

CHEMICAL, MICROBIOLOGICAL AND COMPARATIVE FERMENTATION STUDIES ON DASAMULARISHTA

MUZAFFER ALAM, K.K.S. DASAN, B.RUKMANI and K.K. PURUSHOTHAMAN

Captain Srinivasa Murti Drug Research Institute for Ayurveda, Madras, 600 106, India.

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ABSTRACT: *Dasamularista* was prepared as per the national formulary to study the effect of container on the production of alcohol and the presence of microorganisms during the process of fermentation. *Citraka* (*Plumbago rosea* Linn.) which was one of the ingredient in the drug and subjected to "Sodhana" was also studied. The *Citraka* was impoverished by 50% with respect to plumbagin as a result of sodhana-purification. The drug prepared in glass vessel showed higher amount of alcohol than the earthen pot product. During the process of fermentation two yeasts and one bacteria, *Micrococcus luteus* were observed in the media, the bacterium being a non-alcohol producing organism.

Introduction

Dasamularista is a medicated wine used in the Ayurvedic system of medicine. A detailed study on the pattern of fermentation was carried out by Alam et al (1980).

The following chemical and microbiological studies are reported in this paper.

1. *Citraka* (*Plumbago rosea* Linn.) is an ingredient in the preparation of Dasamularista. The raw material is subjected to "Sodhana". Literature survey showed that in small doses *P. rosea* root has a stimulant action a central nervous system, on plain muscles and on the secretion of sweat, urine and bile. Blood pressure registers a slight fall and peripheral vessels are found to dilate. In large doses the material causes paralysis leading ultimately to death. The minimum lethal dose for frog, mice and rabbit being 0.5, 0.1 and 10 mg/kg respectively of plumbagin, the active principle (Anonymous 1969). The quantity of plumbagin is estimated here in citrak before and after "Shodana".
 2. The effect of container is studied by preparing the drug in an earthen pot and glass vessel.
 3. A detailed microbiological study is carried out in the earthen pot and glass vessel vats during the active phases of fermentation.

Materials and methods

Purification of Citraka: Commercial material citraka was purified as per the procedure described in Ayurvedic formulary, part I (Anonymous 1978). About 100g lime was mixed in 2 litre of water and in it Citraka was soaked for 24 hours. Then the drug was removed and the water was discarded. The same procedure was repeated for another 24 hours.

The plumbagin content was estimated in the purified and unpurified citraka as follows.

The material were washed in cold water to remove foreign materials, cut into small pieces for about 0.5 to 1 cm and dried over filter paper. 1 g material was taken in 50 ml conical flask. 10 ml ethyl alcohol was added and the flask plugged with cotton was kept for 15 hours. 0.5ml as diluted to 10 ml and absorbancy was recorded. A standard curve was prepared with pure plumbagin (80 to 400 µg/10 ml) in ethyl alcohol and the absorbancy was recorded at 418nm on Bausch and Lomb Spectronic 21 Spectrophotometer.

Preparation of Dasamularista

Dasamularista was prepared as detailed in the Ayurvedic formulary (Anonymous 1978)

(i) *Earthen pot:*

3 litres drug mixed solution was taken in an earthen pot of 5 litre capacity which was coated with ghee and smoked with pippali. The pot was covered with lid, sealed with mud and buried in sand upto neck for 30 days.

(ii) *Glass vessel:*

3 litres drug mixed solution was taken in a glass flask of 5 litre capacity. The flask was plugged with cotton and was kept at room temperature in a cupboard for 30 days.

Analytical methods:

pH was recorded on Elico digital pH meter.

Specific gravity, solid content, total sugar and alcohol were determined as reported earlier (Alam et al 1977).

Chromatography

Silica gel thin layer chromatography was carried out in following solvents.

(i) Methanol: Chloroform: : 50:50

(ii) Methanol: Chloroform: : 95:5

The chromatograms were detected by placing the plates in iodine vapours and spraying with sulphuric acid (Sulphuric acid: Water : : 1:1)

Microbiology

A loopful solution on the 5th day of fermentation from earthen pot and glass vessel were inoculated on nutrient agar. Pure colonies were isolated and identified.

