



CODEN (USA): IAJPB

ISSN: 2349-7750

**INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES**Available online at: <http://www.iajps.com>

Research Article

**QUANTITATIVE ANALYSIS OF EMBELIN IN AYURVEDIC
FORMULATIONS VIDAGNASAVA BY UV
SPECTROPHOTOMETRY****Sourabh Jain^{*1,2}, Aakanchha Jain², Asutosh Pal Jain², Nidhi Jain², Vikas Jain^{1,3},
Dharmendra Kumar^{1,4}, R G Singhal⁵**¹ School of Pharmaceutical Sciences, Shobhit University, Modipuram, Meerut.² Bhagyoday Tirth Pharmacy College, Sagar, M.P, India.³ Mahakal Institute of Pharmaceutical Studies, Ujjain, M.P, India⁴ Faculty of Pharmaceutical Sciences, UCSI University, Kuala Lumpur-56000, Malaysia.⁵ School of Basic and Applied Science, School of Pharmaceutical Sciences, Shobhit University, Modipuram, Meerut.**Abstract:**

In this present study a new, simple, rapid, sensitive, precise and economic spectrophotometric method in ultraviolet region has been developed for the determination of embelin (marker compound) in different marketed and In house formulation of Vidagnasava. Embelin showed the maximum wavelength at 294.3 nm and hence the UV spectrophotometric method was performed at 294.3 nm. The samples were prepared by solvent fraction method. Pure embelin obeys Beers law in concentration ranges of 20-120mcg/ml. The content of embelin in ayurvedic formulation was determined by calibration curve $y = 0.007x - 0.116$ $R^2 = 0.998$. The result of analysis has been validated statistically and recovery studies confirmed the accuracy of the proposed method. Hence the proposed method can be used for the reliable quantification of embelin in its ayurvedic formulation.

Key Words: Embelin, Vidangasava, Vidang, Embelia ribes**Corresponding author:****Mr. Sourabh Jain**

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Please cite this article in press as Sourabh Jain et al, *Quantitative Analysis of Embelin in Ayurvedic Formulations Vidagnasava by UV Spectrophotometry*, Indo Am. J. P. Sci, 2016; 3(9).

INTRODUCTION:

Ayurveda is a combination of two sanskrit word AYU means life and VEDA means science and the whole word means "Science of Life". Ayurvedic formulations play a vital role in management of diseases and it gives satisfactory result in the therapeutics [1, 2, 3]. Arishtas and asavas are self-generated herbal fermentations of traditional Ayurvedic system. They are alcoholic medicaments prepared by allowing the herbal juices or their decoctions to undergo fermentation with the addition of sugars. Presence of alcohol in the preparation shows several advantages, like better keeping quality, enhanced therapeutic properties, improvement in the efficiency of extraction of drug molecules from the herbs and improvement in drug delivery into the human body sites [4, 5]. The bulk of knowledge on these fermented decoctions, however, remains lacking in documentation, validation, and determination of marker compounds. Vidangasava is a well-known ayurvedic formulation included in the

ayurvedic formulary of India and mainly it is useful in treating of worm infestations. It expels Intestinal worms and helps to give relief in associated symptoms like stomachache, loss of appetite [6,7]. It contains *Emelia ribes*, *Pluchea lanceolata*, *Piper longum roots*, *Cissampelos pareira*, *Holarrhena antidysenterica*, *Prunus avium*, *Emblca officinalis*, *Woodfordia fruticosa*, *Honey*, *Cinnamomum zeylanicum*, *Cinnamomum tamala*, *Callicarpa macrophylla*, *Elettaria cardamomum*, *Bauhinia variegata*, *Zingiber officinale*, *Symplocos racemosa*, *Piper longum Piper nigrum*,. From all of these crude drugs *Embelia ribes* is marker crude drug in vidangasava [7]. *Embelia ribes* is also known vidang and it is mainly used in the treatment of worms. Embelin is [2,5-Dihydroxy-3-undecyl-2,5-cyclohexadiene-1,4-dione] an active constituent of the vidang [8, 10, 11]. The present study was based on the development and validation of a simple UV spectroscopic method for the estimation of embelin from vidangasava.

