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(ISSN-2321-1563)

+EXPERIMENTAL STUDY

IMPACT OF SAMSKARA ON SHANKHA AND TANKANA- AN ANALYTICAL STUDY

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ABSTRACT:

Background: Though Ayurveda is having its unique analytical approach towards drugs, in present, there is necessity of understanding a drug based on modern technology of analysis too, knowledge of these adopted technologies helps in better understanding and interpretation of the drug. **Aim:** The present study is aiming to find out the physical and chemical changes of the mineral drugs *Shankha*(conch shell) and *Tankana*(borax) after their *Samskara*(processings). **Materials and methods:** As per the classical reference, Shankha and *Tankana* are processed, Samples before and after processing were subjected to analytical study and observations were interpreted. **Results:** Particle size of the *Shankha bhasma* (ash of conch shell) reduced in successive *putas*(incineration). The *Bharjana* (frying) of *Tankana* not only reduces the moisture content, also reduces the particle size lesser than that of *Shankha bhasma*. The XRD study shows changes in the crystallinity of minerals and allotropic modification of the same substance though no much changes in their elemental composition.

Conclusion: The classical processing methods told in Ayurveda will bring about changes in the minerals in its deeper level making it safe, effective, bio-available, which is proven clinically can be better interpreted by different analytical methods.

Key words: *Shankha bhasma*, *Tankana*, Aragonite, Borax, Calcite, X-Ray diffraction, particle size, moisture

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INTRODUCTION

The quality of the pharmaceutical products depends not only on the care taken in its preparation but also in confirming that the material has been correctly identified and then heated and properly processed. A number of purifactory, detoxificatory, incineration procedures were elaborately explained by our ancient *acharyas* (scholars) to make metals and minerals therapeutically useful. All these processes lead to physico-chemical changes, that activate and potentiate the metallic or mineral drug. *Agni*(heat) is the important factor which changes the natural physico chemical properties of the drug which depends on its chemical combination and dissociation which can be brought about by the duration and type of contact of heat. Reverse Pharmacological research has been widely adopted in current Drug Discovery and process Research. It is the high time for systematic observations incorporating instrumentation and technology in each phase of research. Standardisation themes are modified from time to time. New dimensions in instrumentation and electronics has contributed much in research. In the present study, Particle size estimation, Moisture content determination and X-ray diffraction study of *Shankha* and *Tankana* before and after pharmaceutical processing has been done to note the differences and to elicit the impact of *samskara*.

AIMS AND OBJECTIVES:

1. Determination of particle size of *Shankha bhasma* after one, two and three *Gajaputas* (bigger unit of heat), *Ashodhita*(unpurified) and *Shodhita*(purified) *Tankana* by microscopic method.
2. Determination of moisture content of *Ashodhita* and *Shodhita Tankana*.
3. X-ray diffraction Study of samples of Raw *Shankha, Shodhita Shanka, Shankha Bhasma* after each *Gajaputa* , Raw *Tankana* and *Shoditha Tankana*.

MATERIALS OF METHODS:

I. Processing of *Shankha* and *Tankana*:

This was done in the Department of *Rasashastra*, TGAMC, Bellary.

1.Preparation of *shankha bhasma*:

Shankha was purified by making it into pieces and subjected to boiling with *Kanji*(fermented gruel) by means of *Dola yantra*(swing apparatus) for about 3 hours and then washed with hot water[1]. The purified pieces of *Shankha* were taken in *Sharava Samputa*(sealed earthen saucers) and subjected to *Gajaputa.* After self cooling, powdered, given *bhavana*(trituration) with *kumari swarasa*(juice of Aloe vera), kept in

Sharava Samputa and again subjected to *Gajaputa* [2]. It was then given the *Bhavana* of *Kumari Swarasa* and once again subjected to *Gajaputa*, to get good white *bhasma* of *Shankha*[3]

2. *Shodhana*(purification) of *Tankana*:

Fried in dry iron pan till it becomes light and puffed[4].

II. Determination of particle size[5] :

This was done in the Department of pharmaceutics, TVM College of Pharmacy, Bellary.

