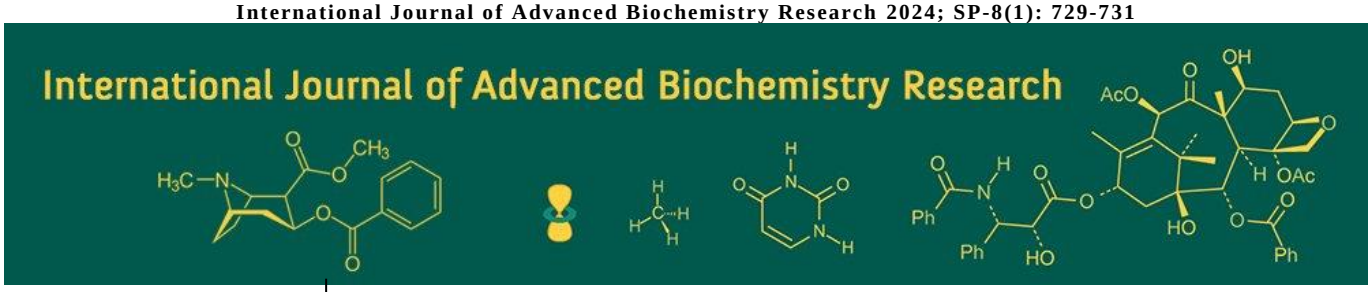

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Vasicine a quinazoline alkaloid from *Justicia adhatoda* L.: Its antioxidant property

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Abstract
Justicia adhatoda plant of Acanthaceae family has been used as an ayurvedic and unani medicine in India for over a long time. It is used in the treatment of several diseases, especially respiratory ailments. The alkaloids in adhatoda are found to have many pharmacological properties. Vasicine, a pyrroloquinoline alkaloid from adhatoda is a major alkaloid that can be potentially used in treatment of various disorders. Antioxidant activity of vasicine is measured in the current study. The results indicate that the inhibition concentration IC_{50} of vasicine is 187 µg/ml.

Keywords: Vasicine, DPPH, adhatoda

Introduction
Justicia adhatoda Linnaeus. plant belongs to the family Acanthaceae. It is a perennial and evergreen shrub of 1–2.5 m high, leaves are simple, 7–19 cm long and 4–7 cm wide with unpleasant smell and bitter taste. The flowers are white, pink or purple. Plants of this genus are widespread throughout the tropical regions of Southeast Asia and throughout the Indian peninsula up to an altitude of 1300 m. It is commonly known as malabar nut. It is also called as *Adhatoda vasica*, *Adhatoda zeylanica Medic*. and the Sanskrit name vasaka. The plant has been used in the indigenous system of medicine in India for over 2000 years (Atal, 1980) [1]. It is a well-known drug in ayurvedic and unani medicine (Manjunath, 1948) [7]. The leaves and flowers are cooked as a vegetable by the Khasi tribe in India.

It is mostly used in the treatment of respiratory diseases like asthma, cough, bronchitis and tuberculosis. Leaves of this plant are used as main source of drug, used for the treatment of a wide variety of diseases and disorders, particularly for the respiratory tract ailments like chronic bronchitis, fever, swellings, asthma, pneumonia, malaria, tuberculosis, cold and cough (Khan, 2017) [5].

The number of alkaloids present in the adhatoda contributed to a vast variety of pharmacological properties of the plant. The major alkaloid found in adhatoda leaves is the quinazoline alkaloid known as vasicine which is also pharmacologically most studied alkaloid. In addition to vasicine, the leaves and roots of adhatoda contain the alkaloids L-vasicinone, deoxyvasicine, vasicinolone, vasicinol, adhatodine, adhatonine, adhavasinone, anisotine and peganine. Studies show that these alkaloids are responsible for adhatoda's bronchodilatory effect (Lahiri and Prahdan, 1964; Atal, 1980) [6, 1].

The 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical is extremely stable and reacts with hydrogen chemicals. It has a UV–Vis maximum absorbance of 517 nm. The approach of DPPH assay depends on the antioxidants scavenging the DPPH which is a free radical. The process involves decolourisation of the solution of DPPH in methanol by reduction process. The antioxidant capacity to reduce the DPPH radical is measured in this test (Baliyan *et al.*, 2022) [2].