MATERIALS AND METHODS:**Drug Samples and Method of Preparation****Table 1: Vidangasava class herbs**

| S. No | Sanskrit name | Botanical Name | Quantity for 100 ml |
|-------|---------------|-----------------------------------|---------------------|
| 1. | Vidanga | <i>Emelia ribes</i> | 781.81 mg |
| 2. | Pippali Mool | <i>Piper longum roots</i> | 781.81 mg |
| 3. | Rasna | <i>Pluchea lanceolata</i> | 781.81 mg |
| 4. | Kutaj Tvak | <i>Holarrhena antidysenterica</i> | 781.81 mg |
| 5. | Patha | <i>Cissampelos pareira</i> | 781.81 mg |
| 6. | Elavaluka | <i>Prunus avium</i> | 781.81 mg |
| 7. | Amlaki | <i>Emblca officinalis</i> | 781.81 mg |
| 8. | Madhu | <i>Honey</i> | 46.90 gm |
| 9. | Dhatakpushp | <i>Woodfordia fruticosa</i> | 3127.27 mg |
| 10. | Tvak | <i>Cinnamomum zeylanicum</i> | 104.24 mg |
| 11. | Ela | <i>Elettaria cardamomum</i> | 104.24 mg |
| 12. | Tejpatra | <i>Cinnamomum tamala</i> | 104.24 mg |
| 13. | Priyangu | <i>Callicarpa macrophylla</i> | 156.36 mg |
| 14. | Kanchanar | <i>Bauhinia variegata</i> | 156.36 mg |
| 15. | Lodhra Tvak | <i>Symplocos racemosa</i> | 156.36 mg |
| 16. | Shunthi | <i>Zingiber officinale</i> | 1240 mg |
| 17. | Maricha | <i>Piper nigrum</i> | 1240 mg |
| 18. | Pippali | <i>Piper longum</i> | 1240 mg |

All these ingredients were procured from the Satbhajaiya Jadi Buti store, Sagar, Madhya Pradesh, India and were authenticated by the department of Botany, Dr. HS Gour University, Sagar. A voucher a specimen no. BTPC/Pcog/15/01 was kept as a reference in the Department of pharmacognosy, BTPC and four brands of vidangasava of reputed companies were purchased from Charka Pharma Medical Agency, Sagar, M.P.

Vidangasava is a fermented liquid preparation made with ingredients in the formulation composition shown in table 1. It contains not more than 10% and not less than 5% of self generated alcohol. Collected raw drug materials cleaned, washed, dried and used for formulation preparation. In house formulation of vidangasava is prepared as per ayurvedic formulary of India.

Chemicals

All the chemicals and solvents were used of A.R. grade, standard embelin (98%) was procured from BI Biotech India PVT. LTD, New delhi.

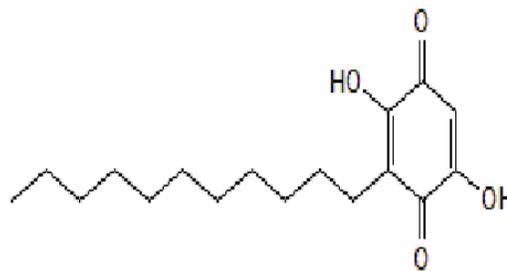


Fig 1: Chemical structure of embelin

Instrumental Specification

- ✓ **Model:** Thermo Scientific
- ✓ **Make:** Evolution 201
- ✓ **Software Version:** UV probe
- ✓ **Path length:** 10 mm
- ✓ **Slit width:** Variable
- ✓ **Blank:** Methanol
- ✓ **Wavelength:** 294

Preparation of standard Embelin solution:

Methanol was used to prepare stock solution of embelin of 1000mcg/ml concentration. Different aliquots were prepared in methanol.