Calibration of eye piece micrometer was done and sample was mounted on the slide and was placed on stage of microscope. Size of each particle was measured in terms of eyepiece division, tabulated and average diameter was calculated.

Calculation: No. of division eye-piece micrometer (x) =21

No. of division of stage micrometer (y) = 14

Calibrated value = <u>y</u> x 10 = <u>14</u> x 10 = 6.6 μm

x 24

III. **Moisture content estimation[6]**:

This was carried out in the Department of Chemistry, Vijayanagar Institute of Engineering College, Bellary.

Accurately weighed sample was taken in the crucible, dried in an Hot air oven at 110^0C for an hour and weighed after cooling the same in a dessicator. Again dried in hot air oven at 110^0C for half an hour and was weighed after cooling the same in a dessicator. Then the weight remained constant, so taken as final weight and moisture content was calculated.

IV. **XRD study:**

For this study, Samples were sent to Ocean Science and Technology cell in Marine Geology and geophysics, Mangalore University.

The procedure carried out for each sample was as follows - Sample was very well grounded to 200 mesh size and air dried. The diffractometer scans were made on randomly oriented samples from 3^0 to 65^0 2θ with a step size of 0.02^0 and 1 second time per step. The 2θ value and intensity of the peak were represented on X and Y axis respectively and peaks were compared with standard x-ray powder diffraction file (X PDF)[7].

OBSERVATIONS AND RESULTS:

Determination of particle size

Particle size of *marita*(incinerated) *shankha* after 1 *gajaputa*.

Average size of the particle =

$\frac{\Sigma nd}{\Sigma n} = \frac{9794.4}{360} = 27.21\mu m$

Particle size of *marita shankha* after 2 *gajaputas*.

Average size of the particles =

$\frac{\Sigma nd}{n} = \frac{6586.4}{360} = 18.3\mu n$

Particle size of *marita shankha* after 3 *gajaputas*.

Average size of the particles =

$\frac{\Sigma nd}{\Sigma n} = \frac{3128.4}{360} = 8.64\mu m$

Report:

1. Average size (mean diameter) of the particles of *marita shankha* after one *gajaputa* = 27.21µm.
2. Average size (mean diameter) of particles of *marita shankha* after two *gajaputas* = 18.3mm.
3. Average size (mean diameter) of particles of *marita shankha* after three *gajaputas* = 8.64µm.

Particle size of *Ashodhita Tankana*:

Average size of the particles

$= \frac{\Sigma nd}{\Sigma n} = \frac{3636.6}{307} = 11.85\ \mu m$

Particle size of *Shodhita Tankana*:

Average size of the particles =

$\frac{\Sigma nd}{\Sigma n} = \frac{2415.6}{306} = 7.89\ \mu m$

Report: 1. Average size (mean diameter) of particles of *Ashodhita Tankana*=11.85 µm.

4. Average size (mean diameter) of particles of *Shodhita Tankana* = 7.89 µm.

Determination of moisture content

1) Initial weight of *Ashodhita Tankana* - 1 g

Final weight of *AshodhitaTankana* 0.798gm

% of moisture content =

$\frac{\text{Diff. in weight}}{\text{Initial weight}} \times 100 = \frac{1-0.798}{1} \times 100 = 20.2\ \%$

2) Initial weight of *Shodhita Tankana* –1 gm

Final weight of *Shodhita Tankana* – 0.99 gm

% of moisture content =

$\frac{\text{Difference in weight}}{\text{Initial weight}} \times 100 = \frac{1-0.99}{1} \times 100 = 5\ \%$

