RESEARCH PAPER

Accepted 27 November 2004
Revised 10 June 2004
Received 23 May 2003
Indian J. Pharm. Sci., 2004, 66(6): 739-744

Interaction of Embelin and Iron in Ayurvedic Formulations

P. SANDHYA AND N. D. GRAMPUROHIT*
C. U. Shah College of Pharmacy, S. N. D.T. Women's University, Sir Vithaldas Vidya Vihar, Juhu, Santacruz (W), Mumbai–400 049.

Ayurveda, the ancient system of herbal medicine is growing popular world wide, but a number of factors such as standardization and stability studies are still at an infant stage for an ayurvedic product. This research paper deals with the study of interaction in some ayurvedic preparations containing *vidang* with *loha bhasma* under different storage conditions. The formulations selected were *Vidangadi Lauha, Candanadi Lauha and Navayasa Churna*. The ratio of *vidang* and *loha bhasma* in these preparations were 1:9 and 1:12, hence test samples containing this ratio and pure *vidang* were subjected to different storage conditions such as, at room temperature, in a dessicator at room temperature and at cold temperature for a period of 6 months. The samples were also evaluated for antimicrobial activity. It was observed that the test samples (except pure *vidang*) showed a decrease in embelin content with time. The embelin content in pure *vidang* remained constant.

Ayurveda, the traditional system of herbal medicine is gaining popularity day by day in our country and even in the Western world. What Ayurveda lacks today is the standardization and stability studies of its formulations. These are some of the relevant features that are yet at an infant stage for an Ayurvedic product. Previous studies from our laboratory showed that *Navayasa Churna*[1] that contained *vidang* and *loha bhasma* as two of its ingredients, had very less embelin content as compared to the fresh sample. This indicated that there could possibly be an interaction of embelin with *loha bhasma* due to which the embelin content might have reduced. This problem needed further investigation. The present research paper describes interaction of embelin with *loha bhasma* (iron) in certain Ayurvedic formulations. The three marketed formulations selected for this study were, *Vidangadi Lauha, Candanadi Lauha and Navayasa Churna*. These are official in Ayurvedic Formulary of India, Part I[2-4]. The ratio of *Vidang* and *Loha Bhasma* in *Vidangadi Lauha* and *Candanadi Lauha* was 1:12 and in *Navayasa Churna* it was 1:9.

***For correspondence.**
Email: drnirmalag@yahoo.com

MATERIALS AND METHODS

The crude drugs and *loha bhasma* were purchased from the local crude drug market at Kalbadevi, Mumbai. Their identity was confirmed by correlating its morphological characters with those given in the literature. Standard laboratory reference samples of *Vidangadi Lauha, Candanadi Lauha and Navayasa Churna* were prepared as per the formulae given in the Ayurvedic Formulary of India, Part I and these preparations were labeled as VL, CL and NC, respectively. Three other test samples containing pure *vidang, vidang* with *loha bhasma* in the ratio of 1:9 and 1:12 were also prepared and labeled as V, V+LB (1:9) and V+LB (1:12), respectively.

All the six test samples were then kept at three different storage conditions that included, room temperature (28-30°), in a desiccator at room temperature (with anhydrous calcium carbonate as desiccant) and cold temperature (6-8°). These stored samples were assayed for iron content, embelin content and antimicrobial activity of embelin over a period of 6 months.

Marketed samples of *Vidangadi Lauha* (Anuja Pharma), *Candanadi Lauha* (Ganesh Ayurvedic Centre) *and Navayasa Churna* (Baidyanath) were purchased from retail shops, Mumbai and were labeled as MVL, MCL and MNC, respectively. These were also analyzed along with the stored laboratory samples.

