SEASONAL AND GEOGRAPHICAL VARIATIONS IN CHEMICAL CONSTITUENTS OF *LEPTADENIA RETICULATA*

Mammen D[1*], Daniel M[2], Sane RT[3]
[1*]Department of Chemistry, M.S. University of Baroda, Vadodara, India.
[2]Department of Botany, M.S. University of Baroda, Vadodara, India.
[3]Guru Nanak Institute for Research and Development, G.N. Khalsa College, Matunga, Mumbai, India.
***Email:** drdenni.mammen@gmail.com

ABSTRACT

Leptadenia reticulata, often referred to as *Jivanti*, has been mentioned to have high therapeutic value in Ayurvedic texts. It has been used in several Ayurvedic preparations as well as a number of formulations such as Leptaden and Speman. The plant being a vital component in these formulations, its quality and consistency is of prime importance. The present study deals with the study of the presence of any major variation in the plant due to change in season or region. Simultaneous comparison of the fingerprints of the plant has been done using HPTLC technique. The aqueous extracts which is known to be most effective have been compared for the plant collected during three different seasons, as well as from three different regions. The technique is simple and cost effective since a number of samples can be screened at once on a single TLC plate. *P*-coumaric acid was used as reference standard with which the HPTLC chromatograms obtained for each sample were compared. *P*-coumaric acid present in each sample was quantified using a calibration graph of the reference standard. The relative standard deviation, limit of detection and limit of quantification were also calculated.

Keywords: *Leptadenia reticulata*, HPTLC, *p*-coumaric acid, geographical variations.

INTRODUCTION

Leptadenia reticulata or *Jivanti* is a twining shrub considered to have a property to bestow health and liveliness to the consumer. Charaka had treated it as an important *rasayana* drug, capable of maintaining youthful vigour and strength. Vagbhata included it among the ten drugs that constitute the *Jivaniya gana* or the vitalizing group[1]. *Jivanti* is cold, sweet, aphrodisiac, rejuvenative and easy to digest. It promotes health and vigour, improves voice, alleviates the three doshas- *vata*, *pitta* and *kapha* and cures eye diseases, haemetemesis, emanciation, cough, cold, dyspnoea, fever and burning sensation. Dysentery, nightblindness, poisonous affections and tuberculosis also are relieved using this drug. The root, which is the most potent part, is used as a component of preparations such as *Jivantyadi ghratam*, *Manasamitravatakam*, *Balarishtam*, *Anutailam*, etc. This plant is found in Gujarat, Sub-Himalayan tracts from Punjab to Sikkim and Khasi hills and throughout peninsular India, ascending up to an altitude of 900 metres. It is also distributed in tropical and sub-tropical parts of Asia and Africa[2].

Though there are other plants claimed to be *jivanti, Leptadenia reticulata* (Retz.) Wight & Arn, is equated with *Jivanti* in Western India. People in Gujarat and Kathiawar use this plant as a pot herb and is considered it to be a good cure for tuberculosis and eye diseases. Therefore, *Leptadenia reticulata* is considered to be the real *jivanti* by many. Ayurvedic Formulary of India also accepts this plant as the true drug[1].

Aqueous extract of the stem of this plant demonstrated vasodilator, transient, inotropic, chronotropic and prolonged hypotensive effect in dogs[3]. A number of studies have been carried out on its galactogogue property. On lactating rats, its ether extract was found to increase lactation[4]. The lactogenic effect of the plant was also studied on Gir cows[5]. Speman, a herbal formulation with *L. reticulata* as one of its nine constituents is said to have beneficial effects on the gametogenic and androgenic functions of the testis in adult mice. It showed an increase in the secretions of the accessory sex organs in the mice[6]. Speman is also shown to increase the serum level of testosterone in young bulls[7]. Another formulation Leptaden, has been shown to provide effective treatment in cases of deficient lactation and lack of lactation in humans[8]. The extract of the leaf shows antibacterial and antifungal activities[9].

