EFFECT OF TIME ON THE FERMENTATION AND STORAGE OF CANDANASAVA

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ABSTRACT: Asavas and aristas are produced by fermentation. The usual fermenting period as per the texts is one month. But the Ayurvedic practitioners believe that prolonged incubation results in increased alcohol content of the products. Candanasava was prepared and studied to examine whether these claims are tenable. Maximum alcohol production (9.8%) in 30 days was reached in the earthen pot. With the progress of time beyond 30 days there was loss of yield, alcohol and sugar. There was rich growth of fungi in the pots.

Candanasava stored in glass bottles did not show any change in any of the measured parameters. There was no increase of alcohol with the prolonged storage contrary to the claims. Chromatographically there was no difference between candanasava as obtained after 30 days fermentation period in earthen pot and the same product stored in glass bottles for three more months.

Introduction:
Asavas and Aristas are Ayurvedic medicines prepared by fermentation. The preparation of aristas is based on decoctions which requires the mixing of coarsely powdered ingredients in water and boiling till reduced to the recommended volume. The cooled decoction is filtered through cloth and in the filtrate are mixed powdered drugs, plant juices honey or jiggery or both. The mixture is taken in an earthen pot, the lid is sealed and is buried in ground for one month. During this period the process of fermentation takes place. The fermented drug is filtered through fine cloth and is used.

The preparation of asavas does not require the preparation of decoctions and instead the coarsely powdered drugs are mixed in water or plant juices followed by honey, or jiggery or both. The rest of the procedure is same as that of the arishtas.

According to the Ayurvedic texts, one month is sufficient for fermentation (Anonymous, 1978). But there is a belief among the Ayurvedic practitioners that the prolonged incubation results in higher quantity of alcohol. Some ayurvedists believe that prolonged incubation should be done by burying the fermenting pot where as others believe that the fermented drug after filtration (one moth as per the text) should be stored for long periods in glass bottles.

The present investigation was carried out to assess the alleged effect of time on the fermentation and storage of candanasava as...
reflected by such pharmacopocial parameters as the final yield, pH value, specific gravity, solid content, sugar content and alcohol percentage.

**Preparation:**

Sarkara and guda (Items No. 26 and 27 respectively) were dissolved in water (Item No.25). Draksa was crushed (Item No. 25). Draksa was crushed (Item No. 24) and was mixed in the solution. The items No. 1 to 23 were coarsely powdered and mixed in the above solution.

### Materials and Methods
Ingredients and preparation of candanasava
Pure and authentic ingredients were used in the preparation of Candanasava