Estimation of iron content:

A spectroscopic method based on the principle that iron forms orange red complex with 1,10-phenanthroline at pH 3.9, with an absorption maximum of 396 nm was applied to estimate the iron content. A standard solution of iron (IP) of strength 0.1 mg/ml was prepared using ferrous ammonium sulphate[5]. The iron content for the test samples was determined at the beginning and at the end of the 6-month storage period. For the sample preparation, an accurately weighed amount of test sample was treated with concentrated HCl. It was then filtered and the filtrate was diluted suitably with distilled water. The pH was adjusted between 3-4 and the absorbance of the colour developed with the reagent was read at 396 nm.

Estimation of embelin content:

Embelin was quantitatively estimated by a HPTLC method. Crude embelin was extracted from the fruits of *Embelia ribes* using n-hexane as solvent. The extracted embelin was purified and its identity confirmed by comparing its mp, UV and IR spectra with those given in the literature and was then used as reference standard. Various solvent systems were tried using precoated silica gel GF_{254} plates of 0.2 mm thickness. The solvent system comprising of n-hexane:n-butanol:4N ammonia (7:1:2) gave best resolution of embelin peaks. For HPTLC analysis, a standard solution of embelin (0.01 µg/µl) was applied as 4 mm bands using a Camag Linomat IV applicator. The plates were developed in a twin trough chamber to a distance of 7 cm. On scanning with Camag TLC scanner III, embelin was located at an R_f of 0.44. *In situ* UV spectrum was recorded and this gave a λmax of 333 nm. This λmax was kept constant for the estimation of embelin in the test samples.

The embelin content was determined in all six test samples every 15 d for 6 mo. For extraction of embelin, accurately weighed amount of samples were kept overnight for maceration with ether. The ethereal layer was decanted and the marc successively washed with ether till the ether layer gave no pink colour with ammonia. The combined extracts were evaporated and the residue obtained was dissolved in 10 ml of chloroform to make test solutions. These test solutions were suitably diluted and applied on HPTLC plates along with standard solution of embelin. The plates were developed and scanned to record the HPTLC chromatograms. The area under the curve of the standard and sample were used to calculate the embelin content in the test samples. The marketed samples were also analyzed for embelin content.

Determination of antimicrobial activity of embelin:

Embelin was found to have good antimicrobial activity[6]. This activity was assayed by agar diffusion method using the microorganisms, *Bacillus subtilis*, *Escherichia coli*, *Shigella dysentrica* and *Staphylococcus aureus*. A standard inoculum of 1×10^6 organisms/ml was used. Cups of 9 mm diameter were bored into previously solidified medium. A standard solution of embelin (1 mg/ml) was prepared in N,N-dimethyl formamide. The antimicrobial activity was determined in the samples of V, V+LB (1: 9) and V+LB (1:12). Embelin was extracted by overnight maceration with ether. The ethereal layer was decanted and the marc successively washed. The combined extracts were evaporated and the residue obtained was dissolved in N,N-dimethyl formamide. An aliquot (0.2 ml) of the test and standard solutions were added into the cups bored on agar plates. The zone of inhibition of the standard and sample was measured and used to calculate the embelin content in the test samples.

Identification of the complex:

Embelin, being a benzoquinolone derivative forms metallic complexes[7]. During the study it was observed that the test samples showed a decrease in embelin content with time. This decrease in embelin content could be attributed to the complex formation between embelin and iron. Preparative TLC was used to isolate the complex from a 6 mo old sample of vidang with loha bhasma. This complex was subjected to spectral (IR spectrum) and elemental analysis.

RESULTS AND DISCUSSION

The percentage of total iron content in loha bhasma was found to be 45.2% w/w. Hence, theoretically the formulations should contain 22.6% w/w and samples of vidang with *loha bhasma* in the ratio of 1:9 and 1:12 should contain 40.8 % w/w of total iron content, respectively. When the test samples were analyzed at the beginning and end of 6 mo the total iron content (%w/w) was found close to the theoretical value (Table 1). Hence it can be concluded that the iron content remained constant during the stability studies at different temperatures. The standard curve for