The plant is known to contain α-amyrin, β-amyrin, ferulic acid, luteolin, diosmetin, rutin, β-sitosterol, stigmasterol, hentriacontanol[10], a triterpene alcohol simiarenol[11] and apigenin[12] have been previously reported from the plant. The aerial parts of the plant are also known to contain pregnane glycosides reticulatin, deniculatin and leptaculatin[13]. The plant also contained tocopherols[14]. Flavonols like quercetin and kaempferol have also been reported to be present in traces in the seeds and pericarp[15]. The plant is reported to contain a triterpenoid leptadenol. It also contains n-triacontane, cetyl alcohol, β-sitosterol, β-amyrin acetate, lupanol 3-O- diglycoside and leptidin[16].

The active principles and other constituents of a number of medicinal plants are bound to fluctuate with seasons and geographic regions[17]. Since *L. reticulata* is widely used at all seasons, it was felt that a study on the seasonal and geographic variations will identify those

changes, if any, occurring in this plant. Therefore, in the present study samples of this plant were collected in April (summer), August (monsoon) and December (winter) during 2008, from Baroda to study the seasonal changes. Similarly geographical variations were studied on plants collected in summer from three states *i.e.* Gujarat, Maharashtra and Kerala.

MATERIALS AND METHODS

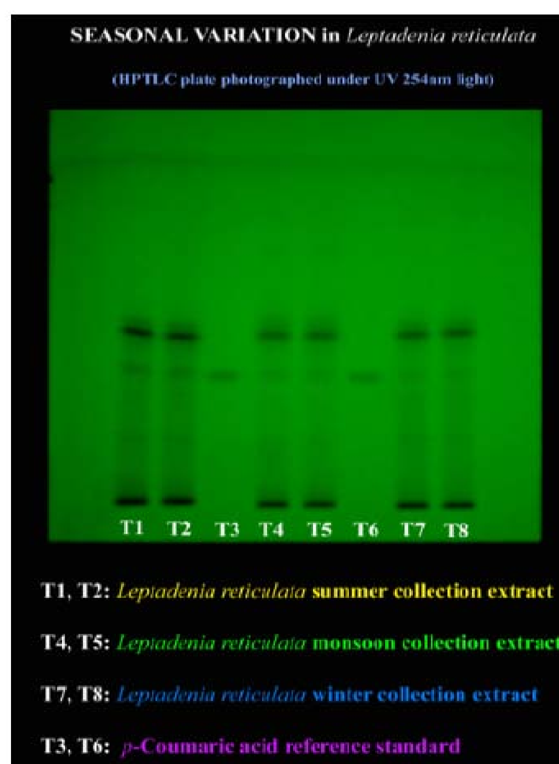
Leptadenia reticulata collected was identified and authenticated at Botanical Survey of India, Pune. The voucher specimen of this plant (No.BSI/WC/Tech/2007/734) is deposited at the Herbarium, B.S.I., Pune. The plant materials were washed, shade dried for a day and then dried completely in an oven at 38°C. The plant materials were coarsely powdered using a rotary grinder and stored in airtight plastic containers and then used for HPTLC analysis.

HPTLC analysis: About 250mg of plant powder was placed in a 20 cm^3 stoppered test tube, to which 10 cm^3 of distilled water was added. The test tube was sealed and subjected to overnight extraction on a rotary shaker. The solution was filtered and the filtrate was subjected to acid hydrolysis in 7% HCl solution. After hydrolysis the solution was cooled and transferred to a separating funnel. Approximately 2 cm^3 of ethyl acetate was added and the separating funnel was shaken thoroughly. The organic layer was separated and the aqueous layer was treated with again with 2 cm^3 of ethyl acetate. The organic layer so separated was pooled with the organic fraction obtained earlier. The pooled ethyl acetate fractions were evaporated on a water bath. The residue obtained was reconstituted in 1cm^3 methanol and used directly for HPTLC analysis. Each of the plant extracts were spotted in duplicate on precoated silica gel 60F254 plates (Merck) using CAMAG Linomat V sample applicator. The mobile phase employed was Toluene: ethanol: hexane (7:2:1, v/v/v). The plates were developed in CAMAG twin trough development chambers (10x10) and visualized under short wave UV (254nm) light. Densitometric scanning of the plates was performed using CAMAG TLC Scanner 3. Spectral scanning of the selected bands on the plates was done in the range 200-400nm in order to obtain the ultraviolet spectra of those specific bands separated on the plates. The reference standard used during analysis was *p*-coumaric acid which exhibits a λmax of 292nm. Varying volumes of the standard was applied on the HPTLC plates and run in the same solvent system. The plates were scanned and their areas were plotted as a function of the concentrations of the standard applied on each band. The calibration graph so obtained was used to quantify the phenolic acids. The relative standard deviation, regression coefficient, limit of detection as well as limit of quantification were then calculated. The software employed for the analysis was WINCATS.

