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AUTHENTICATION OF MORPHOLOGICALLY SIMILAR RHIZOME DRUGS BASED ON TLC FINGERPRINT PROFILES AND VALERENIC ACID CONTENT

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Keywords:

Valerenic acid, rhizomes, *Ferula sumbul*, TLC densitometry

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ABSTRACT: Objective: Present study involved comparison of thin layer chromatograms and quantification of valerenic acid (VA) in four morphologically similar rhizome drugs – *Valeriana jatamansi*, *Nordostachys jatamansi*, *Selinum vaginatum* and *Ferula sumbul*. **Materials and methods:** Ethanol extract of the four drugs were prepared under standard conditions and VA content was determined using TLC densitometry. **Results:** Comparative fingerprint chromatograms of the four drugs were developed and mean content of VA was calculated. *S. vaginatum* contains maximum VA content (360.22 µg/g). **Conclusion:** The four closely allied drugs could easily be differentiated based on thin layer chromatograms and VA content.

INTRODUCTION: In the indigeneous system of medicine, plants have been utilized for the treatment of many ailments, since long. Most people rely heavily on the traditional herbal medicines for preventive as well as curative measures. But many natural drugs have same vernacular names. One of such drugs is "jatamansi".

The four different plant drugs, namely - *Valeriana jatamansi, Nardostachys jatamansi, Selinum vaginatum* and *Ferula sumbul* are either sold under the same common name, or are usually adulterated or substituted by one-another. *Valeriana jatamansi* Jones (syn. *Valeriana wallichii* DC) belongs to the family Valerianaceae.

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Its roots have been traditionally used for treatment of various diseases including sleep disorder, obesity, nervous disorders, epilepsy, insanity, snake poisoning, eye trouble, and skin diseases. The roots show the presence of sesquiterpenoids valerianananoids A-C and essential oil. [1-3]

Nardostachys jatamansi DC belonging to family Valerianaceae is an indigeneous drug found in Himalayan region. The roots and rhizomes have been traditionally used to treat epilepsy, hysteria, syncope, and neurological disorders. [4, 5] It is reported to contain sesquiterpenes (jatamansic acid, jatamansone), lignans and neolignan. [6]

Selinum vaginatum Clark commonly called as bhootkeshi belongs to family Umbelliferae. The roots are used as a nervine sedative and useful in hysteria. It contains coumarins, namely selinidin, angelisin, selinone, orosenol, lomantin and vaginidin. [7, 8]

Ferula sumbul Hook (Syn. *Ferula moschata* Reinsch) of family Umbelliferae, commonly called as sumbul (Hindi) or musk root (English), consists

of cylindrical or tapered pieces of dried roots and rhizomes with bitter taste and slight musky odour. *F. sumbul* has been traditionally used for relieving anxiety, as a sedative in hysteria and other nervous disorders, and as a mild gastro-intestinal stimulant.[9] Coumarins (heraclenol, pabulenol) and bicoumarins (fesmutorin and conferol) isolated from the ethanolic extract of *F. sumbul* roots exhibit anti-HIV activity and inhibit cytokine release. [10]

Morphological similarity among these drugs hinders their correct identification. All the four drugs contain valerenic acid which can be used as an important marker for distinguishing the four drugs. In this study, attempt has been made to validate these drugs on the basis of valerenic acid (VA) content using TLC densitometry.

MATERIALS AND METHODS:

Chemicals and reagents:

The solvents used were of analytical grade. VA was purchased from Sigma Aldrich Laboratories. Roots of *V. jatamansi* and *S. vaginatum* were collected from Mandi region of Himachal Pradesh. *N. jatamansi* was procured locally from Chandigarh. *F. sumbul* roots were procured from RYM exports, Mumbai. All the drugs were authenticated by NISCAIR, New Delhi.

Preparation of standard solution:

VA (2 mg) was dissolved in 1 ml methanol in a 10 ml volumetric flask, and the volume was made upto the mark to obtain 200 µg/ml stock solution.