TABLE 1: ESTIMATION OF IRON CONTENT (%W/W) IN TEST SAMPLES

Test Condition	*Vidangadi Lauha*	*Candanadi Lauha*	*Navayasa Churna*	*Vidang + Loha Bhasma* (1:9)	*Vidang + Loha Bhasma* (1:12)
Room Temp	24.3	24.3	24.1	43.2	42.9
Desiccator	24.1	24.0	24.1	42.9	42.8
Cold Temp	24.3	24.0	23.9	43.8	42.9
After 6 mo					
Room Temp	24.1	24.3	24.3	43.3	42.0
Desiccator	24.1	24.2	24.0	42.9	43.0
Cold Temp	24.1	24.0	24.0	42.8	43.0

The table summarizes the iron content in the test samples at the beginning and end of the study.

iron was found to be linear in the range of 3.2 to 16 ppm. The UV spectroscopic method gave a recovery of 100.2, 100.7 and 100.7% w/w of total iron content for VL, CL, and NC respectively indicating that the method was accurate and precise. The marketed samples, MVL, MCL, and MNC showed a total iron content of 24.3, 24.5 and 20.8 %w/w, respectively.

Vidang fruits showed an embelin content of 2.6 % w/w. Hence, theoretically *Vidangadi Lauha* and *Candanadi Lauha* should have 0.11% w/w and *Navayasa Churna* should have 0.14% w/w of embelin. The mixture of *vidang* with *loha bhasma* in the ratio of 1:9 and 1:12 should have 0.2 and 0.26 %w/w of embelin content, respectively. Embelin in the test samples on 0 d was found similar to the expected theoretical yield. But as the study progressed the content of embelin in all samples except *vidang* reduced with time. The sample of *vidang* showed constant embelin content (Table 2). The other observations made were, the sample of *vidang* with *loha bhasma* had an embelin content of 0.04%

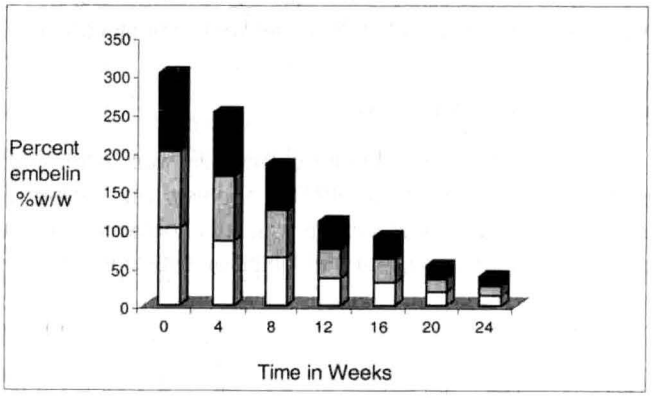

Fig. 1: Graphical representation of embelin content on storage for *Vidangadi Lauha*

The reduction of embelin content in *Vidangadi lauha* is shown graphically by plotting percent embelin content in abscissa and time in weeks in ordinate scale at cold temperature (■), in desiccator at room temperature (■) and at room temperature (□).

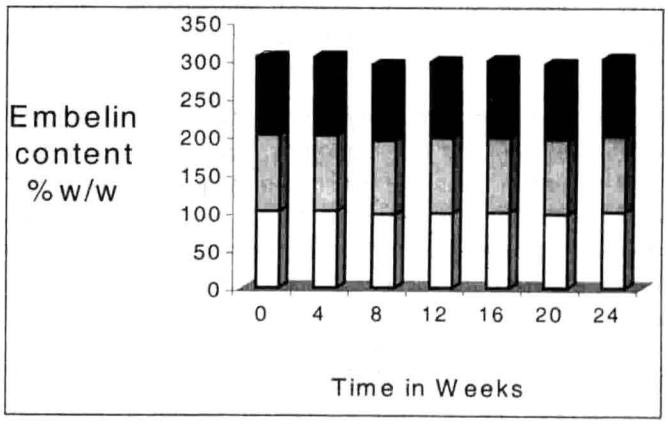

Fig. 2: Graphical representation of embelin content on storage for *Vidang*

The embelin content in *vidang* is shown graphically by plotting percent embelin content in abscissa and time in weeks in ordinate scale at cold temperature (■), in desiccator at room temperature (■) and at room temperature (□).

