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Research Article

EVALUATION OF THE ANTI-INFLAMMATORY ACTIVITY OF VETIVER ROOT EXTRACT IN DAK LAK, VIET NAM

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ABSTRACT

Vetiver grass (Vetiveria zizanioides L.*) is a perennial grass, native to India and now cultivated in many parts of the tropics. Many studies have demonstrated the antioxidant capacity (one of the factors that can lead to inflammation) and the ability to reduce leukocyte migration (important in inflammatory responses) of Vetiver root. However, there is still limited research on this plant involving anti-inflammatory properties in Viet Nam. In this study, we examined the anti-inflammatory activity of the ethanol extract from Vetiver roots through the albumin protein denaturation model. The results show that ethanol extract from Vetiver roots showed good anti-inflammatory activity with* IC_{50} *= 157.63 ± 4.89 µg/mL. The results of this study will expand our knowledge about the anti-inflammatory activity of Vetiver root, contributing to the treatment of inflammatory symptoms in many diseases.*

Keywords: anti-inflammatory; extract; Vetiver grass; *Vetiveria zizanioides* L.

1. Introduction

Inflammation is part of the body's immune response to harmful agents, infections, trauma, or damage to tissues (Calixto et al., 2004; Chen et al., 2018). The manifestations of inflammation commonly seen are swelling, heat, redness, and pain. These inflammatory reactions are created as a mechanism to protect the body or tissues. However, when immune cells become overactive, inflammation can be negatively impacted by the effects of many molecules produced during inflammation (Grivennikov et al., 2010; Gaestel et al., 2009). If the inflammatory response is not controlled, it can progress to multiple chronic inflammatory diseases (Gaestel et al., 2009).

Currently, pain and inflammation are usually controlled with steroid (corticosteroid) or non-steroidal (aspirin) anti-inflammatory drugs. However, they cause many side effects such as allergies, hearing loss, and kidney failure. These drugs also increase the risk of

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bleeding by altering platelet function (Thomas et al., 2000). For centuries, humans have used a variety of biologically active compounds from medicinal plants in raw or purified forms to treat various disease conditions, including inflammatory reactions (Lalrinzuali et al., 2016).

Vetiver grass (*Vetiveria zizanioides* L.) is a perennial grass, native to India and now cultivated in many parts of the tropics. In Vietnam, Vetiver grass is grown mostly in the two provinces of Nghe An and Thai Binh. Many studies have demonstrated the antioxidant capacity (one of the factors that can lead to inflammation) of Vetiver essential oil (Hyun-Jin et al., 2005). Furthermore, Vetiver essential oil has also been shown to reduce leukocyte migration (important in inflammatory responses) (Grover et al., 2021; Lima et al., 2012). These studies show that Vetiver has a lot of potential to extract anti-inflammatory compounds. However, at present, the anti-inflammatory activity of Vetiver has not been fully demonstrated.

Therefore, we decided to investigate the anti-inflammatory activity of Vetiver root extract to provide more data on the biological activity of this species.

2. Materials and methods

2.1. Materials

Five kilograms of Vetiver roots were collected from October to November 2022 in Dak Lak, Viet Nam.

2.2. Method of obtaining extracts from Vetiver roots

Vetiver roots, after being collected, are washed, chopped, and dried at a temperature of 40 - 45ºC. The dried samples were ground into powder and soaked in ethanol at a ratio of 1:10 (g/ml) for 48 hours. After 48 hours, the extract was filtered through filter paper, the residue was removed, the solvent was evaporated, and the extract was collected at 50ºC and 175 mbar pressure. The extract was left to dry naturally, storing it at 4ºC for future use.

2.3. In vitro anti-inflammatory activity assay

Principle: Albumin is a protein occupying a very large proportion of the serum. At temperatures above 50ºC, albumin begins to denature, which is associated with an inflammatory response. The anti-inflammatory activity of the Vetiver root extract was demonstrated through its inhibitory activity on albumin denaturation.

Implementation: The reaction consisted of 150 µL of extract with 150 µL of 5% bovine serum albumin solution. Then, the reaction mixture was incubated at 27ºC for 15 minutes. Next, the reaction mixture was kept at 60ºC for 10 minutes to denature the protein. The sample was measured absorbance spectrophotometrically at 660 nm. The inhibition rate of albumin processing of the extract was calculated according to the following formula:

$$\frac{OD_c - OD_s}{OD_c}.100\%$$

OD_c – The OD value of the negative control

OD_s – The OD value of the extract.

