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HPLC ANALYSIS Of *Adhatoda Vasica* OBTAINED FROM DIFFERENT GEOGRAPHICAL SOURCES

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ABSTRACT

Vasicine is one of the important alkaloid obtained from the plant Adhatoda vasica. In the present study, the drug was collected from five accessions namely Bangalore, Shimoga, Trichy, Kolhapur and Palakkad (Kerala) between August and November. The Methanolic extracts were used for HPLC analysis. The results of the HPLC analysis showed variations in all the accessions. The extract from Bangalore accession showed significantly higher amount of Vasicine (0.93%) and that of Palakkad (Kerala) showed least amount (0.59%) of Vasicine.

Key words: *Adhatoda vasica, HPLC (High Performance Liquid Chromatography), Bangalore, Shimoga, Trichy, Kolhapur, pallakkad.*

INTRODUCTION

Adhatoda vasica is an Ayurvedic medicinal plant, which is a home remedy for several diseases and human requirements. It is mentioned in Veda as an herbal remedy for treating cold, cough, whooping cough and chronic bronchitis and asthma[1-3], as sedative, expectorant, antispasmodic, immunomodulatory[4], hepatoprotective[5] and anti-inflammatory[6]. It is an official drug and is mentioned in the Indian Pharmacopoeia (2007).

The major active ingredients of leaves are quinazoline like the alkaloids Vasicine and Vasicinone, which are used as good expectorant and bronchodilators.

The variations of chemical constituents are observed in plants belonging to the same species grown in different environmental conditions. These variations may occur due to climatic conditions like altitude, temperature, type of soil etc.

The plants collected from different geographical sources have showed similar morphological characters but difference in content of secondary cell constituents. Hence, in the present study, an attempt was made to select an appropriate genotype that produces maximum amount of secondary plant cell constituents. This leads to the production of plants with better market value. Hence, it is very essential to select the correct accession for the particular usage and for further cultivation.

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MATERIALS AND METHODS.

Adhatoda vasica leaves were collected from Bangalore, Shimoga, Trichy, Kolhapur and Palakkad (Kerala) between August and November and were authenticated from FRLHT (Forest for Revitalization of Local Health Tradition), Bangalore. Solvents like methanol of A.R and HPLC grade were used from E-Merck for extraction and Analysis.

Extraction

The leaves were washed, dried under shade and around 15g from each accession were powdered to pass through 40-mesh sieve. The drug was extracted with methanol (50ml x 3) over steam water bath for 3-hours. After cooling, the solvent was removed on a rotary evaporator. The extracts were stored in desiccators and protected from the light.

Chromatographic conditions

Instrumentation

A Shimadzu (Japan) high performance liquid chromatography instrument equipped with Shimadzu two LC-10 ADvp pumps, with SIL 10 ADvp auto sampler, SCL 10 ADP system controller, CTO 10 ADvp column oven in combination with class VP software version 6.03 was used.

A SPD-M10 ADvp photodiode array detector for peak detection and analysis were also used. Solvents were filtered through a Millipore system and analysis was performed on a Merck Silica CN column (250 mm × 4.6 mm I. D. 5 μ size).

The mobile phase used was Buffer: Acetonitrile: Tetrahydrofuran (92:5:3)

{Buffer- 6.55 g of Potassium di-hydrogen phosphate in 500 ml of water and pH adjusted to 2.8 using orthophosphoric acid}

Flow rate- 1.0 ml/min.

Detector wavelength- 280 nm.

Standard Preparation

10mg of Vasicine was accurately weighed and dissolved in 50 ml of methanol, sonicated for

5-10 min, cooled and the volume was made up to 100 ml using methanol.

Sample preparation

All the extracts were equivalently weighed that of reference standard, dissolved in methanol and dilutions were made accordingly.

Procedure

20 µl each of 5 different test solutions and standard Vasicine were injected to HPLC. The chromatogram was scanned up to 15 min. the amount of test samples and references standard were determined according to the formula.