TABLE 2: ESTIMATION OF EMBELIN CONTENT (% W/W) IN TEST SAMPLES

TIW	*Vidangadi Lauha*			*Candanadi Lauha*			*Navayasa Churna*		
	r.t.	Desi	c.t.	r.t.	Desi	c.t.	r.t.	Desi	c.t.
0	0.108	0.108	0.112	0.104	0.105	0.103	0.144	0.145	0.144
4	0.091	0.09	0.089	0.047	0.047	0.047	0.133	0.129	0.128
8	0.068	0.066	0.063	0.027	0.028	0.029	0.093	0.096	0.092
12	0.037	0.041	0.038	0.019	0.017	0.017	0.045	0.048	0.049
16	0.032	0.034	0.031	0.015	0.016	0.016	0.039	0.039	0.037
20	0.019	0.018	0.018	0.013	0.013	0.013	0.028	0.027	0.027
24	0.013	0.013	0.013	0.007	-	-	0.017	0.016	0.016
	Vidang + Loha Bhasma (1:9)			**Vidang + Loha Bhasma (1:12)**			**Vidang**		
	r.t.	Desi	c.t.	r.t.	Desi	c.t.	r.t.	Desi	c.t.
0	0.265	0.268	0.265	0.206	0.204	0.211	2.61	2.62	2.61
4	0.249	0.248	0.252	0.176	0.176	0.177	2.62	2.58	2.65
8	0.185	0.186	0.183	0.123	0.123	0.125	2.52	2.50	2.57
12	0.121	0.117	0.12	0.084	0.088	0.093	2.55	2.58	2.57
16	0.093	0.095	0.096	0.069	0.067	0.071	2.57	2.57	2.59
20	0.076	0.077	0.077	0.047	0.056	0.051	2.52	2.55	2.55
24	0.052	0.051	0.051	0.039	0.038	0.037	2.57	2.60	2.61

TIW – Time in weeks, r.t. – room temperature, Desi – In desiccator at room temperature, c.t. - cold temperature. The reduction in embelin content in the formulation at the end of 6 mo clearly indicates an interaction between embelin and iron.

w/w whereas the formulations had 0.017% w/w of embelin after 6 mo. For the formulation of *Navayasa churna* only 10% of embelin was lost within one month whereas about 50% reduction was seen for *Candanadi Lauha*. These results indicated that other ingredients present in the formulation could have enhanced the degradation of embelin.

The % w/w of embelin in relation to the content present in freshly prepared sample was calculated. A graphical representation of percent embelin content v/s time for the samples of VL and *vidang* is shown in fig. 1 and 2. Similar graphs were also obtained for other samples. The linearity curve of embelin was found to be linear in the range of 6 to 12 ppm. The HPTLC method of analysis gave a recovery of 100, 102.4 and 101.7 %w/w of embelin for VL, CL, and NC, respectively. The embelin content in the marketed samples was below detectable level.

The antimicrobial activity of embelin was determined to correlate the results of HPTLC analysis with biological activity. The sample of *vidang* with *loha bhasma* showed successive decrease in zone of inhibition with time whereas

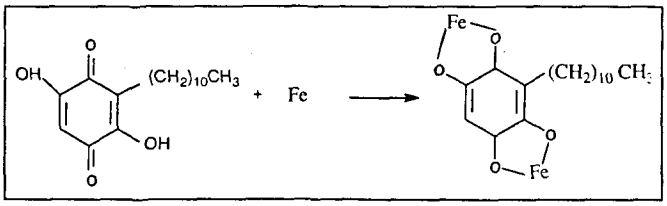

Fig. 3: Molecular structure of the complex formed between iron and embelin.

Iron can react with embelin to form the complex.