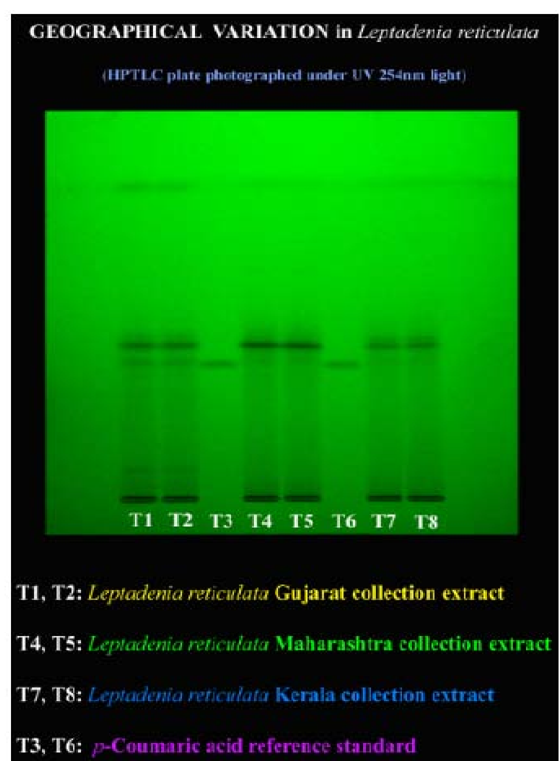
Reagents used: All reagents used during the extraction procedure, sample application as well as for preparation of mobile phases were procured from Qualigens Fine Chemicals, Mumbai, India. Reference standard *p*-coumaric acid has been procured from SIGMA, Mumbai, India.

RESULTS

The HPTLC plate developed after analysis of the extracts of *Leptadenia reticulata* collected during three different seasons is shown in **Plate 1**.



Similarly, the plate developed for analysis of the extracts to study geographical variation is shown in **Plate 2**.



The HPTLC chromatograms of the various extracts were obtained after the densitometric scanning of the two plates. The comparison chromatogram for seasonal variation is given in **Figure A**, while that of the geographical variation was obtained as observed in **Figure C**. Spectral analysis for both seasonal as well as geographical variations were obtained as observed in **Figures B** & **D** respectively. The calibration graph showing linearity for *p*-coumaric acid reference standard was obtained as observed in **Figure E**. Volumes loaded, amount fractions spotted and relative areas obtained for each track on the plate are shown in **Table I**.

Figure A: HPTLC chromatogram comparison showing seasonal variation in *L. reticulata*

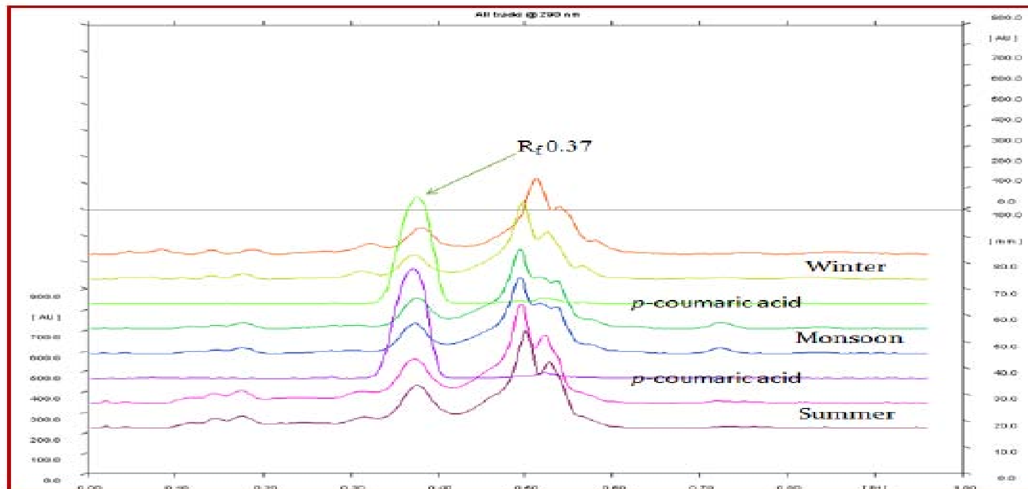


Figure B: Spectra of all bands corresponding to R_f 0.37 on the plate showing seasonal variation. Each spectrum shows a λmax of 292nm.