| S.No. | Ingredient     | Botanical source                        | Part used | Quantity |
|-------|----------------|-----------------------------------------|-----------|----------|
| 1.    | Candana        | Santalum album Linn.                    | Heart wood| 48g      |
|       | (Svetacandana) |                                         |           |          |
| 2.    | Balaka         | Coleus vetiverioides K.C Jacob          | Root      | 48g      |
| 3.    | Musta          | Cyperus rotundus Linn.                  | Rhizome   | 48g      |
| 4.    | Gambhari       | Gymnema arborea Linn                    | Root      | 48g      |
| 5.    | Nilotpala      | Nymphaea stellata Willd.                | Flower    | 48g      |
| 6.    | Priyangu       | Callicarpa macrophylla Vahl.            | Seed      | 48g      |
| 7.    | Padminaka      | Prunus cerasoides D.Don.                | Stem      | 48g      |
| 8.    | Lodhra         | Symploca racemosa Roxb.                 | Stem bark | 48g      |
| 9.    | Manjistha      | Rubia cordifolia Linn.                  | stem      | 48g      |
| 10.   | Rakta candana  | Pterocarpus santalinus Linn.f.          | Heart wood| 48g      |
| 11.   | Patha          | Cissamplelos pareira Linn.f.            | Root      | 48g      |
| 12.   | Kiratatikta    | Swertia chirata Buch.Ham                | Plant     | 48g      |
| 13.   | Nyagrodha      | Ficus bengalensis Linn.                 | Stem bark | 48g      |
| 14.   | Pippali        | Piper longum Linn.                      | fruit     | 48g      |
| 15.   | Sati           | Hedychium spicatum Ham. Ex Smith        | Rhizome   | 48g      |
| 16.   | Parpata        | Molluugo cerviana Ser                   | Plant     | 48g      |
| 17.   | Madhuka        | Madhuca indicaf.J.F. Gmell             | Flower    | 48g      |
| 18.   | Rasna          | Pluchea lanceolata Oliver & Hiern       | Root      | 48g      |
| 19.   | Patola         | Trichosanthes dioica Roxb              | Leaf      | 48g      |
| 20.   | Kamcamara      | Bauhinia variegata Linn.                | Stem bark | 48g      |
| 21.   | Amratvak       | Mangifera indica L.                    | Stem bark | 48g      |
| 22.   | Mocarasa       | Bombax malabaricum DC                  | Gum       | 48g      |
| 23.   | Dhataki        | Woodfodia fruiticosa Kurz.             | Flower    | 768g     |
| 24.   | Draksa         | Vitis vinifera Linn.                    | Dried fruit| 960g    |
| 25.   | Water          |                                         |           | 24.576lit|
| 26.   | Sarkara        |                                         |           | 4.8kg    |
| 27.   | Guda           |                                         |           | 2.4kg    |
Fermentation in an earthen pot:

The mixture was kept for fermentation in earthen pots of 6 litres capacity which were coated with a layer of ghee and smoked with pippali. The solution was kept in six different pots each containing 4.5 litres. The pots were closed with lids followed by sealing with clay smeared cloth.

Clay was pasted around the cloth and pots were buried in sand up to their necks. At 15 day intervals, one pot at a time was opened to examine and analyse the contents.

Fermentation in glass bottles:

Candanasava fermented for 30 days in an earthen pot was filtered through cloth and was kept in transparent narrow mouth glass bottles of one litre capacity closed by screw caps. The glass bottles were thoroughly washed and dried in an over at 110°C for 10 hours rendering them sterile before use. The total number of bottles were six and each was charged with 450 ml. drug. Each bottle was opened at different intervals of time.

Analytical methods:

pH was measured on Elico pH meter. Specific gravity, solid contents, sugar and alcohol were determined as described earlier (Alam et al, 1979).

Chromatography:

Thin layer silica gel chromatography was carried out in the following solvents.

i) Methanol: Chloroform::95:5
ii) N-Butanol: Acetic acid: water:: 63:27:10
iii) N- Butanol: Acetic acid:: 90:10

The chromatograms were detected in iodine and with sulphuric acid: water (1:1)

RESULTS

Fermentation in Earthen pot:

The drug yield in 90 days was 44.4%. The major loss was in the first 15 days and thereafter it was gradual. The pH of the drug dropped from 4.7 to 3.3 up to 45 days and showed increase in the 60th and 75th day samples. In the 90th day sample the pH fell to 3.3. The specific gravity decreased from 1.08 to 1.008 and the solid contents from 22.66% to 4.95%. There was loss of sugar with the progress of time but there was no corresponding increase in alcohol content. This sugar was probably utilized by the non fermenting organisms. The content of alcohol after one month was 9.8% whereas after 90 days it was 7.84%. There was a surface growth of fungus in all the pots and rich growth was noticed as time progressed (Table I).

Fermented asava transferred to glass bottle:

There was no fungal growth. pH, specific gravity, solid contents, total sugar and alcohol did not show any change up to the studied period. There was no change in the volume (Table II).

Chromatography:

Out of the tested solvents for thin layer chromatography the best result was obtained in the solvent system methanol-chloroform
(95:5). The drug on the first day (day of preparation) showed two spots of Rf value 0.95 and 0.73 positive to iodine and sulphuric acid and one set of Rf value 0.073 positive to iodine only. On the 15th day two spots of Rf values, 0.95 and 0.73 were observed positive to iodine and sulphuric acid only one spot of Rf value 0.95. The spots of Rf values 0.73 and 0.073 disappeared (Table III).