The staining procedure used for the identification of he organism are those described in the literature (Peltier et al 1955). All additional media used in the identification of the organism

were prepared as described in “Manual of Methods for Pure Culture Study of Bacteria” published by the society of American Bacteriologists (1950).

Result

The plumbagin standard curve was linear upto the studies concentration (Fig.I). the process of “Sodhana” removed 50% plumbagin (Table – I).

The product from the glass container showed more quantity of alcohol than the product from the earthen pot. The pH was almost the same but there was discernible difference in specific gravity, solid content and total sugar, these differences could be due to the higher amount of available sugar in the glass container (Table-II), where as in the earthen pot a loss due to exudation is always present.

Thin layer silica gel chromatography did not show any difference in the studied solvent systems. Samples from both types of containers showed spots of Rf values 0.90 & 0.71 in the solvent methanol, chloroform (50:50) and the spots of Rf values 0.80, 0.75 and 0.59 in the solvent system methanol, Chloroform of composition 96:5 (Table-III). The spot of Rf Value 0.59 was not positive to iodine. In both the solvents there was development of colour at the point of application indicating the presence of compounds which did not move in these solvents.

The microbiological observations revealed two yeasts and one bacteria in the containers during the active process of fermentation. The organisms were identical morphologically and physiologically in both the containers.

The bacterial cells were spherical, 0.5 μ , in diameter, occurring irregular Clusters. They were aerobic, mobile, gram positive, catalase positive and with inability to ferment glucose and incapable of pigment formation. These characters correspond to family *micrococcus* and the type genus *Micrococaceae*. The organism is identified as *M. luteus* (Cowan, 1974)

TABLE I
Plumbagin in Citraka before and after “Sodhana”

Citraka	Plumbagin mg/g
Before Sodhana (Raw drug)	7.30
After Sodhana (Purified drug)	3.65

TABLE II
Effect of container on the Physico-chemical
character of Dasamularista

Parameter	Earthen pot	Glass Vessel
pH	3.04	3.00
Specific gravity	1.012	1.029
Solid content% (W/W)	8.32	10.20
Total Sugar % (W/W)	3.60	4.46
Alcohol%(V/V)	7.56	10.68

Discussion

The process of “Sodhana” removed the plumbagin from Citraka. The material was 50% depleted with respect to Plumbagin. The glass container showed better results with respect to alcohol production. In our earlier observations also we found the glass vessel to be better in some other parameters as compared to the earthen pot (Alam et al 1977, 1983). The high specific gravity and solid content may be due to high quantity of remaining sugar in the glass vessel compared to earthen pot. The total sugar is less in this preparation compared to our earlier preparations which may be due to quality of the jaggery (Alam et al 1977, 1983).

The high specific gravity and solid content may be due to high quantity of remaining sugar in the glass vessel compared to earthen pot. The total sugar is less in this preparation compared to our earlier preparations which may be due to quality of jaggery (Alam et al 1980). Chromatographically no difference was demonstrable in the preparations from either container in the solvent system methanol, chloroform (50 :50). We observed the same results earlier in other preparations (Alam et al 1977, 1978). The change in polarity of chloroform methanol system did not yield any better result except that the drug prepared in glass vessel resolved into three sulphuric acid positive spots (MeOH: CHCl₃:: 95:5).

Microbiologically there were three micro organisms, one bacteria and two yeasts. The bacteria was found to be a non alcohol generating organism and as identified as *Micrococcus Luteus*. In our earlier results we have reported the presence of *Bacillus sp.* In Dasamularista (Alam et al 1980), but in this preparation it was not present. It may be due to variation of microorganisms in the ingredients from the different geographic regions which is reported by Alam et al (1981) in the dhataki flowers derived from different geographic regions.

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TABLE III

Thin layer Chromatography Rf values of Dasamularista prepared in different containers.

Solvent	RF Value		Colour of the spot	
	Earthen pot	Glass vessel	Iodine	Sulphuric acid
MeOH: CHCl ₃	0.90	0.90	Light brown	Black
50:50	0.70	0.71	Light brown	Black
MeOH: CHCl ₃	0.80	0.80	Light brown	Black
95:5	0.75	0.75	Light brown	Black
		0.59	-	Black

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