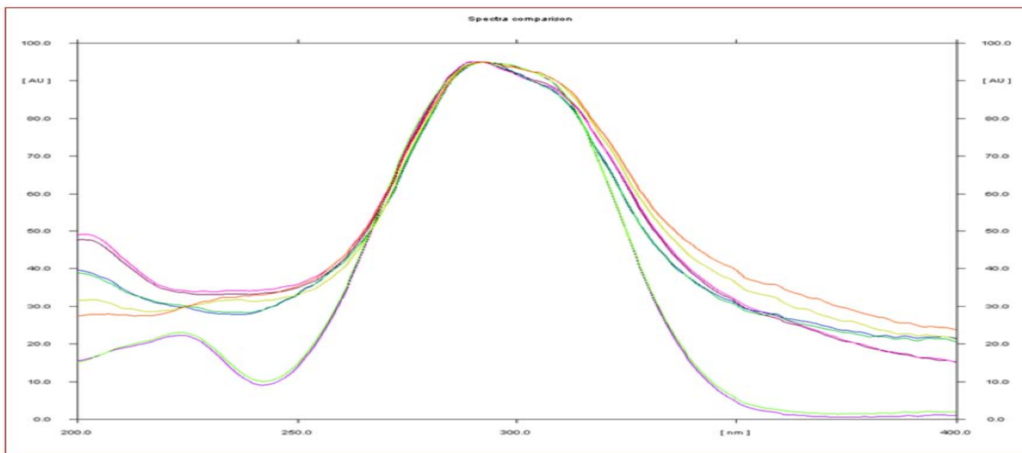


Figure C: HPTLC chromatogram comparison showing geographical variation in *L. reticulate*

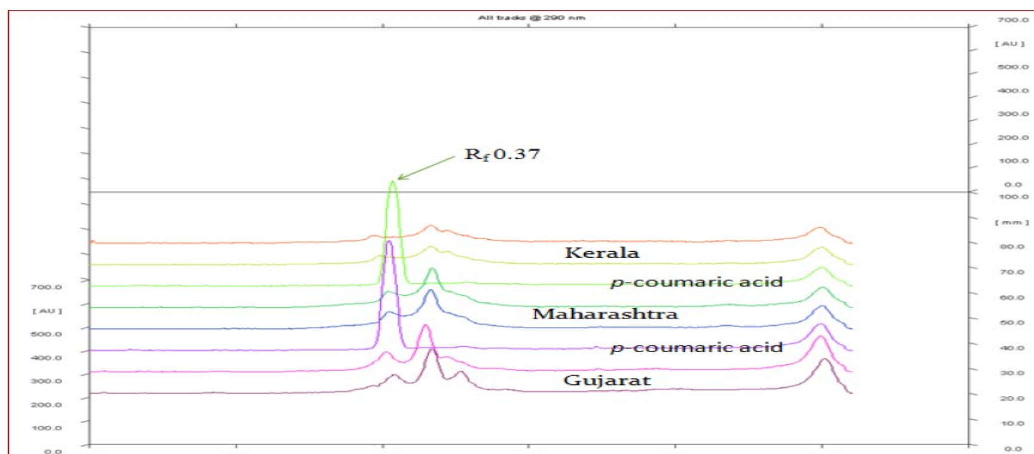


Figure D: Spectra of all bands corresponding to R_f 0.37 on the plate showing geographical variation. Each spectrum shows a λmax of 292nm.