Preparation of sample solution:

Accurately weighed dried powdered drug samples (1 g each), packed in filter paper sachets, were refluxed (2 x 30 min) separately in 50 ml round bottom flasks with methanol (20 ml) on boiling water bath. The marcs were washed twice with methanol. These were then concentrated under reduced pressure, transferred to 25 ml volumetric flasks, and the volumes were made upto the mark. All the samples were prepared in duplicate.

TLC densitometry instrumentation and chromatographic conditions:

A CAMAG HPTLC system (Switzerland) comprising of CAMAG Linomat 5 applicator, CAMAG TLC Scanner 3, CAMAG win CATS software, version 1.3.3, Hamilton syringe (100 µl), CAMAG Reprostar 3, were used for the study. The chromatographic estimation was performed using precoated silica gel 60 aluminium sheets (Merck, Germany; 20 x 10 cm) as the stationary phase. Linear ascending development of the plates to a distance of 80 mm was done using chloroform as the mobile phase in twin-trough CAMAG chamber. Scanning wavelength of 220 nm with a slit dimension of 5.0 x 0.45 mm and scanning speed of 10 mm/sec were employed. Thereafter, the chromatograms were visualized after spraying with 0.5% anisaldehyde followed by heating at 110 ºC.

Linearity plot of VA:

From the stock solution, six working standard solutions were obtained by appropriate dilution with methanol. The concentrations of working standard solutions were 50, 60, 70, 80, 90 and 100 ng/µl. 2 µl sharp bands were apllied on the pre-coated TLC plate, and developed as described above.

Quantification of VA in the root extracts:

Methanol extract of the test samples were applied (5 µl) in duplicate, and chromatograms were obtained under the same conditions as for analysis of VA. Area of the peak corresponding to the R_f value of VA was recorded and the amount present was calculated from the regression equation of the calibration plot.

RESULTS:

Linearity plot of VA:

Linearity plot of VA in the range of 50-100 µg/ml showed a correlation coefficient of 0.996. The regression equation obtained from the plot was used to quantify the content of VA in the drug.

Quantification of VA in the prepared root extracts:

The amount of VA was calculated by comparing the peak area of the standard and the sample solutions. The mean content (% w/w) was highest in *S. vaginatum* (0.0360) and was least in *V. jatamansi* (0.0018), whereas 0.0083 and 0.0026 were found in *N. jatamansi* and *F. sumbul* respectively as shown in **Table 1**.

TABLE 1: VA CONTENT IN THE DRUGS.

Plant drug	VA Content (µg/g; %w/w)
V. jatamansi	18.39; 0.0018
F. sumbul	26.07; 0.0026
N. jatamansi	83.11; 0.0083
S. vaginatum	360.22; 0.0360

DISCUSSION: Since VA is freely soluble in methanol, the plant materials were extracted with methanol. Chloroform, used as mobile phase, shows selective resolution of VA peak without interference with other constituents, as already validated by the developed method.[11] **Fig.1** depicts the fingerprint profiles of *V. jatamansi*, *N. jatamansi*, *S. vaginatum* and *F. sumbul*. As is evident from the chromatograms, the four morphologically close drugs can be differentiated from one another. **Fig. 2** shows chromatogram overlay of the drug samples and reference standard. **Table 1** shows the content of VA in the four drugs. *S. vaginatum* roots contain almost 20 times the content of VA as compared to *V. jatamansi*. Almost five-fold difference is observed in the content of VA in *N. jatamansi* as compared to that in *V. jatamansi.* Substantial difference in the content of VA can be used as a reliable parameter to authenticate the four drugs, viz, *V. jatamansi*, *N. jatamansi*, *S. vaginatum* and *F. sumbul.*

