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Quantitative analysis of Oxidized Pearl Oyster Shell Powder (Muthuchippi Parpam) at various stages of processing

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ABSTRACT:

Background: Marine environment offers a diversity of ecological resources comprising of aquatic plants and animals. Siddha system of medicine had made use of these marine organisms since antiquity. Marine enrolment became a focus of natural products drug discovery research due to its unexplored bio diversity. The potential of marine drugs as pharmaceuticals was introduced by Bergmann in 1950's. Oxidized Pearl oyster shell has been used in the treatment of bronchitis, asthma, osteoporosis, nervous disorders, and musculoskeletal disorders in the traditional medicine. **Aim and Objective:** To identify and quantify the chemical composition and crystallographic structure at various stages of processing oxidized powder of pearl oyster (Muthuchippi Parpam [MCP]). **Methodology:** The crystalline transformations, phase purity and chemical composition of Oxidized pearl oyster shell powder (Muthuchippi Parpam), at various stages of its processing using XRD. **Results:** X-ray diffraction (XRD) analysis revealed a transition from a mixture of Aragonite and Calcite in the raw material to calcite (96%) in oxidized powder of pearl oyster shell. All the study sample showed crystalline structure. **Conclusion:** The study results conclude the concentration of calcium carbonate in the form of calcite increased in end product of oxidized pearl oyster shell powder.

KEYWORDS: Calcite, Muthuchippi parpam, Pearl Oyster Shell, Siddha Medicine, XRD analysis.

INTRODUCTION:

Recently, marine sources have played a crucial role in the discovery of lead components for various pharmacological applications. Oysters, a prominent marine resource, have gained significant attention due to their abundant availability and high protein content [1]. The shells of oysters, make up around 60% of their total weight and are primarily composed of calcium carbonate ($CaCO_3$), accounting for approximately 95% of the shell composition. The remaining portion, constituting 0.1-5%, is an organic matrix consisting of complex macromolecules. This

organic matrix plays a vital role in biomineralization processes, aiding the formation and structural integrity of oyster shells [1]. The organic matrix is composed of a variety of substances, including polysaccharides (primarily chitin), water-soluble and insoluble proteins (such as glycoproteins), lipids, pigments, free amino acids, and short peptides[2]. These components contribute to the unique properties of the oyster shell, making it a subject of interest for various scientific investigations, particularly in the field science and pharmacology [2, 3].

X-Ray Diffraction (XRD) Analysis is a powerful and widely used analytical technique to determine the crystallographic structure, chemical composition, and physical properties of a sample. In XRD, X-rays are directed at a sample, and the angles and intensities of the scattered X-rays are measured. This scattering occurs due to the interaction of X-rays with the atomic structure of the material. The resulting diffraction pattern, which is unique to each crystalline phase, provides crucial deep understanding about the atomic arrangement within the sample. XRD is instrumental in identifying the phases present in a sample, as each phase produces a distinct diffraction pattern [4, 5]. By comparing these patterns with established databases, researchers can accurately identify the crystalline structures of the sample. This technique can also provide detailed information on the unit cell dimensions, atomic positions, and symmetry, which are essential for understanding the structural properties and potential applications. Additionally, XRD allows for the quantification of different phases within a sample, an important aspect in fields such as pharmaceuticals, metallurgy, and materials science. The technique also offers crystallite size, strain, and defects within the crystal structure, all of which are critical factors in determining and improving property of the sample. Importantly, XRD is a non-destructive method, meaning it does not alter or damage the sample, making it ideal for analyzing precious or limited samples. It can be used to determine the preferred orientation of crystals in polycrystalline materials, which is a significant consideration in materials engineering and metallurgy [4,5].

This study aims to assess the changes that occur during the processing of Oxidized pearl oyster shell (Muthuchippi Parpam [MCP]) at various stages, utilizing XRD analysis for comparison. The study will examine the crystallographic alterations and phase transformations that take place throughout the preparation process, providing valuable insights into the structural modifications and potential enhancement of the sample. The findings from this study could have

significant implications for the development and optimization of materials derived from oyster shells, contributing to advancements in pharmacological applications and materials science.

METHODOLOGY:

Ingredients:

- Pearl oyster shell (*Pinctada margaritifera* Linn)
- Aloe vera (*Aloe bardadensis* Miller)
- Sessile joy weed *(Alternanthera sessilis* (L) R.Br. ex DC*)*

Sample preparation:

A fine powder sample was placed in a thin layer on a silicon zero-background holder. Then the sample was recorded for angle 2Θ (Siemens D5005 Diffractometer) using CuKa radiation, Lamda = 1.5406 Angstrom] over the range 10.0 - 90.0[degrees] [6].

