

Optimization of Anti-Inflammatory Activity of *Rauvolfia tetraphylla* L. Crude Extracts Using Response Surface Methodology

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ABSTRACT

Background: The background of this study revolves around *Rauvolfia tetraphylla* and its potential anti-inflammatory properties. To explore the anti-inflammatory activity of crude extracts obtained from the leaf and fruit of *R. tetraphylla*, with the goal of identifying natural compounds that could serve as safer and more effective alternatives to conventional non-steroidal anti-inflammatory drugs. **Objectives:** Aim of the study is to identify the most potent anti-inflammatory activity from *Rauvolfia tetraphylla* and optimizing the synthesis using Response Surface Methodology (RSM). The RSM analysis is to determine the influence of selected independent variables on dependent variables of the anti-inflammatory properties of *R. tetraphylla*, an endangered medicinal shrub. **Materials and Methods:** The anti-inflammatory activity of various crude extracts (hexane, chloroform, ethyl acetate and methanol) derived from the leaf and fruit of *R. tetraphylla*, was investigated using an *in vitro* assay based on denaturation of protein and utilize Response Surface Methodology to optimize the anti-inflammatory activity of the extracts using bovine serum albumin as the test protein. **Results:** The results showed that the ethyl acetate extract of fruit exhibited greatest inhibitory effect. Furthermore, the 3D response surface plot, perturbation plot, and contour plot were successfully obtained, indicating the interdependence of the four factors on the anti-inflammatory activity. By optimizing the parameters, it may be possible to enhance the anti-inflammatory activity, thereby maximizing the plant's therapeutic potential. **Conclusion:** These findings suggest that *R. tetraphylla* leaf and fruit could serve as a potential source of anti-inflammatory agents with better safety and efficacy profiles compared to Non-Steroidal Anti-inflammatory Drugs.

Keywords: *Rauvolfia tetraphylla*, Anti-inflammatory, Protein denaturation, RSM, Protein denaturation.

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Received: 05-07-2023;

Revised: 07-10-2023;

Accepted: 15-05-2024.

INTRODUCTION

The plant *Rauvolfia tetraphylla* is a perennial evergreen shrub belonging to the family Apocynaceae. It reaches a height up to 6 ft. Apocynaceae, also known as the dogbane family, is classified under the order Gentianales.¹ The plant distributed across Mexico, Central America, the West Indies and parts of South America. It has also been introduced and established in various other regions such as India, Australia, Pakistan, Sri Lanka, China, Bhutan, Bangladesh, Indonesia, and Myanmar. In India, it is commonly found in many states including Tamil Nadu, Andhra Pradesh, Karnataka, Kerala, Bihar, Orissa, West Bengal, Madhya Pradesh, Jammu and Kashmir, and Assam.^{2,3} The traditional use

of *Rauvolfia* got attention in the past 300 years ago due to the presence of various pharmacological activities.⁴ The paste of whole plant is used to treat a skin disease which was reported in Kancheepuram, Tamil Nadu;⁵ Bhadrak, Odisha;⁶ Ranga Reddy, Telangana;⁷ Coimbatore and Ooty, Tamil Nadu.⁸ *R. tetraphylla* has been explored for its biological potential through phytochemical analysis,^{9,10} antibacterial activity,¹¹⁻¹³ antioxidant activity,^{14,15} cytotoxic activity,^{16,17} and cardio-protective activity.¹⁸ It is known to possess important medicinal properties such as snake bite, blood pressure, stomach pain, mental disorder, cholera, headache, eye disease, fever, diarrhea, skin diseases, chronic wound, insect bite and dysentery.¹⁹⁻²¹ The phytochemical constituents present in *R. tetraphylla* include indole alkaloids such as ajmalicine, yohimbine, reserpine, demethylserpentine, mitoridine, sarpagine, serpentine, reserpiline, and yohimbine.²² The methanol extracts of the root, stem and leaves contain reserpine.²³ The ethanol extract of aerial parts contains five indole alkaloids known as rauvotetraphyllines A-E (1-5).⁹ Additionally, there are five hybrid monoterpeneoid



DOI: 10.5530/ijper.58.3s.94

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