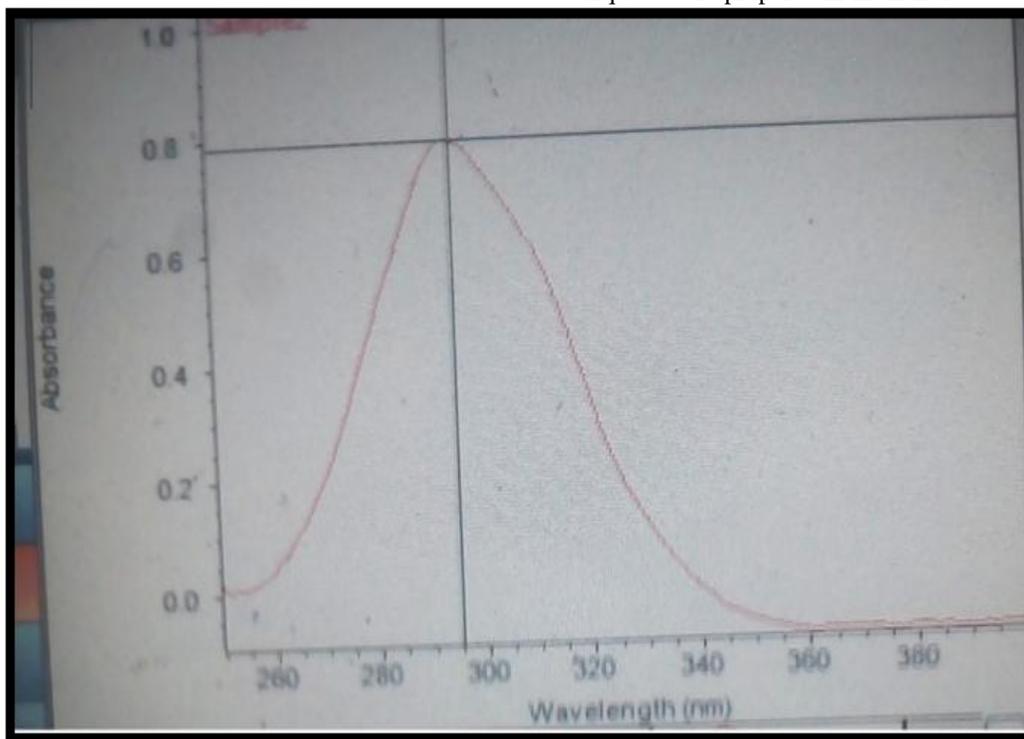


Fig 2: UV Visible spectra Pure Embelin

Preparation of sample solution

50 ml of Vindangarishta and vidangasava of each brand was dried in vacuum to remove the self generated alcohol and the extract was dissolved in 50 ml of water and partitioned with petroleum ether and ether extract was removed and extracted with n-Hexane (50ml×3), chloroform (50ml×3), and ether (50ml×3). Ether extract was filtered, concentrated, weighed and re-dissolved in methanol and volume made up to 100 ml solution was used as test a sample

for estimation purpose. The embelin content in each brand was calculated from the linearity range.

Linearity

Embelin at the concentration levels ranging from 20-120 µg/ml showed linearity between absorbance Vs concentration and obey Lambert Beers law. The equation of regression line were calculated was $y = 0.0077x - 0.116$ and value of $r^2 = 0.998$ was find out for standard Embelin.

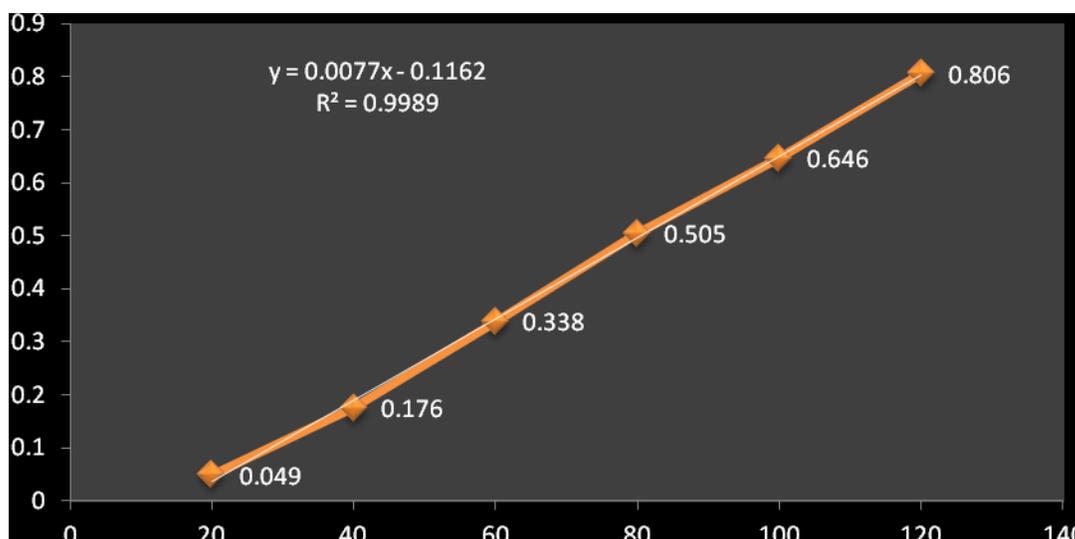


Fig 3: Calibration curve of Embelin by UV Visible spectroscopy at 294nm.