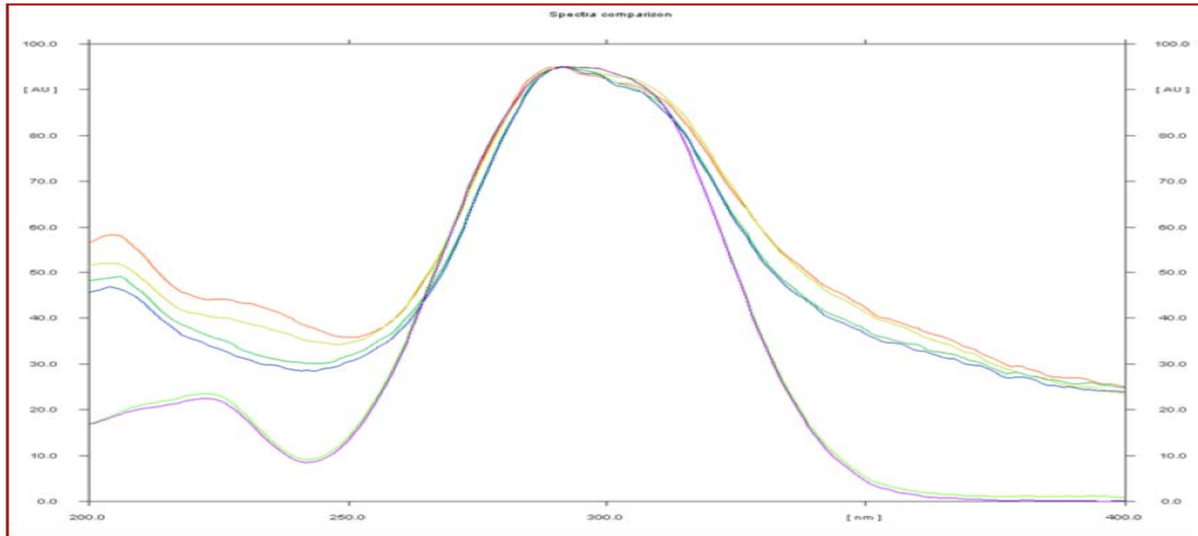


Figure E: Multilevel standard calibration curve obtained using 2.5ppm *p*-coumaric acid reference standard

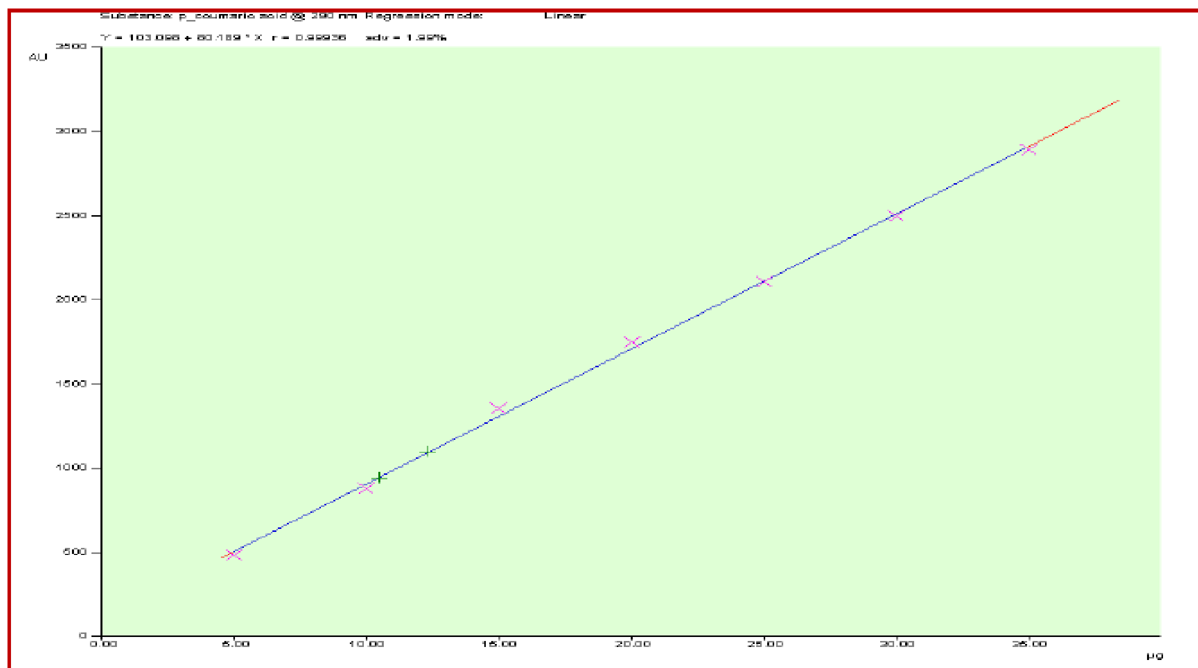


Table I: Multilevel standard calibration curve details for linearity using 2.5ppm *p*-coumaric acid reference standard

