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Quantitative determination of Yohimbine alkaloid in the different part of the *Rauvolfia tetraphylla*

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ABSTRACT

Rauvolfia belongs to the family Apocynaceae. Rauvolfia tetraphylla is economically important medicinal plant because of the presence of various alkaloids. Alkaloid yohimbine is hypotensive a cardiovascular depressant and hypnotic. In our study we have taken the methanol extract of leaves, stem and roots of Rauvolfia tetraphylla and yohimbine as standard. The compression was done by HPTLC. The quantification and identification of yohimbine was done by WINCATS software with densitometric detection (Camag Scanner -3). The present technique allowed different separation profiles that can be useful for phytochemicals characterization of various parts of plants. The linear calibration ranges were 10-1000 µg/ml for yohimbine. Yohimbine was detected only in the leaves of plant in 6.11 %. The HPTLC methods were successfully validated and applied to the quantization of yohimbine.

Key words: Alkaloid, HPTLC, *Rauvolfia tetraphylla*, yohimbine.

INTRODUCTION

An aphrodisiac is defined as any food or drug that arouses the sexual instinct, induces veneral desire and increases pleasure and performance. Although there are many aphrodisiac in the market, there is no scientific evidence that most of them work. Although Yohimbine has been extensively used by men to enhance sex drive and approved by many food and drug authority effects.

Rauvolfia is a genus of evergreen trees and shrubs in the Apocynaceae family. The approximately 85 species in the genus can mainly be found in tropical regions. *Rauvolfia*

tetraphylla and *Rauvolfia serpentina* is the South African quinine plant species. *Rauvolfia tetraphylla* contains a number of bioactive phytochemicals. The *Rauvolfia* species is mainly known for its phytochemical reserpine, which was widely used as an antihypertensive drug. But very little research has been published on the presence of alkaloids yohimbine in the leaves of plant *Rauvolfia tetraphylla*, which is one of the major phytochemical for aphrodisiac application. An alkaloid from the root with 5% acetic acid base was precipitated with ammonia and extracted with chloroform by thin layer chromatography with silica gel, 15 % of gypsum and mobile phase chloroform-ethanol (9:1) system (A. S. Belikovin, 1969). They estimated the total alkaloid contents, which were 3.1 -4.3 % in the roots of *Rauvolfia canescens* and in its epigeal part the alkaloids contents were 1.9- 2.4 %.

Ten indole alkaloids- ajmaline, yohimbine, α -yohimbine, isoreserpine, corynanthine, deserpidine, reserpiline, isoreserpiline, aricine, and a new alkaloid, named lankanescine, have been isolated and identified from *Rauvolfia canescens* by Lakshmi S. R. et al., (2001). J. D. Kohli et al., (1980) studied on pharmacological action of rauwolscine. Rauwolscine (kindly supplied by Mrs. A. Chatterjee, university college of science and technology, Calcutta), an alkaloid of *Rauvolfia canescens* linn, had been found to be a potent adrenolytic compound. It had further been shown to be α -yohimbine.

The objective of our study was the extraction, isolation, identification and competitive quantitative determination of the alkaloid yohimbine in the leaves, root and stem of *Rauvolfia tetraphylla* by HPTLC method.

EXPERIMENTAL SECTION

General experimental procedures:

All solvents used for extraction and chromatography were of high performance liquid chromatographic (HPLC) grade.

Collection and botanical identification of plant:

The plant material viz. root, stem, leaves and seed of *Rauvolfia tetraphylla* (family Apocynaceae) were collected from Divya Nursery near Patanjali Yog Peeth, Haridwar in September 2008 and authenticated by the approved botanist. Voucher specimens were deposited in the herbarium of Divya Pharmacy.

Extraction of selective substance from entire mass of the sample:

The each sample of root, stem, leaves and seed of *Rauvolfia tetraphylla* was taken in methanol for the extraction. 5 g of the root, stem, leaves and seeds were grinded to a homogeneous powder of 20 mesh size. These crushed materials were taken in round bottom flask and pored 10 ml methanol to dissolve the each sample material and Centrifuges each sample at 6000 RPM (Remi KKK-35579). The supernatant was collected and filtered from Whatman no. 4 filter paper for Removal of organic solvent. The filtrate was ready for sampling for obtaining the active phytochemical from the leaves of *Rauvolfia tetraphylla* chromatography.

Chromatography conditions:**HPTLC details for total phytochemicals analysis-****Sample solution:**

5 µl of supernatant solution of each sample was spotted on coated silica gel 60 F₂₅₄ TLC plate by Camag LINOMAT 5.

