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EXTRACTION AND IDENTIFICATION OF ALKALOID-*VASICINE* FROM *ADHATODA VASICA* BY USING THIN LAYER CHROMATOGRAPHY

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ABSTRACT
Adhatoda vasica: Due to its expectorant properties, nees, also known as *vasaka*, is a plant that is widely used in indigenous systems of medicine. In addition, the herb can be used to treat leprosy, asthma, cough, and other skin conditions. *Vasicine* is the main phytochemical present in the plant and is a quinazoline alkaloid. Numerous alkaloids, including vasicinone, deoxy *vasicine*, *vasicinol*, etc., are also present in the *vasaka* plant in addition to *vasicine*. The purpose of the effort is to offer a quick and affordable way for getting *vasicine* out of *Adhatoda vasica* leaves. In this study, a modified version of the conventional acid-base extraction procedure was used to isolate *vasicine* from the leaves of *Adhatoda vasica*. Thin Layer Chromatography was used to further purify the leaf-isolated alkaloid combination. By observing under UV light at 254 nm, standard *vasicine* from Natural Remedy was used to establish the presence of purified *vasicine*. The reference standard's Rf value was 0.55, whereas the purified *vasicine's* Rf value was 0.561 ± 0.039.

KEYWORDS: *Adhatoda vasica*, *vasicine*, quinazoline alkaloid, acid-base extraction, thin layer chromatography.

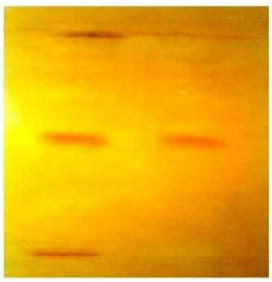
INTRODUCTION
A cough, or tussis in Latin, is an abrupt and forceful exhalation of air from the lungs brought on by an unconscious contraction of the respiratory muscles. It clears debris and fluids from the bronchi and bronchioles as a protective reflex. Three phases make up the cough reflex: inhalation, forced exhalation, and closure of the glottis.

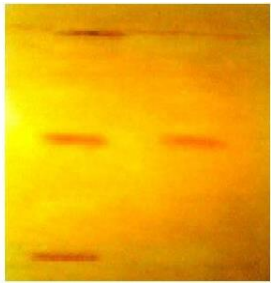
Adhatoda vasica is an Ayurvedic medicinal plant that can be used as a natural cure for a number of illnesses. As a sedative expectorant, antispasmodic, anthelmintic, and herbal medicine for asthma, chronic bronchitis, whooping cough, and chronic bronchitis, it is referenced in the Vedas. The Pharmacopoeia of India (1966) mentions it as an official medicament.

The medication is used in a variety of ways, such as fresh juice, decoction, infusion, powder, alcohol extract, liquid extract, and syrup. The plant is an emmenagogue, and the leaf juice is said to treat glandular tumors, dysentery, and diarrhea.

Bronchitis has historically been treated using a leaf extract. Ayurveda has known about it for 2000 years. The central cough route is the major target of currently marketed cough suppressants. These drugs have considerable adverse effects that limit their usage in humans and are thus extremely unsatisfactory. These side effects include constipation, respiratory depression, dependency, sleepiness, and death. Screening a variety of medicinal plants for promising biological activity is therefore urgently needed. *Adhatoda vasica*, an Acanthaceae-family shrub with simple, opposite leaves and white, pink, or purple flowers, can reach heights of 1.5 to 2.5 meters. The plant is also referred to by its names Justicia adhatoda L. and Adhatoda zeylanica Medic. Additionally, it goes by the popular names Malabar Nut Tree and *Vasaka* in Sanskrit. The plant is well-known in Ayurvedic and Unani medicine, where it is used to cure a variety of illnesses, particularly those affecting the respiratory system.

Quinazoline alkaloids such *vasicine*, vasicinone, and deoxy *vasicine* are found in the plant's leaves. The main secondary metabolite found in *Adhatoda vasica* leaves is the quinazoline alkaloid *vasicine*. Along with vasicinone, another important alkaloid present in the plant, it is a potent bronchodilator that is used to treat respiratory issues.