Track	Standard level	Volume of 2.5ppm standard applied	Amount fraction per track	Area	R_f
1	Standard level 1	2µL	5.0 ng	480.24	0.37
2	Standard level 2	4µL	10.0 ng	875.01	0.37
3	Standard level 3	6µL	15.0 ng	1354.40	0.37
4	Standard level 4	8µL	20.0 ng	1744.85	0.37
5	Standard level 5	10µL	25.0 ng	2106.34	0.37
6	Standard level 6	12µL	30.0 ng	2496.60	0.37
7	Standard level 7	14µL	35.0 ng	2890.67	0.37

Linearity: Varying volumes of 2.5ppm p-coumaric acid standard were applied and run on an HPTLC plate under similar chromatographic conditions as those used for the plant extracts. The plot of the areas of the peaks on each track versus the amount fraction per track gave rise to a calibration plot as shown in **Figure E**. The regression coefficient and relative standard deviation was calculated by the software. The slope and intercept on the Y-axis were also calculated by the software. Based on the value of the relative standard deviation, the **Limit of Detection (LOD)** and the **Limit of Quantitation (LOQ)** were also calculated. Regression coefficient (**r**) was obtained as **0.99930**, Slope as **80.189**, Relative standard deviation as **1.99%**, LOD: **0.0103ng** and LOQ: **0.2481ng**. The amounts of *p*-coumaric acid in each sample quantified on the basis of the calibration graph were found out to be **Summer collection: 7.24-7.75 x 10^{-9}%, Monsoon collection: 5.14-5.80 x 10^{-9} %, Winter collection: 4.20-4.84 x 10^{-9} %, Maharashtra collection: 2.19-3.11 x 10^{-9} %** and **Kerala collection: 2.39-2.89 x 10^{-9}%**

DISCUSSION

Seasonal variation: Similar fingerprints were observed for the samples of *Leptadenia reticulata* **(Plate 1)** collected during summer, monsoon and winter seasons **(Figure A)**. Differences in constituents among the three extracts were quite negligible. All the three extracts showed similar bands at R_f 0.14, 0.18, 0.37, 0.48 and 0.52. However the monsoon sample showed the presence of an additional band at R_f 0.72, which was specific only to that season, and absent in the other two seasons. Likewise extracts of the plants collected during the summer and the winter seasons showed an extra band at R_f 0.32. Since the bands at R_f 0.37 matched with that of the *p*-coumaric acid which was used as reference standard, the UV spectra of all those bands were recorded and then overlaid **(Figure B)**. The near exact overlay of the UV spectra of the samples with that of the reference standard as well as the display of the λmax of each of the overlaid spectra at 292nm indicated that all of the bands at R_f 0.37 were those belonging to the *p*-coumaric acid. Thus it can be concluded that *p*-coumaric acid is present in the plant in all seasons.

Samples collected during all the three seasons showed hardly any variation in constituents. This indicates that climate does not affect the chemical spectrum for the plant. The advantage of this observation is that the collection of the plant can be done at any time of the year.

Geographical variation: The study on geographical variations of *Leptadenia reticulata* revealed that the fingerprints of the three extracts of the plants collected from Gujarat, Maharashtra and Kerala were very similar to each other **(Plate 2)**. The bands were very few in number as compared to those observed during the seasonal variation study **(Figure C)**. The prominent bands observed in all the three samples were those at R_f 0.37, 0.49 and 0.54. The bands at R_f 0.37 in all the samples matching with that of *p*-coumaric acid reference standard were subjected to spectral analysis. The λmax of each sample was found to be 292nm, which matched with that of the reference standard **(Figure D)**. This indicated the presence of *p*-coumaric acid in all the three samples of the plant collected from three different regions.

The absence of variations in samples collected from the three different regions indicates that the environmental conditions did not affect the chemical constituents. Minor variations, however were observed between the three samples.