Candanasava stored in glass bottles also showed only one spot of Rf value 0.95 positive to iodine and sulphuric acid, throughout the period.

**Discussion**

The prolonged incubation in the fermentation pot resulted in the loss of sugar, alcohol and the yield. These observations corroborate our earlier findings (Alam et al 1977, 1978). The change in pH after 15 days may be due to concentration of acidic compounds in the fermenting medium caused by the loss of water or the continued production of acid metabolic products by the surface fungus and the fermenting Bacillus. The subsequent slight increase (60th and 75th day) may be due to concentration of alkaline compounds due to loss of water or the neutralization of acids by the principles released from the drug powders of the interaction with the pot shell which is responsive to acids. In the 90th day sample the pH again dropped which may be due to metabolic action of the organisms in the vat.

There was a copious growth of fungi in the earthen pots with the progress of time. The reduction in sugar may be due to its utilization by the non alcohol fermenting organisms.

The maximum alcohol production attained in 30 days in earthen pots remained constant upto 45 days. Later on 1.17% alcohol was lost either by evaporation or by its being broken down.

The prepared asava stored in glass bottle did not show any change in any of the measured parameters except a 0.02% fall in solid contents. No fungal growth was noticed in any of the bottles.

Chromatographically earthen pot or glass bottle did not show any change.

**TABLE –I**

| Parameter                    | 0      | 15     | 30     | 45     | 60     | 75     | 90     |
|------------------------------|--------|--------|--------|--------|--------|--------|--------|
| Yield % (V/V)                | 100    | 77.7   | 66.6   | 58.6   | 49.6   | 48.9   | 44.4   |
| pH                           | 4.4    | 3.2    | 3.3    | 3.3    | 3.45   | 3.5    | 3.3    |
| Sp. gravity                  | 1.08   | 1.022  | 1.015  | 1.015  | 1.008  | 1.008  | 1.008  |
| Solid content % (W/W)        | 22.65  | 9.35   | 7.25   | 6.70   | 4.70   | 2.60   | 4.95   |
| Total Sugar% (W/W)           | 26.65  | 5.65   | 3.75   | 3.26   | 2.16   | 1.85   | 1.62   |
| Alcohol% (V/V)               | -      | 9.1    | 9.8    | 8.10   | 8.10   | 7.84   |
TABLE – II
Analytical value of candanasava stored in glass bottles:

| Parameter                  | At the time of bottling | 60 days | 90 days |
|---------------------------|-------------------------|---------|---------|
| Yield % (V/V)             | 450                     | 450     | 450     |
| pH                        | 3.30                    | 3.30    | 3.30    |
| Sp. gravity               | 1.015                   | 1.014   | 1.014   |
| Solid content % (W/W)     | 7.25                    | 7.24    | 7.23    |
| Total Sugar% (W/W)        | 3.75                    | 3.8     | 3.8     |
| Alcohol% (V/V)            | 9.80                    | 9.80    | 9.80    |

TABLE – III
Thin layer chromatography Rf values of Candanasava prepared by prolonged incubation in earthen pots and stored in glass bottles

| Fermentation   | Incubation/storage Days | Colour of the spot | Iodine | Sulphuric acid |
|----------------|-------------------------|--------------------|--------|----------------|
| Earthen pot    | 0.95 0.73 0.073 0.95    | Brown Black        | Brown  | Black          |
| Glass bottle   | 0.95 Not tested 0.95    | Brown Black        | Brown  | Black          |

Conclusion

The prolonged incubation of candanasava in earthen pots did not cause any increase in the content of alcohol. The period of 30 days prescribed in the texts as sufficient for most of the drugs (Alam et al 1979) has been found to be applicable to candanasava. Prolonged storage upto 90 days of candanasava in glass bottles did not result in the betterment of or alteration of any of the analytical values.

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