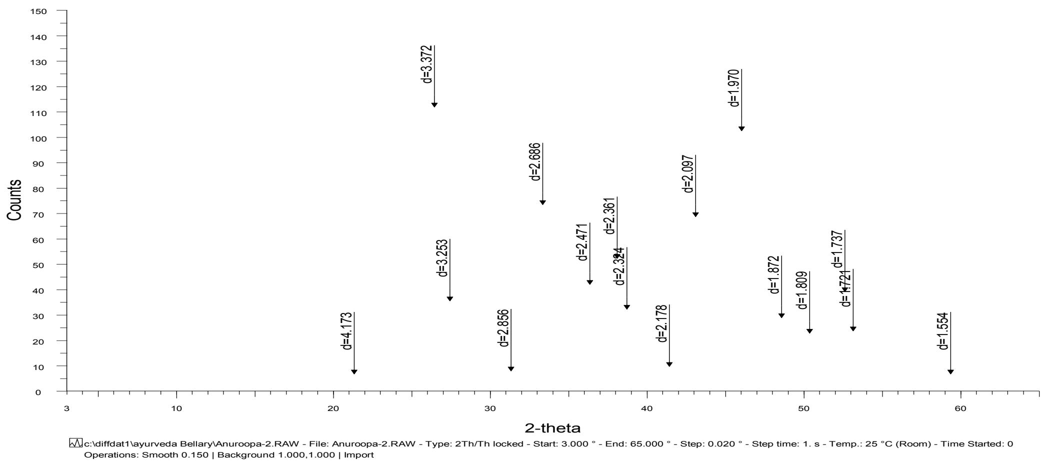
Report :

1. Moisture content of *AshodhitaTankana* = 20.2%.

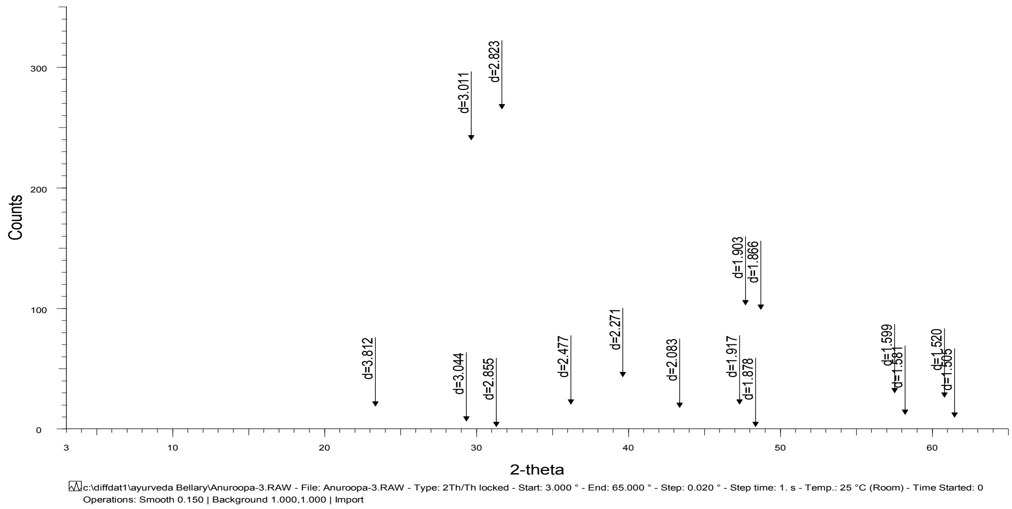
2. Moisture content of *Shodhita Tankana* = 5 %.

