# Standardisation of an Ayurvedic formulation "Sanjivani Vati"

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Sanjivani vati is official in Ayurvedic formulary of India and is prescribed for the treatment of cough and fever. It is a polyherbal preparation containing ten ingredients. In this research paper, an attempt has been made to develop standardisation methods for some of the ingredients of Sanjivani vati. Quantitative estimation of tannins was done by Folin Denis method using gallic acid as standard. Embelin and Piperine were estimated by reverse phase HPLC. A standard laboratory reference sample of Sanjivani vati and two marketed samples were evaluated as per the developed method. The methods developed were validated in terms of linearity, accuracy, precision and recovery. Results indicated that only one marketed sample complied with all the standards prescribed and its content of tannin, piperine and embelin were equivalent to standard reference values.

**Key words:** Embelin, piperine, standardisation, tannins

## INTRODUCTION

Herbal medicines are generally available as a mixture of more than one plant constituent. It is important to quantify the maximum possible number of markers in such herbal formulations through which the quality of the formulation may be assessed. Sanjivani Vati, a poly herbal preparation containing ten ingredients, is prescribed in Ayurvedic formulary of India, for the treatment of cough and cold. It contains one part each of vidang (*Embelia ribes*), nagara (*Zingiber officinalis*), krsna (*Piper longum*), pathya (*Terminalia chebula*), amla (*Emblica officinalis*), bibhitaka (*Terminalia bellerica*), vaca (*Acorus calamus*), gudduci (*Tinosporia cordifolia*), bhallataka (*Semicarpus anacardium*), and visa.(*Aconitum heterophyllum*).[1] As it is difficult to estimate each and every ingredient for its chemical constituent, few of the main ingredients of Sanjivani Vati have been identified and standardised. The formulation describes the presence of triphala, vidang and pepper the principal constituents of which are tannins, embelin and piperine, respectively. It was thought worthwhile to evaluate the content of these three ingredients in the formulation. A reference sample of Sanjivani Vati (SVR) prepared in the laboratory and two marketed formulations M1 and M2 were chosen for the standardisation.

## MATERIALS AND METHODS

### Procurement of Crude Drugs

The crude drugs were purchased from the local crude drug market, Kalbadevi, Mumbai and their identity was confirmed by correlating their morphological and microscopical characters with those given in literature.[2] They were dried below 60°C, powdered, sieved through 80# and stored in air tight containers. Standard laboratory reference sample of Sanjivani Vati was prepared as per the formula given in Ayurvedic Formulary Part -1 and labeled as SVL.

### Instruments

U.V.- visible Spectrophotometer (ELICO-SL159) with matched quartz cuvettes was used for the spectrophotometric determination. HPLC was performed in a system consisting of Spinchrom with Shimadzu CC-10AT VP pumps, using a fixed wavelength guided by a programmable UV visible detector (Shimadzu SPD-10AVP) at 254 nm; chromatograms were analyzed using Spinchrom software provided with the system. The chemicals and solvents used were either Hi media HR-grade or HPLC grade.

### Estimation of Tannins

A spectrophotometric method based on the principle that tannin forms coloured complex with phosphotungstomolybdic acid was applied to estimate the tannin content individually in Haritaki, Bibhitaki and Amla.[3] A standard solution of tannic acid of strength 1mg/ml was prepared. 5ml of this solution was made up to 100 ml to give 50 μg/ml tannic acid solution.

### Linearity Studies

Linearity of tannic acid was studied in the concentration range of 3 to 30μg/ml. To each flask containing the requisite concentration; Folin-Denis reagent (0.5 ml), sodium carbonate solution (1 ml) and distilled water (up to 10 ml) was added. The absorbance was read at

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700 nm within 30 mins of the reaction, against the reagent blank. A triplicate analysis was carried out. The linear regression line, which showed the plot of absorbance vs. concentration, was then recorded.

**Estimation of Embelin and Piperine**

Quantitative estimation of embelin and piperine was carried out by HPLC. Pure samples of embelin and piperine were isolated from the fruits of *Embelia ribes* and *Piper nigrum* Linn respectively.[4,5] The purity of piperine and embelin was identified by comparison of their m.p, U.V. and I.R. spectra with the standard values.[6-9] These were then used as reference standards. Quantitative HPLC was performed on a C18 column using different solvent system comprising of Methanol, Acetonitrile and Water. The mobile phase consisting of acetonitrile: water (20:80) was found suitable for embelin and that of acetonitrile: water: acetic acid (48:52:1) for piperine. The wavelength used was 254 nm and a flow rate of 1ml/min was set. Standard stock solution of 0.1 mg/ml of each embelin and piperine was prepared in the respective mobile phases.

**Linearity Studies**

The linearity study of embelin was assessed in the concentration range 1-7ppm and that of piperine in the range 1-5ppm. 10 µl of each solution was injected into the HPLC system and chromatographed under optimised condition. A retention time of 2.79 mins for embelin and 14.98min for piperine was obtained. The linearity curves were prepared and were assessed in terms of slope, intercept and correlation coefficient values.

