MICROBIOLOGICAL EVALUATION OF *LOHASAVA*

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Abstract

Through the use of selected microbial species and by employment of microbiological procedures, *lohasava*, an Ayurvedic medicinal preparation containing iron has been evaluated for its therapeutic potency. The microbiological procedures have been shown to be suitable for the chemical and biological evaluation of the medicine.

Introduction

Lohasava is a popular, iron-containing Ayurvedic tonic preparation renowned for its efficacy against anaemias, dropsy, chlorosis and as a general restorative after chronic illness, especially malaria; claim has also been made in its favour for checking diarrhoea and as a remedy against diseases of the spleen. As an indigenous product, it is manufactured by almost all the pharmacies and is available throughout the country. Although there is no authentic record about the magnitude of its production, two of the manufacturers have indicated its manufacture as amounting to 640 and 1,000 gallons annually. Information gathered also seems to indicate that the preparation is made through a fermentation process for which a liquor containing honey, jaggery, iron filings, ginger, pepper, myrobalan, etc., is made use of and finally the debris filtered off. Despite such large scale production and so widespread consumption of this preparation for which known amounts of well-known ingredients are used, there has not been any attempt made at either laying down standards for the choice of its raw materials, its contents or establishing its potency. It was in this context that attempts were made at assessing the potency of this medicine and the experiments carried out and results recorded are presented in this paper.

It is evident from the name of the preparation itself and the claims made in its behalf as a therapeutic that iron forms the principal and effective ingredient. The role of iron in the nutrition and metabolism of animals and microorganisms has been recently reviewed to need any further mention here.[1, 5] The sensitivity of *Clostridium lacto-acetophilum* and its ready response to even minute amounts of this element in all its chemical combinations provided an

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TABLE I

Results of analyses of *lohasava*

	1	2	3	4	5	6	7	8	9
Manufacturer's code Batch No. name and packing	Z 1028 16 oz.	Z 1093 16 oz.	AP 5577 16 oz.	AP 125622 16 oz.	S_I * 8 oz.	S_{II} * 8 oz.	M_I * 8 oz.	M_{II} * 8 oz.	NP * 8 oz.
Colour	Dark brown with turbidity	Dark brown with turbidity	Dark brown with turbidity	Dark brown with turbidity	Dark brown with turbidity	Dark brown with turbidity	Black with ppt.	Black with ppt.	Dark brown with turbidity
Sp. gr.	1.103	1.093	1.124	1.12	1.005	1.076	1.0	1.014	1.129
Ref. index	1.3752	1.3716	1.3887	1.3846	1.367	1.3	1.338	1.339	1.386
Alcohol content ml./per 100 ml.	7.1	6.2	7.77	4.86	11.7	10.0	7.1	7.5	7.55
Solid value g per 100 ml.	30.2	25.96	32.32	30.9	20.34	23.64	3.1	10.56	35.8
pH	3.6	3.9	4.5	4.6	4.15	4.1	3.6	3.4	4.3
Acidity ml. 0.1 N NaOH per 100 ml.									
Total	26.4	113.4	119.0	111.2	88.74	122.9	187.6	229.1	236.0
Fixed	25.3	44.46	58.0	52.2	53.13	58.0	48.07	107.3	66.7
Volatile	1.1	68.76	61.0	59.0	35.61	64.9	139.53	121.8	169.3
Sugars g per 100 ml.									
Total	28.56	24.64	32.0	30.0	18.26	16.15	0.875	9.13	30.0
Reducing	27.06	24.64	32.0	30.0	18.26	16.15	0.875	9.13	30.0
Sucrose	1.5	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil

* Batch No. not mentioned

	1	2	3	4	5	6	7	8	9
Vitamins mg. per 100 ml.									
Thiamine	0.020	0.035	0.036	0.030	0.031	0.025	0.023	0.036	0.08
Riboflavin	0.05	0.25	1.0	0.8	1.0	1.1	Nil	0.01	2.0
B_{12}	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil	Nil
Folic acid	0.12	0.15	0.2	0.18	0 125	0.10	0.08	0.06	0.25
Pyridoxine	Nil	Nil	0.5	0.8	0.1	0.2	Nil	Nil	0.5
Calcium panto-thenate	Nil	Nil	0.2	0.14	0.12	0.10	Nil	Nil	0.15
Ascorbic acid	4.5	3.5	4.7	5.88	2.35	3.5	3.5	2.35	6.47
g. per 100 ml.									
Nitrogen	0.029	0.006	0.0426	0.038	0.0015	0.028	0.0007	0.001	0.058
Protein N × 6.25	0.18	0.0375	0.2663	0.2375	0.0094	0.175	0.0044	0.0062	0.362
Ash	0.36	0.5	1.36	0.98	0.3	1.22	0.24	0.54	1.74
Calcium	0.152	0.1	0.2413	0.1706	0.14	0.149	0.12	0.145	0.147
Iron	0.062	0.206	0.164	0.049	0.0446	0.119	0.0279	0.165	0.363
Phosphorus	0.0035	0.0034	0.088	0.095	0.036	0.055	0.04	0 055	0.049
Magnesium	0.002	0.001	0.025	0.002	0.0015	0.002	0.0018	0.0023	0.003
Sulphur	0.023	Nil	0.18	0.1	0.05	0.03	0.01	0.021	0.18