X-ray diffraction Study

XRD of Sample No. 1 (*Ashodhita Shanka).*

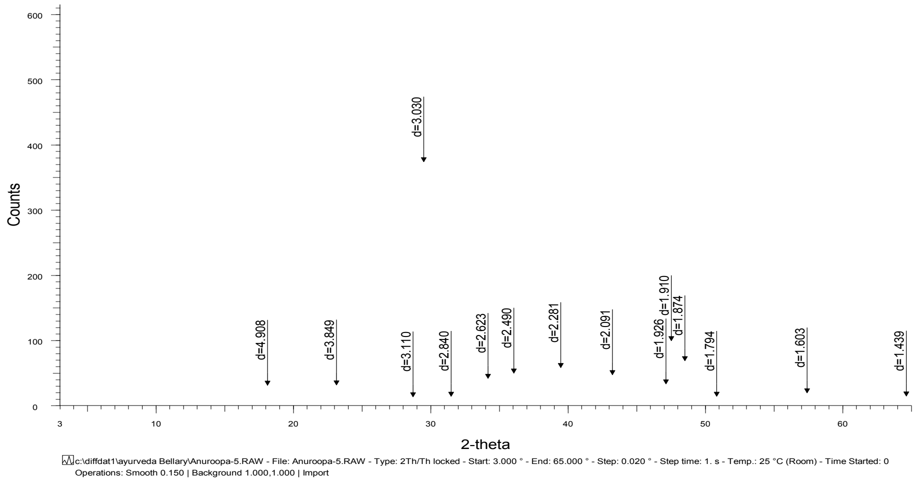


XRD of Sample No. 2 *(Shodhita Shanka)*



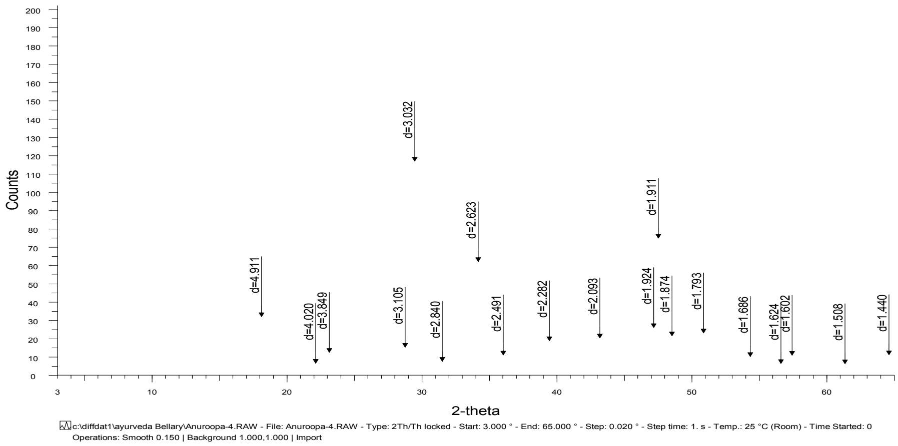
XRD of Sample No.3

(*Shanka Bhasma* After 1*Gajaputa*)