Development system:

Toluene: ethyl acetate: diethyl amine (70: 20: 10) solvent systems given the good separation.

Stationery phase:

Pre coated silica gel 60 F₂₅₄ TLC plate of 0.2 mm thickness

Sample development in mobile phase:

The plates were immersed vertical in development tank. Development was started and spots were allowed to migrate along with mobile phase after 1 cm on to the stationery phase up to 10 cm distance. The plates were then taken out from the development chamber.

Detection:

The typical chromatogram was evaluated for detection of the position of the solutes in UV at 254 and 366 nm and further by densitometry scan. The analysis was done in triplets for the validation and reproducibility of the method. Image of the chromatographic plate was taken at UV 366 nm by the Camag REPROSTAR 3 for visual documentation.

RESULTS AND DISCUSSION

We have screened the total phytochemicals present in root, stem, leaves and seeds of plant *Rauwolfia tetraphylla*. The summary of HPTLC results for total phytochemical is presented in fig. 1 to fig.2. Track 1st was stem part of the plant and had 12 bands, track 2nd was leaf extract of the plant and had 9 bands includes the band of yohimbine at Rf .56, track 3rd fruit extract of the plant and had no bands, track 4th was our reference sample yohimbine and had 4 bands, track 5th root sample had 5 bands and track 6th was the solvent sample which had no bands at 366 nm. Alkaloid yohimbine detected in good amount in track 2 (leaves of the plant) at Rf 0.56. The spectrum of control sample of yohimbine was matched with the band at Rf 0.56 of track 2nd in densitometry scan (fig.2).

Certain species are the source of valuable emetics and cathartics. The species *Rauwolfia tetraphylla* and *R. serpentina* has received special attention as the source of tranquilizing drugs. Among the purified alkaloids obtained from *Rauwolfia tetraphylla* and *R. serpentina*, reserpine was, perhaps, the one most used as tranquilizing agents. Yohimbine was the principal indole alkaloid derived from the bark of the yohimbe tree (*Pausinystalia yohimbe*). It was observed that a good amount 6.11% of yohimbine was present in the leaves of *Rauwolfia tetraphylla* whereas yohimbine was absent in the stem, seeds and root of the plant *Rauwolfia tetraphylla*.

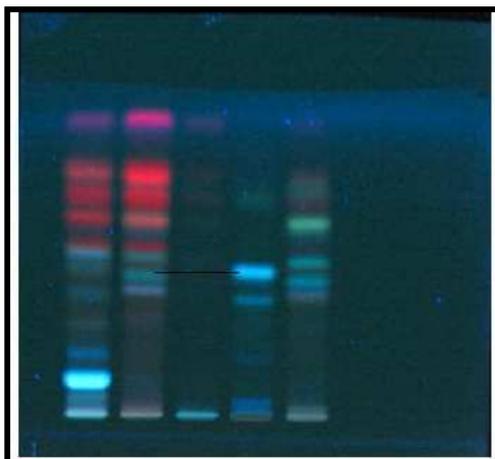


Fig.-1:-Track-1to Track -6 represents the fingerprinting Chromatogram of Stem, Leaf, Seed, Control(Yohimbine) and root parts of Plant *Rauwolfia Tetraphylla*

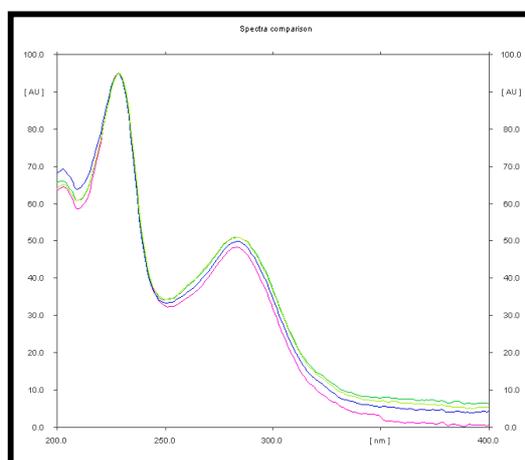


Fig. -2:- The spectra of band at Rf .56 of track 2 (the leaves of plant *Rauwolfia tetraphylla*) was matched with the control sample of yohimbine.

In HPTLC analysis Yohimbine was found as the major active principle in the leaves of *Rauwolfia tetraphylla*. *Rauwolfia tetraphylla* leaves were contain maximum phytochemicals with major alkaloid yohimbine than other parts of the plant. Small amount of dry leaves have good amount of yohimbine and could be used as a good aphrodisiac (fig.1).

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