TABLE 3: DETERMINATION OF ANTIMICROBIAL ACTIVITY (EMBELIN CONTENT % W/W) IN TEST SAMPLES

TIW	Organism: *Bacillus subtilis*								
	Vidang + *Loha Bhasma* (1:9)			*Vidang* + *Loha Bhasma* (1:12)			*Vidang*		
	r.t.	Desi	c.t.	r.t.	Desi	c.t.	r.t.	Desi	c.t.
0	100	100	104	104	108	100	112	112	108
4	91.8	91.8	91.8	83.6	87.7	87.7	100	100	100
8	71.4	75.5	75.5	71.4	75.5	67.3	100	100	95.9
12	46.9	46.9	46.9	51.0	42.8	42.8	108	100	100
16	42.8	38.7	34.6	42.8	30.6	34.6	100	100	100
20	22.4	22.4	26.5	26.5	18.3	22.4	100	100	100
24	10.2	14.2	10.2	14.2	10.2	14.2	104	100	104
				Organism: *Escherichia coli*					
	Vidang + *Loha Bhasma* (1:9)			*Vidang* + *Loha Bhasma* (1:12)			*Vidang*		
	r.t.	Desi	c.t.	r.t.	Desi	c.t.	r.t.	Desi	c.t.
0	95.59	93.39	93.39	97.8	91.18	91.18	100	100	100
4	73.55	82.37	77.96	82.37	73.55	77.96	100	100	100
8	69.14	66.94	69.14	75.76	69.14	64.71	104.4	100	100
12	60.33	55.72	58.12	62.53	55.92	55.92	104.4	100	100
16	49.31	47.1	42.7	53.72	42.7	42.7	104.4	100	106.6
20	33.88	31.68	29.47	33.88	29.47	29.47	100	108.8	100
24	22.86	22.86	25.06	25.06	20.66	22.86	100	100	100

TIW – Time in weeks, r.t. – room temperature, Desi – desiccator in room temperature, c.t. - cold temperature. The reduction in antimicrobial activity of the test samples at the end of 6 mo indicates the loss of potency of embelin due to its interaction with iron.

in *vidang* powder the zone of inhibition was constant. The results for only *B. subtilis* and *E. coli* were presented in Table 3. Similar results were obtained for the other two organisms tested. The percentage of embelin on the basis of microbial assay in comparison with fresh sample was calculated. The results showed a decrease from 100% to 23%.

The decrease in embelin content could be attributed to the fact that embelin forms a complex with iron. The comparison of IR spectra of pure embelin and the complex showed the disappearance of the hydroxyl group and the presence of only the ketone group of the complex. The elemental analysis revealed the elements C-49.9%, H-6.3%, O-16.5% and the residual ash of 27.3%. These results matched with content calculated theoretically from the assumed molecular formula of the complex (fig. 3). The antimicrobial activity for the complex was also tested against the same test organisms. No zone of inhibition for the complex was observed.

From this investigation it is evident that embelin forms a complex with iron, thus reducing the content of free embelin in the formulations with time. Hence, in order to

retain the activity of embelin it is essential to administer a freshly prepared formulation. However, further investigations are necessary to find out *in vivo* availability of embelin from the formulations.

REFERENCES

1. Eapen, S. and Grampurohit, N.D., **Indian Drugs**, 2002, 39, 101.
2. The Ayurvedic Formulary of India, Part I, 1st Edn., Government of India, Ministry of Health and Family Planning, 1976, 20.
3. The Ayurvedic Formulary of India, Part I, 1st Edn., Government of India, Ministry of Health and Family Planning, New Delhi, 1976, 224.
4. The Ayurvedic Formulary of India, Part I, 1st Edn., Government of India, Ministry of Health and Family Planning, New Delhi, 1976, 226.
5. Vogel, A.L., In; Jeffrey, G.H., Basset, J., Eds., Vogel's Textbook of Quantitative Chemical Analysis, 5th Edn., ELBS with Longmann, London, 1989, 690.
6. Rodrique, S., **Fitotherapia**, 1994, 65, 47
7. Rao, C.B. and Venkateshwarulu, V., **Current Sci.**, 1960, 29, 136.