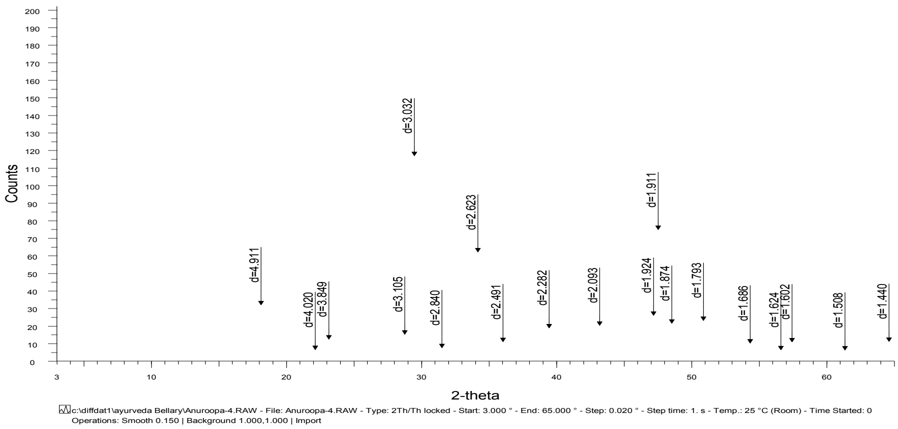


XRD of Sample No. 4

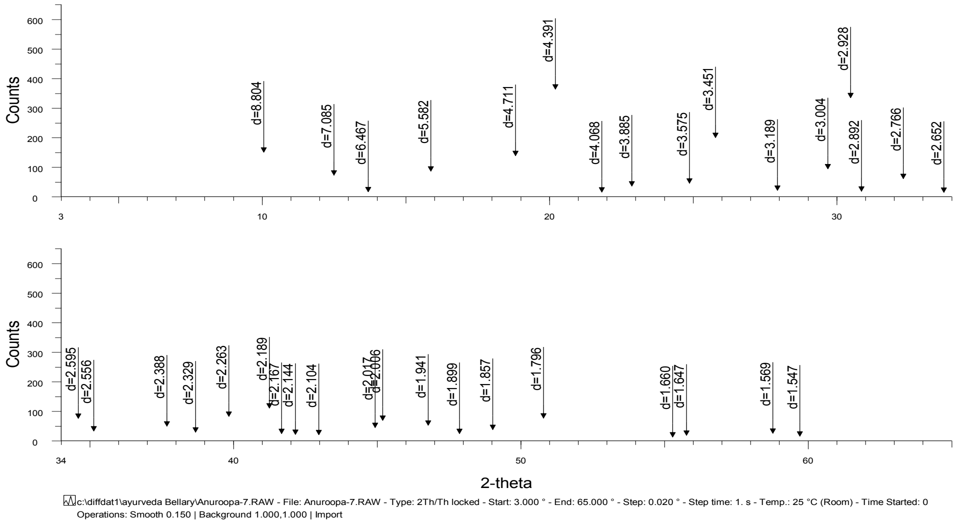
(*Shanka Bhasma* After 2 *Gajaputas*).



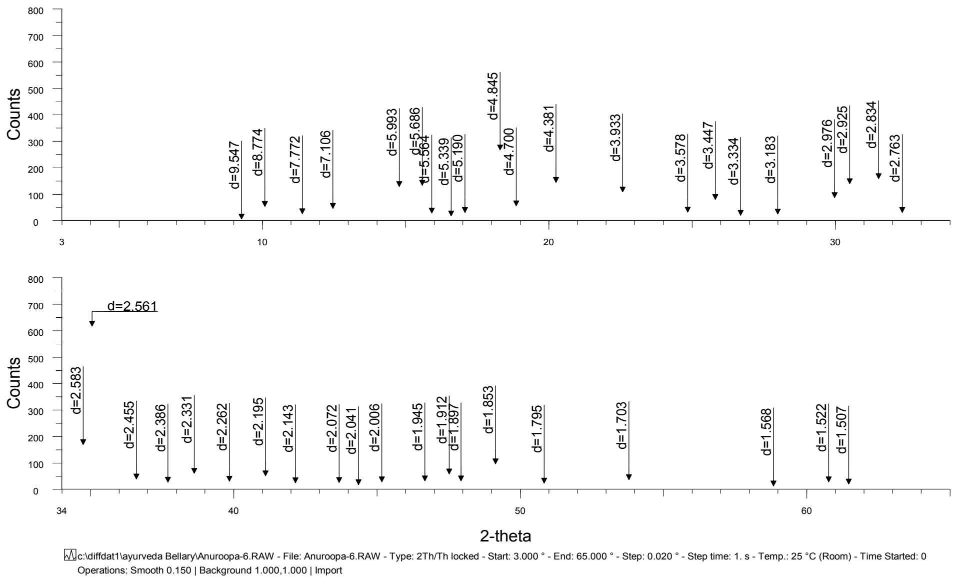
XRD of Sample No. 5 (*Shanka Bhasma* After 3 *Gajaputas*)



XRD of Sample No. 6 (*Ashodhita Tankana*)



XRD of Sample No. 7 (*Shodhita Tankana*)



Report: Minimum 3 strong peaks are required for the identification.

Sample No 1. 3.393 (d), 2.699(d), and 1.976(d) strong peaks of the sample matched with 3.393(d), 2.699(d) and 1.976(d) peaks of the standard Aragonite respectively. Hence XRD identification of *Ashodhita Shankha* was Aragonite (CaCO3) and the system of crystallization is orthorhombic.

Sample No.2. 3.372(d), 2.361(d) and 1.970(d) strong peaks of the sample matched with the 3.394(d), 2.370(d) and 1.976(d) respectively. Hence XRD identification of *Shodhita Shankha* was Aragonite (CaCO3) and the system of crystallization is orthorhombic.

Sample No.3. 3.011(d), 1.896(d) and 1.86(d) peaks of the standard Magnesium calcite respectively. Hence XRD identification of *Marita Shankha* after one *Gajaputa* was Magnesium calcite (Mg $CaCO_3$) and the system of crystallization is Trigonal.