The present study sets at rest the speculations on the probable variations in chemical constitutions induced by the seasons and environment. Not every plant can be cultivated everywhere. Even some plant is able to grow in other regions, the phytochemicals of the plant growing in two different regions are found to be different. To take the example of '*guggul*', a very important drug in the oleo-gum resin of a xerophytes growing in the xeric regions of Kutch, India. This resin contains a large amount of guggulusterols, lignans and flavonones. Since the resins fetched high dividends, the farmers of nearby Kheda district tried to cultivate it in fields supplied with plenty of water. Though the plants grew well, there were no guggul exudations. In Kutch the plant had to produce resin to retain water due to extreme heat and for wound healing purpose. But in Kheda district where temperature was low and climate was moist, the plant did not require to produce resins. It is also known that *Cannabis sativa*, the narcotic plant, produces cannabinoids only in tropical countries. Thus it is implicit that plants produce various secondary metabolites as a result of their interaction with the environment[17].

Comparison of chromatograms of the various extracts of *Leptadenia reticulata* collected during three different seasons, and from three different regions, hardly showed any difference. This could mean that the phytochemicals present in the plant are quite similar. This could mean that change of season or region does not affect the synthesis of phytochemicals in the plant. Thus the plant can be considered to show enough consistency irrespective of the time or place of collection. This is a very positive result as far as standardization of the plant material is concerned. Similar analysis need to be carried out for all other plants to certify its consistency before they are used to prepare formulations.

REFERENCES

1. Sivarajan V V and Indira B. Ayurvedic Drugs and their Plant Sources. Oxford and IBH Publishing Co. Pvt. Ltd, Delhi. (1994).
2. Anonymous. Wealth of India- Volume III, CSIR, New Delhi.
3. Anjaria JV, Deshmankar BS, Verma SCL and SP Saxena. Studies on Leptadenia reticulata. Part I.

Pharmacological actions of aqueous extract. Indian J Med Res; 48: (1960).457-464.

4. Anjaria JV, Varia MR, Janakiraman K and OD Gulati. Studies on Leptadenia reticulata. Lactogenic effects on rats. Indian J Expt Biol; 13: (1975). 448-449.
5. Anjaria JV, Kamboya SH,Vaishnav VN and OD Gulati. Studies on Leptadenia reticulata. Lactogenic effects on Gir cows. GUJVET; 8: (1974);25-27.
6. Jaytilak PG, Pardanani DS, Murthy BD and AR Sheth. Effect of an indigenous drug (Speman) on accessory reproductive functions of mice. Indian J Expt Biol; 2: (1976); 170-173.
7. Sud SC, Atheya UK and SR Chaudri. Effect of Speman and Tentex forte on blood testosterone levels in bulls. Indian J Ani Res; 17: (1983); 59-62.
8. Habla A and A Sitaratna. Leptaden: A clinical trial of its value as a galactogogue. The Antiseptic; 69: (1972); 331-333.
9. Patel RP and AS Dantwala. Antimicrobial activity of Leptadenia reticulata. Indian J Pharm; 20: (1958). 241-244.
10. Krishna PVG, Venkata RE and RDVenkata. Crystalline principles from the leaves and twigs of Leptadenia reticulata. Planta Med; 27: (1975). 395-400.
11. Subramaniun PS and AJ Lakshmanan. On the constituents of Leptadenia reticulata: Occurence of simiarenol. Indian J Chem; 15B: (1977). 180.
12. Sastry BS, Vijayalakshmi T, Venkata RE and RDVenkata. Chemical constituents of the stem bark of Leptadenia reticulata. Indian drugs; 22: (1985). 611-612.
13. Srivastav S, Deepak D and A Khare. Three novel pregnane glycosides from Leptadenia reticulata W&A. Tetrahedron; 50: (1994). 789-798.
14. Anjaria JV, Mankad BN and OD Gulati. Isolation of stigmasterols and tocopherols from Leptadenia reticulata. Indian J Pharm; 36: (1974). 148.
15. Subramanian SS and AGR Nair. Flavonoids of some Asclepiadeceous Plants. Phytochemistry; 7: (1968). 1703-1704.
16. Noor F, Ahmed A, Imtiazuddin SM and B Khan. Leptidin from Leptadenia reticulata Phytochemistry; 32: (1993). 211.
17. Daniel M. Herbal Technology- Concepts and Approaches. Satish Serial Publishing House, New Delhi. (2008).

****