Formula

[Content off Vasicine= standard area/ sample area X standard dilution /sample dilution X % purity]

RESULTS AND DISCUSSION

Under the chromatographic conditions described above the retention time of Vasicine were around 5.461 and the chromatogram of standard Vasicine and that of test samples are shown in the figure 1-6 respectively. The analysis indicated the presence of optimum amount of Vasicine in Bangalore (0.93%) and Trichy (0.89%). The content of Vasicine was found to be 0.74%, 0.65% and 0.59% in Kolhapur, Shimoga and Palakkad respectively (Table 1).

Table-1: Percentage of content present in Adhatoda vasica leaves from different Geographical origin.

Sample	Fig no	Injection volume	Retention time (min)	Area	Content of vasicine
Standard Vasicine	1	20µl	5.461	3686582	-
Bangalore	2	20µl	5.493	2479947	0.93%
Shimoga	3	20µl	5.536	1044138	0.65%
Trichy	4	20µl	5.515	1867842	0.89%
Kolhapur	5	20µl	5.504	2575055	0.74%
Pallakad (Kerala)	6	20µl	5.525	1720883	0.59%

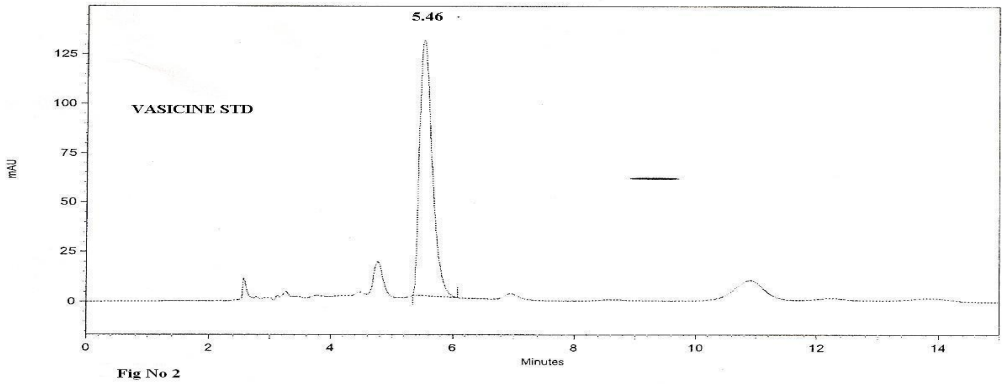


Fig No 2

(A)

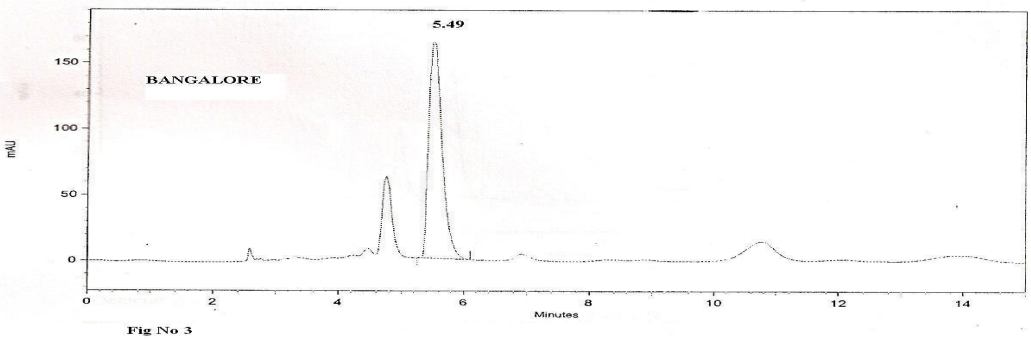


Fig No 3

(B)