Sample No 4. 3.032(d), 1.911(d) and 1.874(d) strong peaks of the sample matched with the 3.039(d), 1.917(d) and 1.876(d) of the standard calcite respectively and 2.623(d), 4.911(d) and 1.924(d) strong peaks matched with the 2.62(d), 4.91(d) and 1.92(d) of the standard calcium oxide hydrate respectively. Hence *Marita Shankha* after two *Gajaputas* was identified as calcite (CaCO3) and calcium oxide hydrate ($CaO.H_2O$) and the system of crystallization is Trigonal.

Sample No.5 3.030(d), 1.910(d) and 2.281 (d) strong peaks of the sample matched with the 3.039(d), 1.917(d) and 2.283(d) of the standard calcite respectively and 2.623(d), 4.908(d) and 1.926(d) strong peaks matched with the 2.62(d), 4.91(d) and 1.92(d) of the standard calcium oxide hydrate respectively. Hence *Marita Shankha* after three *Gajaputas* was identified as calcite (CaCO3) and calcium oxide hydrate ($CaO.H_2O$) and the system of crystallization is Trigonal

Sample No.6. 4.845(d), 3.933(d) and 2.834 (d) strong peaks of the sample matched with the 4.845(d), 3.933(d) and 2.834(d) of the standard Borax respectively and 2.925(d), 4.381(d) and 3.447(d) strong peaks matched with the 2.92(d), 4.38(d) and 3.44(d) of the standard Tincalconite respectively. Hence *Ashodhita Tankana* was identified as Borax ($Na_2B_4O_{7.}$ $10H_2O$) and Tincalconite ($Na_2B_4O_{7.}$ $5H_2O$) and the system of crystallization is Monoclinic.

Sample No.7. 2.928(d), 4.391(d) and 8.804 (d) strong peaks of the sample matched with the 2.92 (d), 4.38(d) and 8.75(d) of the standard Tincalconite respectively. Hence *Shodhita Tankana* was identified as Tincalconite ($Na_2B_4O_{7.}$ $5H_2O$) and the system of crystallization is Trigonal.

DISCUSSION:

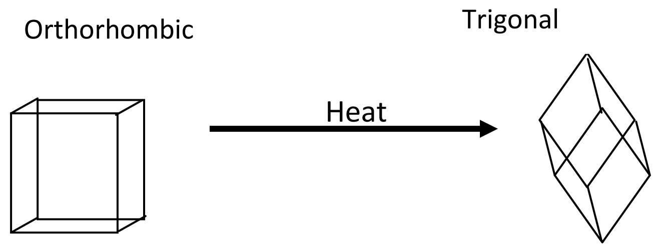
Discussion on particle size: The particle size is proportional to optimal biological activity. Marana reduces the particle size of particular drugs. By microscopic method, the average size of the particle after one *Gajaputa* was 27.21μm, which was a fine state of particles reduced due to the effect of *Marana*. After second *Gajaputa*, average particle size was 18.3μm, which further reduced by another *Gajaputa*. After third *Gajaputa*, mean size of the particle of *Shankha Bhasma* was 8.64 μm and majority (8.05%) of the particles were in the minimum range (mean diameter of 6.6μm), showing that as the number of *putas (*unit of heat) increases, particle size reduced and it was mean size of 8.6μm satisfied both *Rekhapoornatva*(fineness*)* and *Varitaratva*(lightness) test. The mean particle size of *Tankana* before *Shodhana* was 11.85μm and after *shodhana* was 7.89μm, inferring that the process of *Bharjana* (frying) also reduced the particle size. It is interesting to note that the average particle size of the *Shodhita Tankana* is lesser than that of three *Gajaputa marita shankha bhasma,* which indicates *Bhasmikarana* (process of incineration) of *Tankana* is not required to reduce its particle size.

Reduction in the particle size during marana may due to the following reason : Solid crystal at a rest has packed particles which are closed together in a lattice fore and vibrate in their fixed portion. But when temperature increases, the particle grain (crystalline area) increase and Vibrate more strongly, occupies more spaces this causes solid to expand. Due to increase in intra atomic distance, electrostatic forces get weakened. Due to continuous heating, particles get enough energy to break forces holding them together and to get reduce in their sizes.