Diclofenac is an anti-inflammatory that will be used as a positive control in the experiments.

2.4. Statistical analysis

The percentage of albumin protein denaturation, standard deviation, and IC_{50} value of the extract were processed on Microsoft Excel 2016. The experiments in the project were performed at least three times, and the results were statistically significant when $p < 0.05$. Data are presented as mean ± standard deviation (SD).

3. Results and discussion

3.1. Determination of the IC_{50} value of Diclofenac

Diclofenac is a nonsteroidal anti-inflammatory drug widely used to treat pain and to help relieve symptoms of arthritis such as inflammation, swelling, stiffness, and pain in the joints. In this project, Diclofenac was used as a positive control for the experiments.

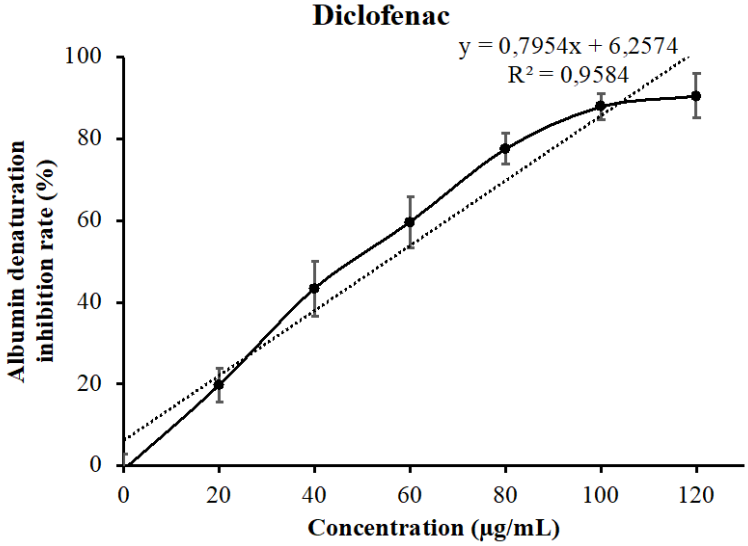

Figure 1. *Diclofenac anti-inflammatory activity*

Diclofenac's anti-inflammatory activity was investigated by the group in the concentration range from 0 to 120 µg/mL. The results are shown in Figure 1.

Figure 1 illustrates that the anti-inflammatory activity of Diclofenac increased linearly with concentration with a high correlation ($R^2 = 0.9584$). The results show that the IC_{50} value of Diclofenac is 55.76 ± 2.35 µg/mL. Therefore, Diclofenac at a concentration of 55.76 µg/mL will be used as a positive control for future experiments.

3.2. Determination of the anti-inflammatory activity of ethanol extract from Vetiver roots

Inflammation is a protective response to the body to eliminate exogenous and endogenous harmful substances produced by damaging stimuli (Calixto et al., 2004). However, if the inflammatory response is not controlled, it can progress to a variety of chronic inflammatory diseases (Grivennikov et al., 2010). Many studies have proven that

protein denaturation is one of the causes of rheumatoid arthritis (Gaestel et al., 2009). Therefore, in this study, the bovine serum albumin variable model was used to investigate anti-inflammatory activity.

In this study, the anti-inflammatory activity of the ethanol extract from Vetiver roots was investigated at the concentration range from 0 to 240 µg/mL. The results are shown in Figure 2.

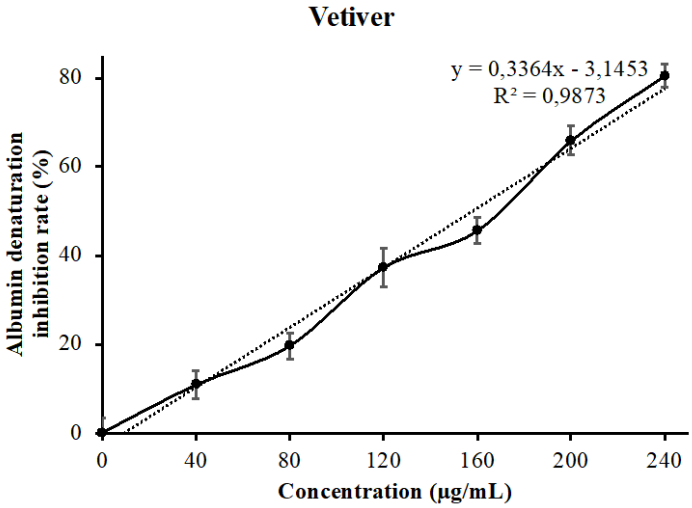

Figure 2. *Anti-inflammatory activity of the ethanol extract from Vetiver roots*

Figure 2 shows that the anti-inflammatory activity of the ethanol extract from Vetiver roots increased linearly with concentration with a high correlation (R^2 = 0.9873). The IC_{50} value of the extract to be 157.63 ± 4.89 µg/mL.

