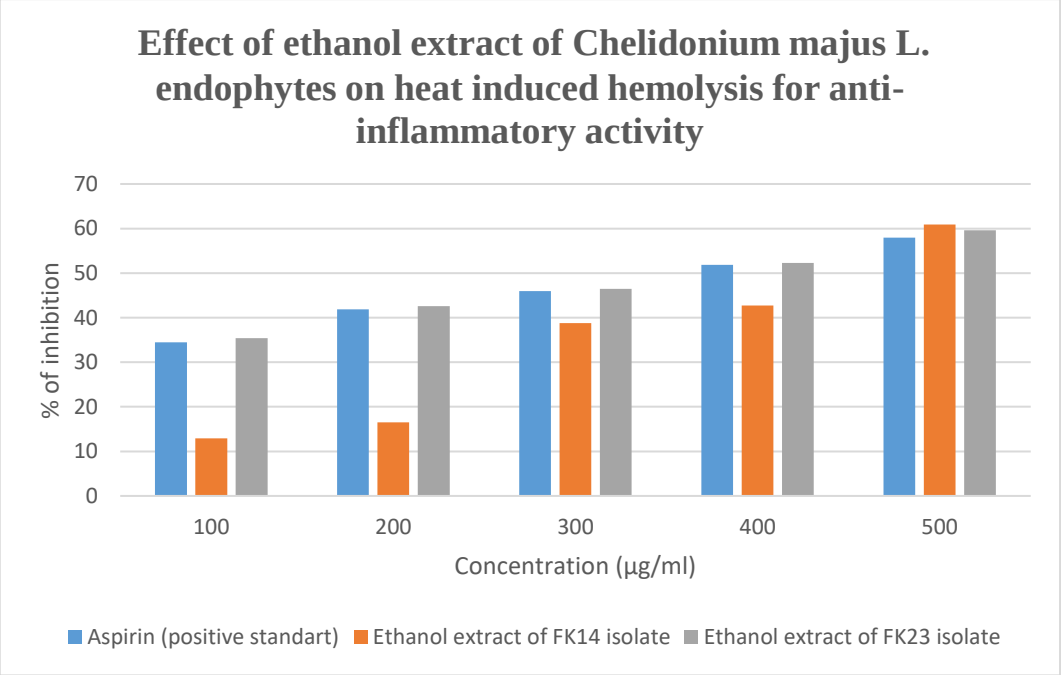


Fig.3 **Effect of ethanol extract of Chelidonium majus L. endophytes on heat induced hemolysis for anti-inflammatory activity**

On heat induced hemolysis for anti-inflammatory activity method, the activity of isolate FK23 was 59.6%, and isolate FK14 was 60.9%, which exceeds the same indicator of aspirin, equal to 57.9%(Fig.3).

These results indicate a significant anti-inflammatory potential of fungal isolates FK23 and FK14 in comparison with widely used anti-inflammatory drugs. Thus, it was revealed that celandine endophytes demonstrate pronounced anti-inflammatory potential, which opens up prospects for the creation of new medicines based on these natural microorganisms.

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