Discussion on Moisture content (Loss on drying at 11^{0}C): Moisture content of *Ashodhita Tankana* was 20.2% and that of *Shodhita Tankana* was 5%, showing that the *shodhana* process reduced the moisture content of *Tankana*. Reduction in moisture content reduced the chance of microbial contamination, decomposition due the undesired chemical changes. *Shodhana* of *Tankana* by *Bharjana* caused dehydration and made the *Tankana* bloomed and puffed. So untoward effects of *Ashodhita Tankana* like *Vanti* (vomiting), *Bhranti* (giddiness) were not observed after *shodhana* as the excessive *kleda* (moisture) was removed. Heating of *Tankana* evaporates the water of crystallisation, made it to swell and changed it to the form of fragile masses. Hence *Shodhita Tankana* will attain *Sukshma* (minute), *Laghu* (light) *guna* (property) and helps to reaches the target area. The net loss in weight of *Tankana* is due to the loss of water molecules in it. So moisture content is an objective analytical marker in standardization of *Tankana*.

Discussion on X – ray diffraction: X – ray diffraction leads primarily to the identification of crystalline compound from their diffraction patterns. In the present study, XRD pattern of *Ashodhita Shankha* showed that, the observed 14 peaks were matching with that of standard Aragonite ($CaCO_3$). On comparison, 3 strong peaks were enough to consider *Ashodhita shankha* as Aragonite. In the XRD pattern of *Shodhita Shankha*, same peaks as that of *Ashodhita Shankha* were observed with slight reduction in the d-values, inferring the influence of *Shodhana* over the intra atomic structure, where the interplanar spacing of the atoms has reduced. Both *Ashodhita* and *Shodhita Shankha* crystallizes in the Orthorhombic system, but observed the change in the crystalinity of the phase after *shodhana*. This changes can attribute to the object of *Shodhana* which is preliminary procedure makes metals and minerals suitable for *marana*.

XRD pattern of *Marita Shankha* after one *Gajaputa* showed that 12 peaks observed were matching with that of standard Magnesium calcite. Mineralogical, Aragonite changes into calcite on heating. This is observed in the present study, that *Shodhita Shankha* (XRD identification Aragonite) after subjecting it into one *Gajaputa* changes to magnesium calcite. Here the polymorphic form of $CaCO_3$ crystal from orthorhombic system changes to the another polymorphic form of Trigonal system of crystallisation, also changes in the cleavage and cleavage fragment shape. Hardness from 3.5 to 4 reduced to 3, which may be due to the effect of heat.



X-RD pattern of *Marita Shankha* after two *Gajaputas* showed the presence of two compounds viz. calcite ($CaCO_3$) and calcium oxide hydrate ($CaO\ H_2O$), 11 peaks were matching 3 with the standard calcite and 5 peaks with the Standard calcium oxide hydrate. Chemically, calcium carbonate is converted into calcium oxide by heating $CaCO_3 \rightarrow CaO + Co_2 \uparrow$ and CaO readily absorbs moisture from the atmosphere to form calcium oxide hydrate or calcium hydroxide. In the present study, XRD pattern of *Marita Shankha* after two *Gajaputa* showed also the presence of CaO inferring the process of oxidation of $CaCO_3$ during 2^{nd} *Gajaputa* in which the mineral $CaCO_3$ dissociates into its oxide form. The oxide form, was not present *Marita Shankha* after one *Gajaputa*, was identified after 2^{nd} *Gajaputa* may be because trituration with *Kumari Swarasa* prior to 2^{nd} *Gajaputa* might have accelerated the process of oxidation, helping in breakdown of particles of minerals and small amount of CaO not combined with CO_2 to form $CaCO_3$. The hydrate form may be because of CaO Combining with the water molecule in the atmosphere, forming calcium hydroxide.

