

Physicochemical Investigation of Vidanga Berries

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Abstract: Herbal medicines are being used as a effective source for treatment of many diseases since ancient times. Embelin, is an active phytoconstituent obtained from fruits of *Embelia tsjeriam cottam*, commonly known as Vidanga. Vidanga consists of phytoconstituents which is used o cure various severe diseases. Thus there is a need to justify the Quality of the herbal drugs which are highly used nowadays. Standardization methods hence act as an tests performed to ensure the safety of health. Also the phytochemical investigation is performed to make the use of important chemical constituents to its fullest. In this work we have performed various evaluation tests as per Ayurvedic Pharmacopia which were found to be within the limits. An effort was made further to optimize the best extraction solvent. UV spectroscopic scanning was done from 200-400nm of which λ_{max} was found to be 291nm with a good peak shape and height for chloroform. HPTLC studies revealed a better peak height for Toulene:ethyl acetate and better separation of constituents at chloroform:methanol for chloroform solvent. Thus Chloroform was found to the best solvent for extraction of vidanga berries.

Keywords: Vidanga, *Embelia tsjeriam cottam*, Phytochemical, Standardization

1. Introduction

Embelin (2, 5-dihydroxy-3-undecyl-p-benzoquinone), is found to be the active principle of *Embelia tsjeriam cottam* commonly known as Vidanga. Vindanga is official in Indian pharmacopeia 2014.⁽¹⁾ and Ayurvedic Pharmacopia ⁽²⁾. *Embelia tsjeriam cottam* belonging to the family Myrsinaceae, is a climber found in the Western Ghats of Lonavala and also seen in the southern states of Maharashtra, Karnataka, Kerala, Tamil Nadu and Andhra Pradesh upto an altitude of 1600m. The fruits of this plant contain (2.5-3.1%) embelin also known as second solid gold of India⁽³⁾. *Embelia tsjeriam cottam* is reported to possess a wide spectrum of biological activities. The fruits, leaves and roots are used to cure various diseases Embelin (2, 5-

dihydroxy-3-undecyl-p-benzoquinone), is found to be the active principle of *Embelia tsjerium*. Quinone constitutes one of the well known groups of naturally occurring organic compounds. One of the major attractions among researchers towards quinone compounds is their color and biological activities¹. Benzoquinones are the simplest representatives of quinone grouEmbelin shows diverse pharmacological activities including chemo prevention in hepato-carcinogenesis observed in Wistar rats ⁽⁴⁾, antifertility effects⁽⁵⁾, antiobesity⁽⁶⁾, antitubercular⁽⁷⁾, antibacterial⁽⁸⁾

In this present work, an effort has been made to study the physicochemical characteristics of vidanga berries and test the various phytoconstituents present in it.



Figure 1: Vidanga berries

Synonyms:

Common name: Malabar Embelia
Hindi: Babrang, Baibrang, Bayabirang, Bhingi, Baya Birang
Marathi: Ambati, Ambuti, Kokla, Waiwarung,vavding
English- Vidanga
Sanskrit : Jantughna, Krmighna, Vella, Krmihara, Krmirip

2. Material and Methods

Plant material:

The dried berries of *Embelia tsjeriam cottam*(vidanga) and Embelin marker were procured from Yucca enterprises, Mumbai. Authentication was done by Agharkar Research Institute, Pune

Reagents and chemicals:

Methanol AR Grade, Ethyl acetate AR Grade.,Toluene AR Grade,n-hexane,Chloroform, Distilled Water.

Preparation of Standard stock solution:

Standard stock solution of Embelin was prepared separately by dissolving 10mg of marker in 10ml of methanol to get concentration of 1000µg/ml. From the respective standard stock solution, working standard solution was prepared containing 100µg/ml of Embelin separately in methanol

Selection of mobile phase and chromatographic conditions:

Chromatographic separation studies were carried out on the working standard solution of Embelin(100ug/ml). Toulene: Ethyl acetate and Chloroform:Methanol , were chosen as the mobile phase, which was reported and was found to have good resolution and acceptable peak parameters.