MATERIALS AND METHODS

1. Collection: *Adhatoda vasica* leaves were collected from the medicinal garden at Sanjay Ghodawat University, Kolhapur School of Pharmaceutical Sciences. Ms. Pushpalata Sherekar verified the plant specimen's authenticity, and a voucher specimen was deposited at the herbarium.

2. Chemicals and Standard- Merck provided analytical-grade chemicals and reagents. Merck provided TLC plate silica Gel 60 F254.

3. Extraction of alkaloid *vasicine*- The leaves were air dried for two weeks at room temperature and were made into fine powder, which was kept in air tight containers. 10 gm of leaf powder was soaked in 100ml chloroform in 1:10 ratio for a period of 48 hours and was subjected to vigorous shaking intermittently. The filtered extract was concentrated using a rotary evaporator to obtain semi solid crude extract. To the concentrated extract, 100 ml of 0.01 N HCl was added and stirred for 4 hours. The acid treated extract was filtered to obtain a clear solution which was extracted with 100 mL chloroform thrice. The aqueous layer was collected in a beaker and the organic layer containing lipids and other impurities was discarded. To the aqueous layer collected 5% ammonia solution was added untilthe pH reached 9.5. The basified solution was extracted with 100 mL chloroform thrice. The bottom layer was collectedand was concentrated to obtain yellowish-brown colored amorphous residue.

Isolation and Purification of *vasicine*

Thin Layer chromatography was carried out to separate *vasicine* from the unwanted components. Mobile phase was prepared as Ethyl acetate: Methanol: Ammonia in the 8:2:.2 ratio. Preparative TLC was carried out and alkaloid spots were detected under 254 nm UV light. Orange spots could be detected on spraying Dragendroff's reagent.

Stock solution of *vasicine* was prepared by dissolving 1 µg of accurately weighed standard in 1 ml methanol. Thin layer chromatography was carried out using ethyl acetate: methanol: ammonia in 8:2:0.2 ratio, and the presence of *vasicine* was checked under UV light of 254 nm wavelength. On spraying the plate with Dragendroff's reagent, orange-colored spots appeared indicating the presence of *vasicine*.

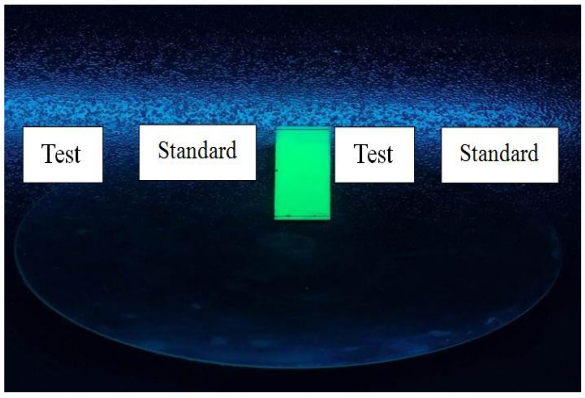


Figure: The presence of vasicine was examined using UV light at a wavelength of 254 nm.

Figure- When Dragendroff's reagent was sprayed onto the plate, orange-colored spots developed, confirming the presence of *vasicine*.

RESULTS AND DISCUSSION

On conducting Thin Layer Chromatography presence of *vasicine* could be detected by TLC. Standard *vasicine* showed and Rf value of 0.55 and the purified sample showed a similar Rf value of 0.55 hence confirming that the compound Extracted and Identified from *vasaka* leaves was *vasicine*.

Calculation- $R_f = \frac{\text{Distance traveled by solute}}{\text{Distance traveled by solvent}}$

$R_{f} = \frac{2.2}{4}$

$Rf = 0.55$

CONCLUSION

From the above study it can be concluded that *vasicine*, the major alkaloid of *Adhatoda vasica* can be extracted from the leave of the plant by acid base extraction, in a cost-effective manner And Identification by Thin Layer Chromatography.

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