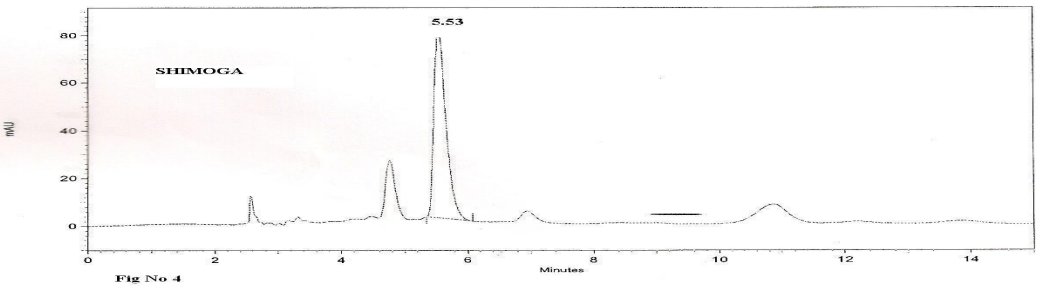
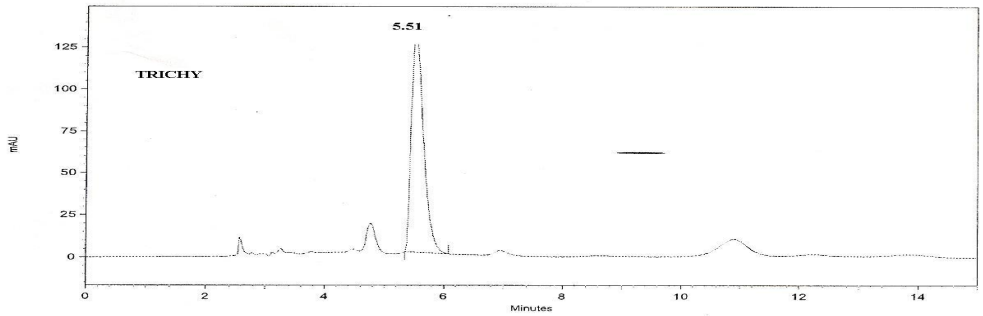


Fig No 4

(C)



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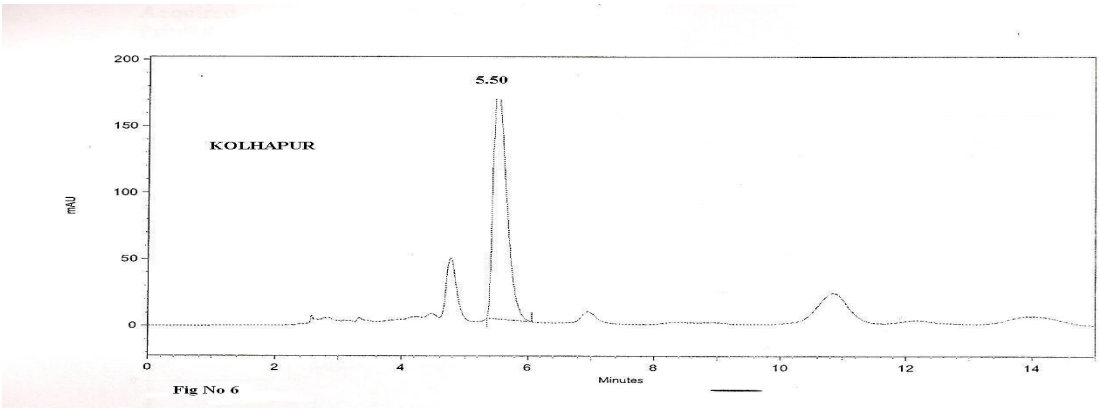

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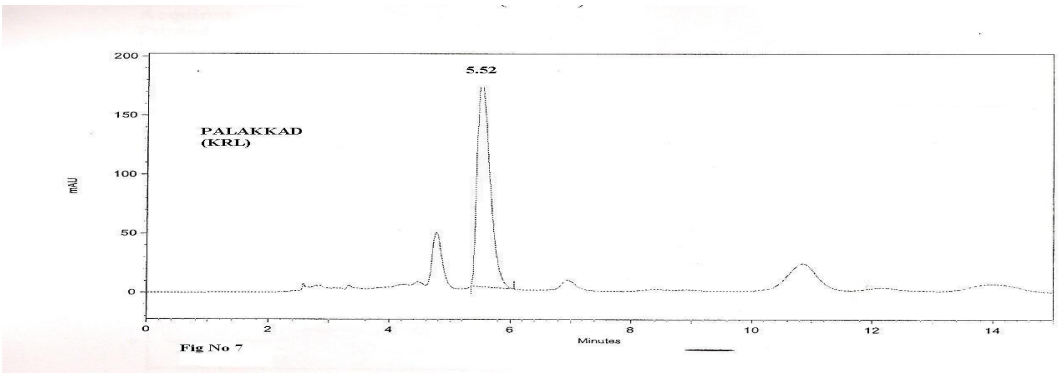

(F)

Fig No 1: HPLC chromatogram of (A).Standard Vasicine, Leaves extracts from (B). Bangalore, (C). Shimoga, (D). Trichy, (E). Kolhapur and (F). Palakkad (Kerala) accession.