Recovery study

The values of amount added, amount recovered and % recovery is shown in Table 2. All readings were taken in triplicates.

Table 2: Recovery study of Embelin (n=3)

| Standard | Recovery level % | Standard in (µg/ml) | Amount found | % Amount recovered | SD |
|----------|------------------|---------------------|--------------|--------------------|------|
| Embelin | 50 | 50 | 50.32 | 101.60 | 0.89 |
| | 100 | 100 | 98.23 | 97.09 | 0.67 |
| | 150 | 150 | 149.41 | 99.60 | 0.91 |

Precision study

Precision study of analytical method was developed by multiple measurement of homogeneous sample [9]. These intraday and inter-day precision was utilized to study the changeability of the process (Table. 3).

Table 3: Intermediate assay precision of embelin (n=3)

| Intraday Precision | | | | Inter-day Precision | | | |
|--------------------|--------------|----------------|-------|---------------------|--------------|----------------|-------|
| Conc. (mcg/ml) | Amount Found | % Amount found | % RSD | Conc. (mcg/ml) | Amount Found | % Amount found | % RSD |
| 50 | 49.09 | 98.18 | 0.74 | 50 | 50.12 | 100.24 | 0.77 |
| 100 | 99.09 | 99.09 | 0.65 | 100 | 101.23 | 101.23 | 0.89 |

Assay result:

Concentration of Embelin from four brands and in house vidangasava formulation was calculated by linear regression $y = 0.007x - 0.116$ $R^2 = 0.998$

Table 4: Determination of Embelin from four brands and in house vidangasava formulations.

| Formulation | Mean absorbance | Concentration ($\mu\text{g/ml}$) |
|-------------|-----------------|------------------------------------|
| In House | 0.184 | 420.85 |
| Brand-A | 0.159 | 390.57 |
| Brand-B | 0.228 | 491.42 |
| Brand-C | 0.148 | 370.12 |
| Brand-D | 0.132 | 350.71 |

RESULT AND DISCUSSION:

Embelin obeys Beer Lambert's law in concentration range 20-120 $\mu\text{g/ml}$ at the λ_{max} 294 nm. The correlation coefficient (r^2) was calculated where the (r^2) value 0.998 indicates the good linearity among absorbance and concentration.

The concentration of Embelin present in different brand of vindangasava formulations and in house formulation were found to be 420.85 $\mu\text{g/ml}$ (In house formulation), 390.57 $\mu\text{g/ml}$ (Brand-A), 491.42 $\mu\text{g/ml}$ (Brand-B), 370.12 $\mu\text{g/ml}$ (Brand-C) and 350.71 $\mu\text{g/ml}$ (Brand-D) respectively (Table.4). These result showed that concentration of embelin significantly higher in brand B as compare to other brand.

The recovery studies were performed at three levels by adding known amount of embelin with pre-analyzed sample of embelin in vidangasava. The experiment was repeated three Times at both level (Table.2) and result shows 101.60%, 97.09 % and 99.60 % which prove reproducibility of the result. The % standard deviation (%SD) value was found to be 0.89, 0.67 and 0.91 respectively. In interday precision study % relative standard deviation (%RSD) value were found to be 0.74, 0.65 and intraday precision, % relative standard deviation (%RSD) value were found 0.77, 0.89 for embelin (Table.3) .The low value of slandered deviation showed that, the method is precise. From the data it is obvious that the present method of UV spectrophotometric determination of embelin is simple, precise, accurate, and suitable for routine analysis of embelin in vidangasava.

CONCLUSION:

Vidangasava is ayurvedic fermented formulation which contains fifteen herbal drugs. In all of these herbal drugs vidang is marker plant which is mainly used as anthelmintic. Embelin is natural benzoquinone derivative which is isolated from vidang (*Embelia ribes*). The present method designed to estimate marker embelin from vidangasava and result showed that method was simple, precise, robust, rugged and accurate and can be used for the routine analysis of embelin from ayurvedic fermented formulation.

COMPETING INTERESTS:

The authors declared that there is no conflict of interests exists.

ACKNOWLEDGEMENT:

Authors are thankful to Management of Bhagyodaya Tirth pharmacy college, Sagar for providing the facility to carry out research work.

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