**Determination of Gallic Acid, Embelin and Piperine in the Formulation**

**Gallic acid content**

The gallic acid content was determined in the sample of Haritaki, Bibitaki, Amla, SVR, M1 and M2. For sample preparation, an accurately weighed amount of the test sample was refluxed below 60°C with distilled water (75 ml) for 30 mins for complete extraction of tannins. The extract was filtered through sintered glass funnel (G-3) by vacuum filtration. The filtrate was centrifuged at 200 rpm for 20 min. The supernatant volume was made up to 100ml with water in case of individual crude drugs and 25ml for SVR and marketed samples. For analysis, the blue coloured complex was developed in the similar manner as in calibration curve, replacing the tannic acid with tannin extracts and absorbance read at 700 nm. The content of tannins was estimated using the calibration curve of tannic acid and is represented in Table 1.

**Embelin and piperine content**

The embelin content was determined for vidang, SVR and marketed samples. An accurately weighed amount of the test sample was macerated overnight with diethyl ether (25 ml). The ether solution was decanted and the marc successively washed with ether till the last washing gave no pink colour with ammonia. The ether was evaporated and the residue was dissolved in 10ml of chloroform to make the test solution. These test solutions were suitably diluted for HPLC analysis.

The piperine content was determined for the fruits of pippali, SV and marketed samples. For extraction of piperine accurately weighed amount of the test sample was refluxed with dichloromethane for an hour. The contents were cooled, filtered and the residue obtained was re-extracted with dichloromethane. The combined filtrates were evaporated and the residue thus obtained was dissolved in 100 ml of ethyl acetate. Appropriate dilutions were made and were used for HPLC analysis using the optimised chromatographic conditions. The embelin and piperine content in the test solution was calculated using the standard curve and is given in Table 1.

**Percent Recovery Studies**

To an accurately weighed amount of test sample, a known amount of gallic acid, embelin or piperine was added individually in each sample. The samples were analysed and the percent recovery was calculated from the amount of drug detected. The result of all validation studies is given in Table 2.

## RESULT AND DISCUSSION

The tannin content was analysed by UV spectroscopy using gallic acid as standard. A 2% w/w tannin content was observed in Haritaki, Bibitaki and Amla. Analysis of

**Table 1: Result of analysis of Sanjivani Vati**

| Sample | Tannin content % w/w ± SD | Embelin content % w/w ± SD | Piperine content % w/w ± SD |
|---|---|---|---|
| SVL | 2.095±0.002 | 2.768±0.0019 | 1.4763±0.0019 |
| Marketed (M1) | 0.6187±0.003 | 1.680±0.0133 | 1.2474±0.0133 |
| Marketed (M2) | 1.929±0.002 | 2.494±0.0014 | 1.0199±0.0014 |

SD: Standard deviation

**Table 2: Result of validation of Sanjivani Vati**

| Analytical method | Linearity | Coefficient of variation | Accuracy (% Recovery) | Precision (Standard deviation) |
|---|---|---|---|---|
| UV-Vis. spectroscopy for tannin content | 3-30 µg/ml | 0.9978 | 99.85 | 0.003 |
| HPLC for embelin content | 1-7 ppm | 0.9873 | 99.79 | 0.0055 |
| HPLC for piperine content | 1-5 ppm | 0.9988 | 99.87 | 0.0053 |

the laboratory reference sample showed tannin content of 2.095%, while varying results were observed for the marketed samples. Comparative study of the tannin content of the marketed preparation revealed the tannin content of M1 as 0.6187% w/w and M2 as 1.929% w/w. It was observed that the sample M1 contained low amount of tannins which could be attributed to amount of crude drug added or the quality of the material used. The calibration curve for tannic acid was found to be linear in the concentration range of 3-30 μg/ml. Further the correlation coefficient indicated good linearity; the method was found to be accurate, precise and reproducible.

The fruits of vidang are reported to contain 2.6–3.2% w/w of embelin.[10] HPLC analysis of vidang showed an embelin content of 3.23% w/w which was in accordance with the reported value. Analysis of laboratory sample gave an embelin content of 2.768% w/w. The marketed sample M1 and M2 showed an embelin content of 1.680 and 2.494% w/w respectively. Linearity studies showed that embelin was linear over a concentration range of 1-7 ng, while percent recovery studies indicated a recovery of 99.87% of embelin, thus proving the accuracy and precision of the analytical method.

The fruits of pippali gave a piperine content of 1.52% w/w which was within the range given in literature i.e., 1–2% w/w.[11] Analysis of the marketed preparations revealed that formulation M1 and M2 contained 1.2474 and 1.0199% w/w of piperine respectively Linearity studies showed that piperine was linear over a concentration range of 1-5ng, while percent recovery studies indicated a recovery of 99.87% of piperine, thus proving the accuracy and precision of the analytical method.

This study was focused on quantitative estimation of tannins, embelin and piperine in Sanjivani Vati by using modern methods of analysis. From this research paper it can be concluded that the marketed formulation M2 complied with all standards prescribed, whereas M1 had low amounts of tannin and embelin content. This variation could be attributed to the amount of crude drug added or the quality of the material used. The developed method was found to be accurate and precise, and hence, could be used for the routine analysis of these drugs in ayurvedic formulations.

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