Shahwar *et al.*, (2012) [8] determined the antioxidant value of vasicine at various concentrations. The highest activity was observed at 300 µg/ml with IC_{50} 212.3 ± 1.9 µM.

Materials and Methods

Plant material: The leaves (Figure 1) were collected from Pharmacovigilance Laboratory for Animal Feed and Food Safety (PLAFFS), TANUVAS, Madhavaram Milk Colony, Chennai – 51. were authenticated from Captain Srinivasa Murthy Central Ayurveda Research Institute, Anna nagar, Chennai-600 106. The extraction procedure was carried out at PLAFFS.

Chemicals: All the reagents and chemicals for extraction and assays were of analytical grade. Silica gel and florisil of mesh size 60 -100 was used. Thin Layer Chromatographic (TLC) plate silica Gel 60 F254 from Merck was used. The vasicine standard was obtained from Natural remedies, Bangalore. 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Method: in the present study two different extraction protocols were followed. First method included collection of fresh leaves and drying. The dried leaves were ground to powder and was soaked in chloroform at 1:100 ratio for 48 h. It was filtered and evaporated to obtain a green coloured semisolid crude residue. This was subjected through acid base extraction by dissolving the residue in 0.1 N HCl for four hours. This was subjected to separation in a separatory funnel with 100 ml chloroform. The aqueous layer was discarded and the remaining solution was basified (pH 9.5) with 5% ammonia method by Sreelekshmi *et al*. (2021) [10].
Fresh leaves extraction was done by crushing the leaves using pestle and mortar. These ground leaves in a steel vessel were autoclaved at 121 °C temperature, 15 lbs pressure for 30 min. The crushed leaves were then squeezed through 4 layers of muslin cloth to obtain a juice (Soni *et al*., 2008) [9]. This juice was used in isolation of alkaloids by column chromatography using chloroform as mobile phase and the elute was collected.
The elute obtained from both the methods of extraction was concentrated and dissolved in methanol for preparative thin layer chromatography. The mobile phase used was ethyl acetate, methanol and ammonia at 8:2:0.2 ratio. The alkaloid spots on the TLC turned orange on spraying Dragendroff's reagent (Figure 2) and it was observed under UV light at 254 nm (Duraipandiyan *et al*., 2015, Keesara and Jat, 2017 and Sreelekshmi *et al*., 2021) [3, 4, 10]. The alkaloid was scraped and quantified using spectrophotometer at 545 nm.

Antioxidant assay

Different concentrations of vasicine extracted (10, 20, 40, 60, 80 and 100 µg/ml) was assayed for antioxidant activity against the standard ascorbic acid (1 mM). Vasicine in methanol was added to DPPH in methanol at 1:1 ratio and incubated at room temperature in the dark for 30 minutes. The absorbance of this mixture was measured at 517 nm using UV-VIS spectrophotometer. The free radical scavenging activity was calculated as

$$\% \text{ DPPH inhibition} = \frac{A-B}{A} \times 100$$

Where A is the absorbance of control (DPPH in methanol) and B is the absorbance of the vasicine extract.

Results and Discussion

Extraction: In the present study, first method of extraction from dry leaves produced 1.1 µg/ml of vasicine while second method involving fresh leaves extraction gave 6 µg/ml of vasicine. Similar studies by Soni *et al*., (2008) [9] observed that the fresh leaves produced about 3.46 mg/ml and dry leaves gave 1.92 mg/ml of vasicine.

DPPH assay: Antioxidant activity of vasicine extracted from Justicia adhatoda was measured by DPPH free radical scavenging method and their scavenging activity was compared with the standard antioxidant ascorbic acid. The DPPH free radical scavenging activity of the vasicine is shown in Table-1. Vasicine extracted showed dose dependent activity. Among the six different concentrations used in the study (10 to 100 µg/ml) vasicine showed scavenging activity of 29% at 100 µg/ml. On the other hand, ascorbic acid showed 84% activity at 100 µg/ml. % scavenging activity or % inhibition was plotted against concentration and from the graph IC_{50} (Inhibition concentration 50) value was calculated by linear regression analysis (Figure 3). IC_{50} value of vasicine was 187 µg/ml. While Shahwar *et al.*, (2022) [8] found that the IC_{50} of vasicine was 212.3 µM. The standard ascorbic acid had 51% inhibition activity at 50 µg/ml concentration.