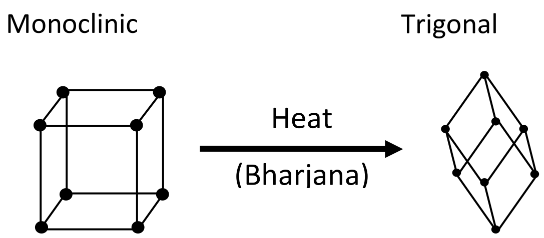
XRD pattern of *Marita Shankha* after three *Gajaputa* also showed the presence of two both calcite and calcium oxide hydrate. 9 peaks matching with standard calcite and only 4 with standard calcium oxide hydrate. XRD patterns of *Marita Shankha* after three *Gajaputa* was similar to that of *Marita Shankha* after two *Gajaputas* with slight alteration of d value indicating the minimum change of interplaner space of the atoms. In this pattern the peaks of calcium oxide hydrate are not stronger, hence the percentage of the same is quite low, may be due to reformation of calcium carbonate from the calcium oxide hydrate which combines slowly with the atmospheric CO_2 or CO_2 within the *sharava samputa* liberated during dissociation to form calcium carbonate.

$Ca(OH)_2 + CO_2 \rightarrow CaCO_3 + H_2 \uparrow$

Hence, the process of *marana* can be interpreted as the procedure in which the metals and minerals are reduced to ashes, in which not only physical changes observed, also chemical changes occurs. Though main element remains constant, there will be changes in its allotrophic form, also in its crystallinity. These changes may be the reason for the drug to become safe, effective and bio available.

XRD pattern of *Ashodhita Tankana* showed that, 25 peaks were matching with the standard borax and 6 peaks with the standard Tincalconite. *Tankana* is nothing but the mineral Borax and XRD identification of *Tankana* was also Borax, along with small concentration of Tincalconite, pseudomorph of Borax. It is natural phenomenon of chemical reaction of Borax altered to the mineral Tincalconite from dehydration, without changing the shape of the crystal. It was observed from the XRD pattern that the material crystallised in monoclinic system showing that only water molecules are lost to form pseudomorph Tincalconite without altering the crystallization. Small concentration of Tincalconite indicates only the surface of Borax exposed to environment has altered.

XRD pattern of *Shodhita Tankana* showed 32 peaks matched with the standard Tincalconite. On heating Borax ($Na_2B_4O_7$ $10H_2O$) above 60^0C, it change to Tincalconite ($Na_2B_4O_7$ $5H_2O$) i.e. from decahydrate form to pentahydrate form. The difference is in the number of oxygens, hydroxides and water molecules. The XRD identification of *Shodhita Tankana* was Tincalconite and observed that not even a single peak matching with the standard Borax, showing that the whole Borax has changed to tincalconite with change in the system of crystallisation from Monoclinic to Trigonal and marked reduction in hardness from 2-2.5 to 1.



Hence the process of *Shodhana* of *Tankana* done to make the drug fit for internal administration can be interpreted using XRD study. The process not only reduces its moisture content, but also changes its crystallinity. Hence after *shodhana* the untoward effect seen before processing will not be observed later, also it becomes highly effective and stable.

CONCLUSION

- *Shodhana* of *Rasoushadhis* is an essential step which will modify the raw drug into its safe, bioactive therapeutic form.
- Particle size of the *Shankha bhasma* reduced in successive putas supporting to their *Rekhapoornatva* and *Varitaratva lakshanas*. . The mean particle size of *Tankana* reduced after *shodhana* and is lesser than that of *shankha bhasma,* which indicates *Bhasmikarana* of *Tankana* is not required to reduce its particle size.
- Moisture content of *Tankana* reduced markedly after *shodhana* which reduces the chances of microbial contamination and make the drug stable and devoid of untoward effects
- XRD study shows that the classical processing methods told for Shankha and Tankana will bring about changes in the minerals in its deeper level. Though main element remains constant, there will be changes in its allotrophic form, also in its crystallinity, making it safe, effective and bio-available.

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Cite this article as: Anuroopa HK, Shankara Gouda, Madhav Diggavi. Impact of samskara on Shankha and Tankana- an analytical study, *J of Ayurveda and Hol Med (JAHM)*.2016;4(3):12-23

Source of support: Nil

Conflict of interest: None Declared.