Selection of Detection Wavelength:

100 mg of each extract was weighed and dissolved in corresponding solvent in 10 ml volumetric flask to obtain stock solution of extract (10mg/ml). Further 1ml stock solution was diluted to 100 ml using corresponding solvent, so that strength of final working solution was 100mcg/ml. The solution of each extract was centrifuged at 1500 rpm for 20 minutes.From the standard stock solution further dilutions were done using methanol and scanned over the range of 200-400 nm and the spectrum was obtained. It was observed that the marker showed considerable absorbance at 291 nm

3. Result and Discussion

3.1 Physicochemical investigation

3.1.1 Plant Material

The dried berries of Embelia tjseriam cottam(vidanga) and Embelin marker were procured from Yucca enterprises, Mumbai. Authentication was done by Agharkar Research Institute, Pune.

3.1.2 Evaluation Tests of Berries powder

Table 1: Evaluation of Powder

S. No	Prameter	Specification	Observation	Result
1.	Preliminary test			
1.1	Colour	Brownish	Brownish	Passes
1.2	Odour	Odourless	Odourless	Passes
1.3	Taste	Sweet	Sweet	Passes
2.	Chemical test			
2.1	Alkaloid	Brown ppt	Brown colour ppt in aqueous layer	Passes
2.2	Foreign Organic matter	NMT 2%	1.2%	Passes
2.3	Total Ash	NMT 6%	4%	Passes
2.4	Acid insoluble ash	NMT 1.5%	1.5%	Passes
2.5	Alcohol soluble Extractive	NLT 10%	11.2%	
2.6	Water soluble Extractive	NLT 9%	10.4%	Passes

3.1.3 Extractive Value

50gm Fruit powder of Vidanga was accurately weighed and dispersed in 100 ml Ethanol. The extraction was carried out for 5 cycles. The extract was concentrated on water bath at 50°C and powder form was obtained .Extraction was carried out in the following solvents with reference to paper⁽⁹⁾using Water, Methanol, Ethanol, Petroleum Ether, Ethyl Acetate, N-hexane, Chloroform .High percentage of extractive value in Ethanol solvent shows presence of more Polar components than non-polar ones. Ethyl acetate and ethanol shows high percentage extractive value than non-polar solvent. Percentages of extractive values are shown in Table no.5 below.

Table 2: Percentage of extractable matter

Sr. no	Extract	Percentage	Polarity
1.	Water	6%	9
2.	Methanol	7%	5.1
3.	Ethanol	9%	5.1
4.	Pet. Ether	11%	4.5
5.	Ethyl acetate	4%	4.4
6.	N-hexane	6%	4.3
7.	Chloroform	5.6%	4.1

3.2 Evaluation of Extracts

a) UV spectrum of Extracts:

The supernatant solution was used for determination of UV spectrum. Multiwavelength scan was done. The absorbance at λmax was used as quality control parameter to check quality of powder. UV spectrum of extracts in diff solvents is as shown in Fig.5.1