Table 1: Antioxidant inhibition percentage of vasicine at various concentrations

Vasicine concentration	DPPH inhibition %
10 µg/ml	11
20 µg/ ml	15
40 µg/ ml	17
60 µg/ ml	27
80 µg/ ml	28
100 µg/ ml	29

Fig 1: Plant *Justicia adhatoda L.*

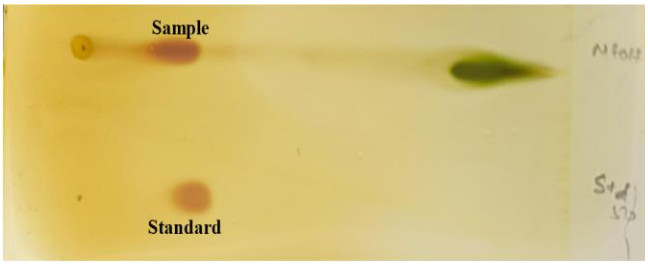

Fig 2: TLC plate of vasicine against the reference standard vasicine

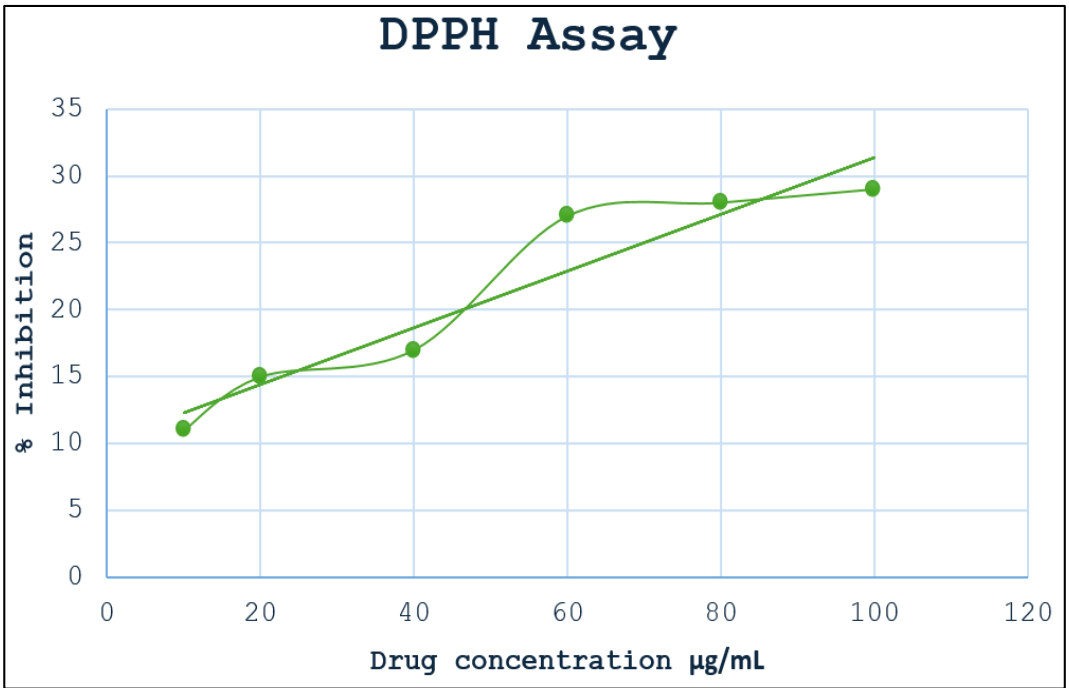

Fig 3: Graph depicting the linear regression of DPPH assay of vasicine extracted

Conclusion

The results from the current study indicate that vasicine extracted from *Justicia adhatoda* had free radical scavenging activity. IC_{50} value of vasicine in the present study was found to be 187 µg/ml. The phytochemicals were found to act as antioxidants against the negative impacts of oxidative stress. They either work as antioxidants directly or stimulate cellular antioxidant enzymes.

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