RESULTS:

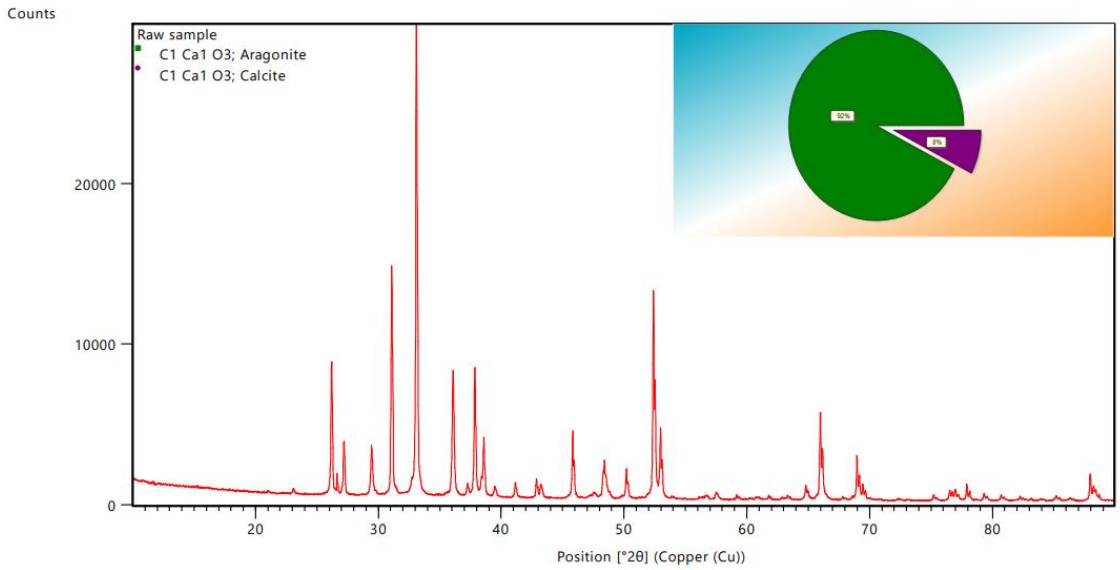


FIGURE – 1: SAMPLE – 1 RAW PEARL OYSTER SHELL (MUTHUCHIPPI)

Table – 1: XRD Analysis of Raw Pearl oyster shell (Muthuchippi)

REFERENCE CODE	SCORE	COMPOUND
98-015-7992	58%	Aragonite
98-003-7241	45%	Calcite

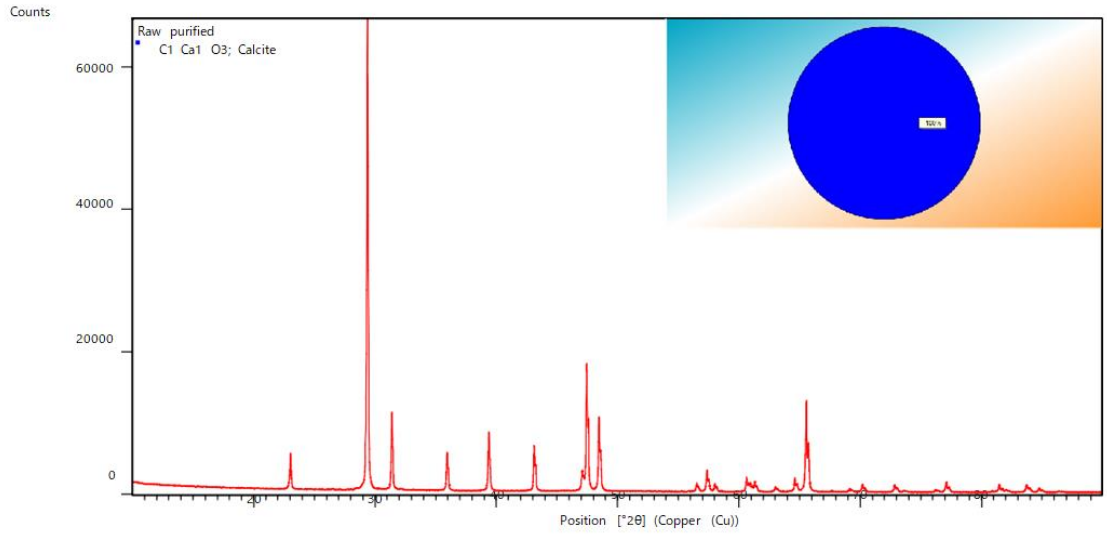


FIGURE – 2: SAMPLE – 2 PURIFIED PEARL OYSTER SHELL (MUTHUCHIPPI)

Table – 2: XRD Analysis of Purified Pearl Oyster Shell (Muthuchippi)