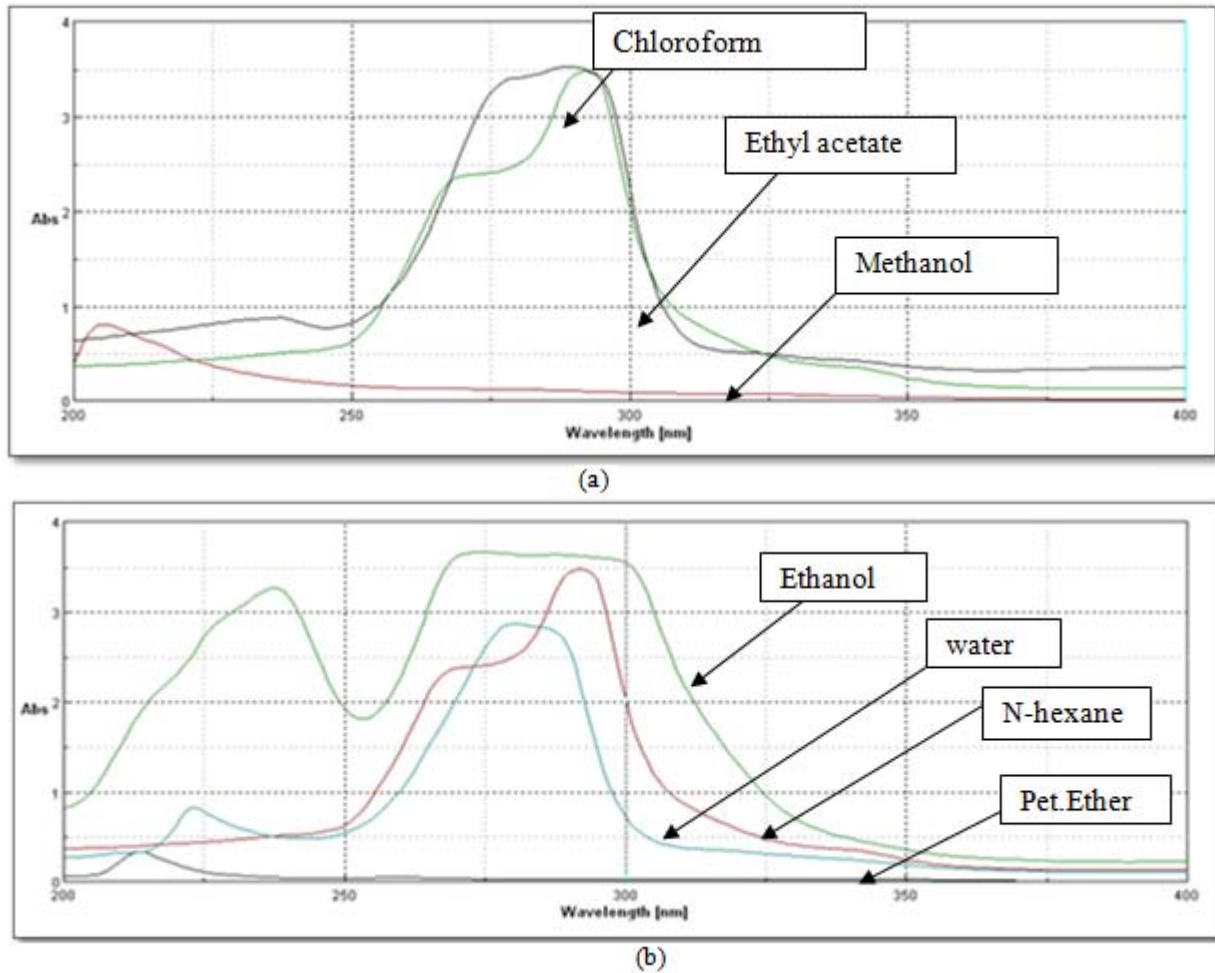


Figure 1: UV spectrum of solvent extracts (A) and (B)

b) HPTLC of extracts

HPTLC of extracts obtained using Methanol, Ethanol, Chloroform, Ethyl acetate, Dichloromethane, n-Hexane as solvents using different solvent system on TLC plates pre-coated with silica gel 60 F254 (10 × 10 cm with 250µm layer thickness) from E. Merck, Germany. The samples were applied onto plates as a band with 6mm width using applicator (Camag, Switzerland). Linear ascending development was carried in a twin trough glass chamber (20 × 20cm) using solvent system. Plates were then observed under UV lamp at 254nm. Results of various extract as shown in Table.3. HPTLC plate of solvents extract using mobile phase Toulene: Ethyl acetate and Chloroform:Methanol as shown under UV chamber at Fluorescent mode as shown in fig.2 and 3.