easy and reliable tool for determining the availability of iron in the preparation. Furthermore, all the previous experience has been in favour of using microbiological techniques for the purposes of evaluating Ayurvedic medicinal preparations.[2-4]

EXPERIMENTAL PROCEDURES AND RESULTS

Chemical analyses of commercial samples of *lohasava* were undertaken with a view to establish the various constituents that go to make-up the product and to determine the ranges in which they generally occur. The methods employed for sugars, acids, vitamins, etc., and for a general microbiological evaluation have been mentioned in an earlier publication.[8] Since iron happens to be the principal ingredient of therapeutic value in this preparation special attention was paid to its determination and a microbiological method of great sensitivity and reliability was made use of for the purpose.[7] The results of the various analytical tests carried out are presented in Tables I and II.

TABLE II

Microbiological assay of iron in *lohasava* samples using *Clostridium lacto-acetophilum*

No.	*Lohasava*		Dilution of *lohasava* in 100 ml. medium	Hilger reading Absorbancy	Concentration of iron in mg./100 ml. *lohasava*	By chemical analysis
1	Z 1093	.....	1 ml.	0.733	200	206
			0.5 ml.	0.774	195	
2	AP. 125622	.....	2.0 ml.	0.810	45.1	49.82
			1.0 ml.	0.830	42.2	
3	S_I	.....	2.0 ml.	0.817	40.3	44.68
			1.0 ml.	0.839	41.6	
4	N. P.	.....	1.0 ml.	0.706	353	363.7
			0.5 ml.	0.747	341	
5	Z. 1028	.....	3.30 ml.	0.731	60.6	62.55
			2.45 ml.	0.750	61.22	
6	Control	.....	Nil	1.0	.....	.....

As in the case of *drakshasava*[8] records of growth responses of the six bacterial species, *viz.*, *Saccharomyces cerevisiae*, *Bacillus subtilis*, *Lactobacillus casei*, *Staphylococcus aureus*, *Escherichia coli* and *Corynebacterium carotenogenum* were compiled in various media designed for the purpose. The composition of media used was on the lines detailed previously[8] with this difference that whereas in those studies *drakshasava* was put to the test, in this instance,

lohasava was substituted. The details of the results recorded are not presented in this paper as they do not add materially much to the present discussion.

Discussion

The chemical analyses of the different samples of *lohasava* revealed that the composition of the preparation, even with respect to its principal ingredient iron, varied from sample to sample, and what is more, for different batches manufactured by one and the same pharmacy. It was also surprising to find that one of the samples obtained for analysis had a very heavy precipitate of iron at the bottom of the container indicating thereby that the product was totally unsatisfactory for the purposes for which it was intended because the iron therein was not in a stable solution. What was even more surprising was the finding that the iron contents varied within very wide limits, one sample registering as low as 27.9 mg./100 ml., as compared to another which recorded as high an iron content as 363.7 mg./100 ml. This points to the need for specifying the minimum level for iron in the preparation and to the need for incorporation, into the product, a stabilizer at the time of processing and/or for stressing this need at the time of laying down standards for its manufacture.

The microbiological investigations led to the conclusion that the preparation, in general, is deficient in amino acids, vitamins and minerals. The only ingredients the preparation contained in great measure and which account for its total solids were the sugars and the alcohol, the latter accounting for its preservation for long periods of time. Inasmuch as the preparation represents a product of spontaneous fermentation, there is no agreement even in the values obtained for alcohol in the samples examined. However, the pH values of the preparation fall within a limited range varying from 3.4 to 4.6.

The most significant conclusion that can be drawn from this study pertains to the usefulness of *Clostridium lacto-acetophilum* for the assay of iron contained in this preparation. The organism's response which is specific to only iron, [6] to even as low as 1 p.p.m. of the element, offers a quantitative measure. Furthermore, even though the preparation contains a large number of other ingredients, both organic and inorganic, none seem to interfere in the estimation of iron by this method which is both simple and suitable as a routine test for a large number of samples. Above all, this microbiological procedure yields results which compare well with those secured by the employment of standard chemical methods as well as that aimed at determining the biological utility of iron contained in *lohasava*. [9]

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