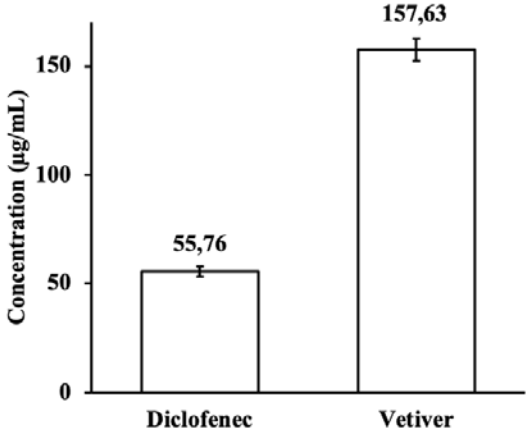

Figure 3. *Comparison of IC_{50} values of Diclofenac and Vetiver extract*

(Statistically significant difference with p-value < 0.01)

Moreover, when comparing the IC_{50} value of Vetiver extract with Diclofenac, it is seen that the activity of Diclofenac was about 2.8 ± 0.14 times higher than that of Vetiver extract (Figure 3). This proves that the compounds in Vetiver extract have high anti-inflammatory potential. The anti-inflammatory activity of Vetiver may be due to the presence of several

compounds that are derivatives of vetiverol and vetivones that have been investigated previously (Grover et al., 2021).

This result is also consistent with some other studies on the anti-inflammatory activity of Vetiver grass. For example, Vetiver essential oil was able to inhibit the migration of leukocytes to the site of carrageenan-induced inflammation (Gabrielle et al., 2012). Moreover, Vetiver essential oil exhibits potent anti-proliferative activity and also significantly inhibits collagen III production by fibroblasts, which may reduce the release of inflammatory mediators and thus reduce the inflammatory process (Han et al., 2017).

4. Conclusion

In this study, we examined the anti-inflammatory activity of the ethanol extract from Vetiver roots through the albumin protein denaturation model. The results showed that the ethanol extract from the roots of Vetiver had good anti-inflammatory activity with IC_{50} = 157.63 ± 4.89 µg/mL. Further studies on the anti-inflammatory activity of Vetiver grass will help in the treatment of inflammatory complications in many diseases.

❖ ***Conflict of Interest:*** *Authors have no conflict of interest to declare.*

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ĐÁNH GIÁ HOẠT TÍNH KHÁNG VIÊM CỦA CAO CHIẾT TỪ RỄ CỎ VETIVER TẠI ĐẮK LẮK, VIỆT NAM

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TÓM TẮT

Cỏ Vetiver (Vetiveria zizanioides L.*) là loại cỏ lâu năm, có nguồn gốc từ Ấn Độ và hiện được trồng ở nhiều nơi ở vùng nhiệt đới. Nhiều nghiên cứu đã chứng minh cỏ Vetiver có khả năng kháng oxy hóa (yếu tố có thể dẫn đến viêm) và giảm sự di chuyển của bạch cầu (một trong các giai đoạn của phản ứng viêm). Tuy nhiên, ở Việt Nam, các nghiên cứu khoa học về khả năng kháng viêm của cỏ Vetiver còn nhiều hạn chế. Trong nghiên cứu này, chúng tôi kiểm tra hoạt tính kháng viêm của cao chiết ethanol từ rễ cỏ Vetiver thông qua mô hình biến tính protein albumin. Kết quả cho thấy cao chiết ethanol từ rễ cỏ Vetiver có hoạt tính kháng viêm tốt với IC_{50} = 157.63 ± 4.89 µg/mL. Kết quả nghiên cứu này sẽ mở rộng hiểu biết của chúng ta về hoạt tính kháng viêm của rễ cỏ Vetiver, góp phần điều trị các triệu chứng viêm trong nhiều bệnh lí.*

Từ khóa: kháng viêm; cao chiết; cỏ Vetiver; *Vetiveria zizanioides* L.