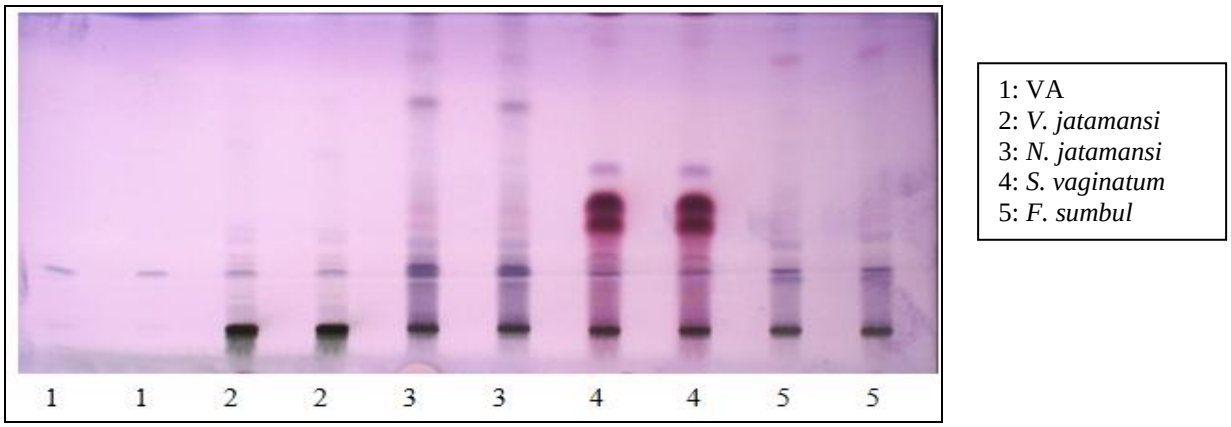

FIG. 1: TLC FINGERPRINT PROFILES OF VA AND THE DRUGS AFTER SPRAYING WITH 0.5% ANISALDEHYDE REAGENT

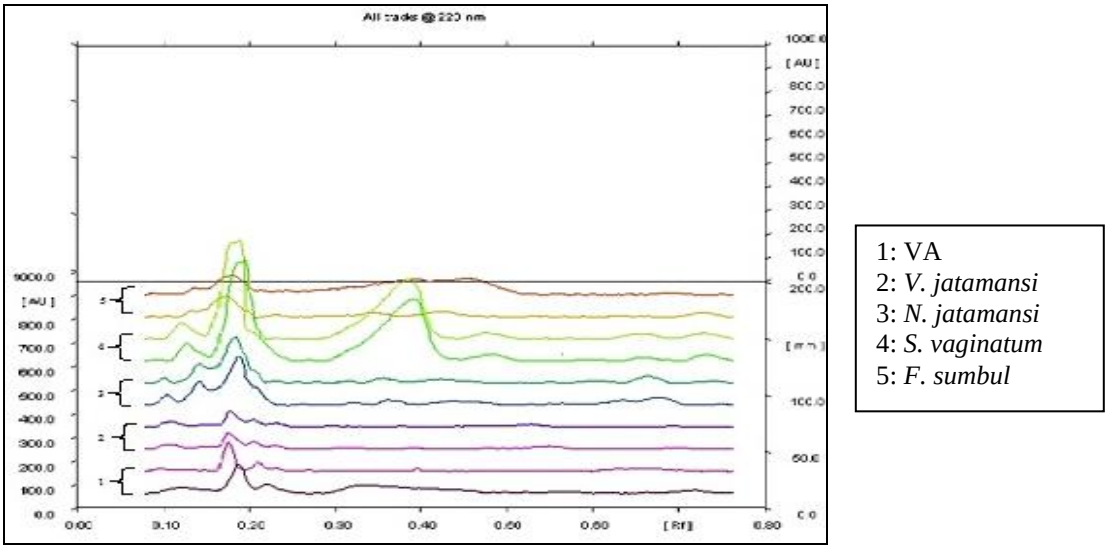

FIG.2: CHROMATOGRAM OVERLAY OF VA ALONG WITH THE DRUGS SAMPLES.

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CONFLICT OF INTEREST: The authors have no conflict of interest.

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