REFERENCE CODE	SCORE	COMPOUND
98-015-8258	86%	Calcite

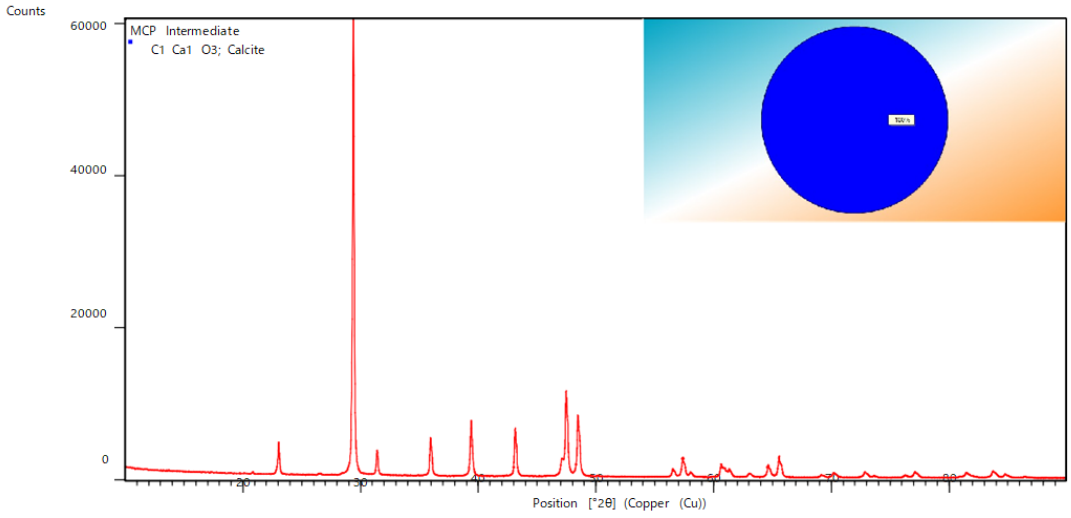


FIGURE – 3: SAMPLE – 3 INTERMEDIATE OF PEARL OYSTER SHELL (MUTHUCHIPPI PARPAM)

Table – 3 : XRD Analysis of Intermediate of Pearl Oyster Shell (Muthuchippi Parpam)

REFERENCE CODE	SCORE	COMPOUND
98-016-6364	85%	Calcite

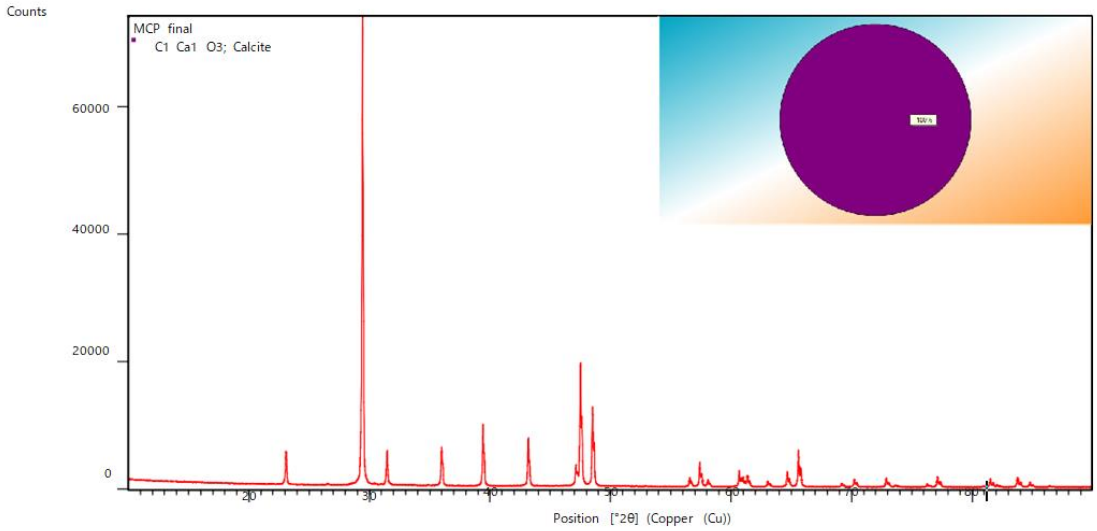


FIGURE - 4: SAMPLE – 4 OXIDIZED PEARL OYSTER SHELL POWDER (MUTHUCHIPPI PARPAM)

Table – 4 : XRD Analysis of Pearl Oyster Shell Muthuchippi Parpam

REFERENCE CODE	SCORE	COMPOUND
98-015-8258	96%	Calcite

DISCUSSION:

The XRD analysis revealed sharp peaks at various stages of pearl oyster shell oxidation, indicating the crystalline nature of the sample. The patterns of different samples at various stages of processing oxidized pearl oyster shell (*Pinctada margaritifera*). Figure 1 (Raw Sample): This image shows a PXRD pattern with two identified major phases: Aragonite and Calcite with a range of 58% and 45% respectively. The corresponding pie chart indicates the phase composition, with Aragonite being the dominant phase. (Table - 1). Figure 2 (Purified Sample): This image shows a PXRD pattern with a single identified phase: Calcite. The pie chart shows 86% Calcite, indicating successful purification. (Table - 2). Figure 3 (MCP Intermediate

Sample): This PXRD pattern is similar to the Raw Purified sample, showing Calcite as the only phase present, with a 85% composition in the pie chart. (Table - 3). Figure 4 (MCP Final sample): This pattern is similar to the previous two, with Calcite being the only phase present, and the pie chart also showing 96% Calcite. (Table - 4)

The Raw Sample contains both Aragonite and Calcite, with Aragonite as the major phase. The Purified, MCP Intermediate, and MCP Final samples are all composed majorly of Calcite, indicating that the purification and processing stages successfully converted the Aragonite phase and left Calcite as the sole phase. Aragonite forms in the ocean through inorganic precipitation, creating marine cements or free crystals in the water column. Inorganic precipitation of aragonite in caves can occur in the form of speleothems. Aragonite is common in serpentinites where magnesium-rich pore solutions apparently inhibit calcite growth and promote aragonite precipitation. Aragonite is highly heat unstable and converted to calcite when heated [7].

This analysis suggests that the purification and processing steps were effective in isolating calcite from the raw material. Calcite is a stable polymorph of calcium carbonate. It has three structures, prismatic (both short and long), (2) rhombohedral, and (3) scalenohedral.[8]

X-ray diffraction (XRD) analysis of pearl oyster shells, particularly from species like Pinctada margaritifera, has been a subject of various studies aimed at understanding their unique biomineralization processes. These shells are primarily composed of calcium carbonate in two crystalline forms: aragonite and calcite. These are structurally different but the chemical composition is almost similar. The nacreous layer, known for its iridescence, is primarily aragonite, while the prismatic layer is composed of calcite [9].

Studies have revealed that the formation of these crystalline structures is influenced by precursor phases such as amorphous calcium carbonate (ACC). Advanced XRD techniques, combined with other methods like Raman spectroscopy and scanning electron microscopy, have showed the transformation of ACC to crystalline forms. These studies have helped to map the spatial distribution of these phases and understand the role of organic matrices and elements like magnesium in the crystallization process [10]. Similarly, another study revealed anisotropic lattice distortions in the aragonite crystals of the nacreous layer, indicating that the crystals are not perfectly uniform. The prismatic layer, composed of calcite, also showed unique crystallographic orientations. These findings suggest that the structural properties are finely tuned at the molecular level to enhance mechanical strength [11]. Another study focused on the

role of organic macromolecules in controlling the polymorphism (crystal form) of calcium carbonate in oyster shells. This XRD analysis revealed that the nacreous layer of the shell primarily consists of aragonite crystals, which are organized in a hierarchical manner. The study showed that specific macromolecules in the shell matrix play a crucial role in determining whether calcium carbonate crystallizes as aragonite or calcite. This research contributed to understanding the mechanisms by which organisms influence crystal formation [12].

Additionally, the occurrence of non-nacreous pearls, which consist entirely of calcite, has also been characterized using XRD. These pearls display different microstructural patterns compared to the nacreous ones, highlighting the diversity within even closely related mineralized structures [13].

Calcite is one of the form of Calcium carbonate, $CaCo_3$ is helpful in the treatment of musculoskeletal disorders, gastrointestinal ailments, pancreatitis, etc Oxidized pearl oyster shell powder contains 96% of calcite [14]. Further studies could explore the bioavailability and therapeutic efficacy of Oxidized pearl oyster shell powder (Muthuchippi Parpam), correlating its crystallographic properties with pharmacological outcomes. Advanced techniques like Raman spectroscopy and scanning electron microscopy could be employed to gain deeper knowledge into the microstructural characteristics and interactions within the formulation. Additionally, exploring the integration of Oxidized pearl oyster shell powder (Muthuchippi Parpam) in modern pharmacological applications could pave the way for novel therapeutic strategies, combining traditional wisdom with contemporary science.

CONCLUSION:

The XRD analysis of oxidized form of pearl oyster shell (Muthuchippi Parpam) at various stages of its formulation has provided clear understanding on the crystalline transformations and phase purity of the material. The transition from a mixture of Aragonite and Calcite in the raw sample to a purified and processed state of 96% Calcite through subsequent stages highlights the effectiveness of the purification and processing steps. These findings underscore the importance of XRD in confirming the crystalline nature and chemical composition of traditional Siddha formulations.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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