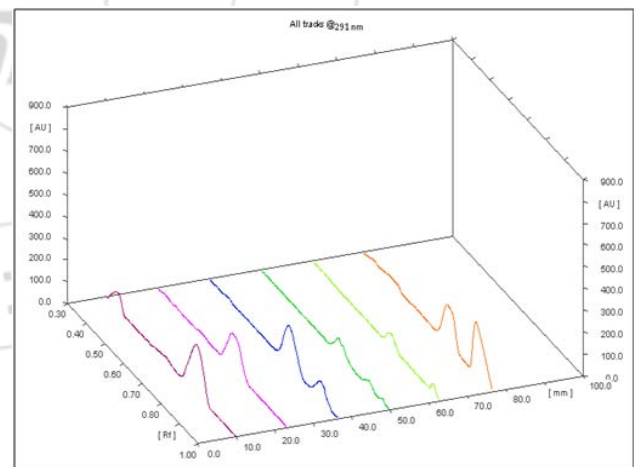


Figure 2: Densitogram of diff. solvents extracts in Toulene: Ethyl acetate(6:4)
 Track- 1.ETOH 2. Ethyl acetate, 3. Chloroform, 4. N-hexane, 5. Pet ether, 7. Water

Table 3: Rf value of various solvents in Toulene:Ethyl acetate(6:4)

Sr. no.	Extracted solvent	Band observed 291nm
1.	Ethanol	0.75
2.	Ethyl acetate	0.70
3.	Chloroform	0.74,0.92
4.	N-hexane	0.80,0.9
5.	Pet ether	0.80
6.	Water	0.78,0.95

Table 4: Rf value of various solvents in Chloroform:Methanol(6:4)

Sr. No.	Extracted solvent	Band observed at 291nm
1.	Ethanol	0.20
2.	Ethyl acetate	0.20,0.30
3.	Chloroform	0.20,0.32,0.85
4.	N-hexane	0.12,0.25
5.	Pet ether	0.20
6.	Water	0.28,0.42,0.76

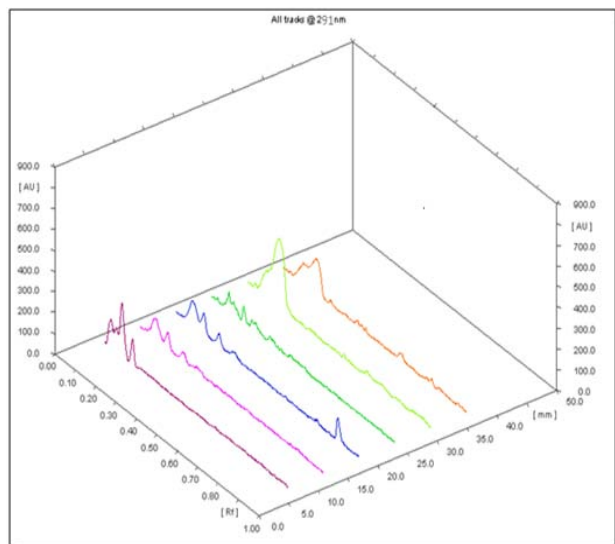


Figure 3: Densitogram of diff. solvents extracts in Chloroform:Methanol (6:4)

Track- 1. ETOH 2. Ethyl acetate, 3. Chloroform, 4. N-hexane, 5. Pet ether, 7. Water

4. Conclusion

Physicochemical Investigation of Vidanga berries was done successfully. The tests revealed the presence of various Alkaloids, Glycosides, Tannins. Ash value was determined which was found to be within the limit as per standards of Ayurvedic Pharmacopoeia. Chloroform showed a good UV spectroscopic absorbance as compared to all other solvents. HPTLC studies also showed the presence of various phytoconstituents in chloroform in both the mobile phases. Toulene:Ethyl acetate showed a good peak height and higher separation was observed when Chloroform:methanol was used as mobile phase which was clearly differentiable with a better separation distance between each constituent. Thus chloroform can be preferable used as an solvent for extraction of Vidanga berries.

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