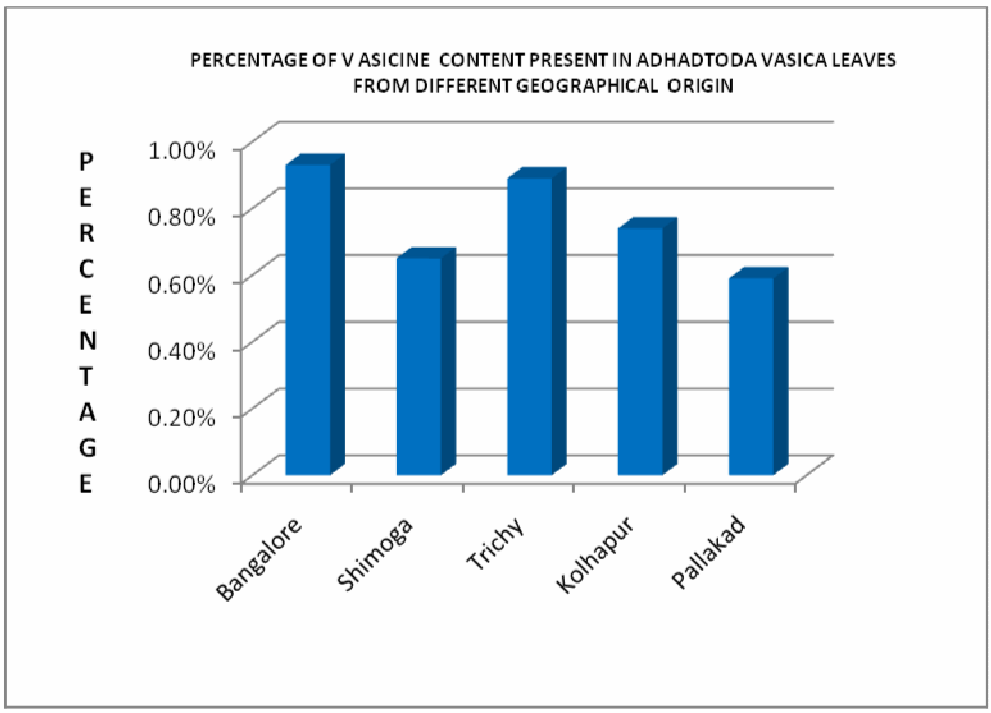

Figure-2: Percentage of Vasicine Present in *Adhatoda Vasica* leaves from different Geographical sources.

REFERENCES

1) *Nadkarni AR, Nadkarni KM.* ***Indian Materia Medica****. Popular Prakashan, Bombay, 1976, 42.*

2) *Kirtikar KR, Basu BD.* ***Indian Medicinal Plants****. Volume-Periodical Experts, Vivek Vihar, Delhi, 1975:18902.*

3) ***Wealth of India****, Volume-8, C.S.I.R, New Delhi, 1982, 31.*

4) *Jinyvarghese K, Karpe ST, Kulkarni SR. Immunostimulant activity of Adathoda vasica , Lawsonia inermis and Alkanna tinctoria.* ***Indian Drugs****. 2005;42(6):345-352.*

5) *Bhattacharyya D, Pandit S, Jana U, Sen S, Tapas KS. Hepatoprotective activity of Adhatoda vasica aqueous leaf extract on D-galactosamine-induced liver damage in rats.* ***Fitotherapia****. 2005;76(2):223-225.*

6) *Chakraborthy A, Brantner AH. Study of alkaloids from Adathoda vasica Nees on their anti-inflammatory activity.* ***Phytother Res****. 2001;15:532-534.*

Article History:----------

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