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Original Article

Pharmacognostic Identification of Adulterated Marketed Samples of *Maranta arundinacea* L. Starch: A Case Study

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ABSTRACT

Introduction: *Maranta arundinacea* L. (arrowroot) starch is readily available in the market owing to its soaring demand in the pharma and food industry. Despite its cost in extraction methods and minimal yield, it is marketed at cheaper rates. The unclear picture of its authenticity, quality and the high chances of adulteration are still unexplored. This case study is planned to identify and authenticate genuine and adulterated samples of Maranta Starch (MS) procured from various vendors in comparison with a Botanical Reference Standard (BRS) and In-House preparation (IH). **Materials and Methods:** The BRS, IH and nine commercial samples (labeled as MS 1 to 9) were subjected to a systematic approach of powder microscopy, preliminary physiochemical assays and X-ray Diffraction (XRD). **Results:** On gross examination, the texture, color, odor and taste of samples showed minute differences, however, the powder microscopy and iodine test demonstrated significant differences. The samples which reported more deviation were tested and confirmed for the presence of inorganic matter through an ash test. This was further studied for XRD analysis, which reported major adulterants in the samples like dolomite ($CaMg(CO_3)_2$), cordierite ($Al_4Mg_2Si_5O_{18}$), quartz (SiO_2) and Zinc Sulphide (ZnS). **Conclusion:** This case study reveals the extensive adulteration of marketed MS in the South Indian market, particularly Chennai.

Keywords: Herbal drug analysis, X-ray diffraction, kūkai nīru, Siddha drug.

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INTRODUCTION

The traditional system of medicine has gained a lot of popularity in recent years since the susceptibility to side effects is low compared to that of synthetic remedies.[1] Owing to the recognition and demand for herbal medicines, the WHO proposed herbal drug safety monitoring and standardization guidelines in 2004.[2] On a familiar note, there are two categories of adulteration, one being intentional/direct and the other, unintentional.[3] Drugs are adulterated/substituted at different stages of processing. The analysis of raw drugs being adulterated is comparatively less challenging when compared to that processed drugs due to their altered form. Usually, powdered drugs are adulterated to decrease their cost which in turn affects the drug potency.[4] One such processed plant substance is starch, which forms the major source of carbohydrates. Starch is usually obtained from different parts of a plant such as the root, seed, tuber, leaves, stem, etc.,[5] Starch finds its application in many industries like food, pharma industry, etc.,[6,7] This study assesses the market samples of Maranta Starch (MS) in comparison with a Botanical Reference Standard (BRS) and In-House preparation (IH).

The starch extracted from rhizomes of *Maranta arundinacea* L. (East Indian Arrowroot) is highly demanded in the South Indian market due to its exceptional use in the AYUSH Pharmaceutical sector.[8] MS is the water-soluble starch, which is termed kūkai nīru in Tamil, is a long time included in South Indian traditional culinary practices and healthy recipes, especially in infant nutrition. In Siddha medicine, it is highly recommended as a functional food for infants.[8-10] There is a long history of culinary practice that is relied on MS. Various dishes prepared from it, especially the gruel or porridge form called kūkaimā kañci is indicated in gastritis and pediatric diarrheal diseases, owing to its virtue to soothe irritated bowels. Nowadays it is also being used in the food industry as a flavorless thickening agent for cooking purposes.[9]

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Traditional preparation of MS involves the process of grinding into a paste, repeated filtration through cloth, precipitation and sun drying. The yield of pure MS obtained through this method is very less and therefore it is more expensive than any other edible starches in the market.

In spite of the considerable time taken for starch extraction and the cost behind it, the majority of the pharmaceutical and food industries still rely on local vendors who supply the starch at lower prices. The marketed products under the label of MS are very popular; however, the genuineness of the quality is still under concern. For economic benefits, fraudulent practices of adulteration among the different vendors may include the addition of other cheaper and substandard plant starches or sometimes whitening agents like Talc to improve the colour and lustre of the product that makes it appear similar to the original.

As part of the pharmacognostic approach, the studies on MS are limited and there are no comparative market sample analyses of MS so far.[11,12] For the purpose of this case study, we followed a method that focused on a Pharmacognostic approach coupled with analytical methods like iodine test, ash value and XRD that affirms the authenticity of the sample, identification of starch and the adulterant materials in the sample.

MATERIALS AND METHODS

Procurement of BRS

MS which was authenticated and retained in the raw drug repository of the Department of Pharmacognosy, Siddha Central Research Institute (SCRI), Chennai was considered as the RS.

Preparation of IH

Fresh whole plants of *M. arundinacea* (Figure 1) were procured from a local farm and the rhizomes were separated, cleaned, and sliced in the in-house pharmacy SCRI. The slices were crushed to prepare the paste and rubbed through a sieve cloth tied in a vessel filled with potable water. The starch settled was collected after sun drying and it was powdered and preserved for studies.

Procurement of Market Samples

Nine branded market samples of MS were procured from different vendors and labelled MS1 to 9 (Figure 2).

Organoleptic studies

MS 1 to 9 and IH were studied for organoleptic characters. Their colour, odour, texture and taste were studied and documented in comparison with BRS.[13]

Powder microscopy

A pinch of the sample was taken and mounted with glycerine and observed under the Nikon Eclipse E200 microscope with Zeiss Axiocam ERc5S under bright field light. The observations were documented.[14]

Iodine test

A pinch of the sample was taken in an embryo cup and to it, 2-3 drops of iodine solution were added and mixed well and the changes were documented.[15]

Preliminary phytochemical analysis

Tests were done to detect the presence of starch, carbohydrates, sugar, saponin, triterpenoids, steroids, alkaloids, protein, amino acids, flavonoids, phenol and tannins in all the samples.

Ash value

All the samples were subjected to an Ash test to determine the amount of inorganic non-combustible material.[15]

XRD Studies

The samples of MS that had high ash value were analyzed with X-ray Diffraction (XRD) for identifying the mineral source (PXRD-Aeris PANalytical, Netherlands).

RESULTS

All the powders were odourless. Colour examination of the powders revealed three categories namely, dull white (IH, MS1 and 4), white (BRS, MS3 and 5) and bright white (MS2, 6, 7, 8 and 9) (Table 1). According to the texture, the samples were

Figure 1: Whole plant and rhizome of *Maranta arundinacea* L.

segregated under four categories such as very fine/soft (MS2, 6, 7, 8 and 9), soft (BRS, MS3 and 5), granular (MS1 and 4), mixture of fine, coarse and crystalline powder (IH) (Table 1). The solubility of the sample in the mouth also varied namely 'completely soluble in the mouth' (BRS, MS2, 6, 7, 8 and 9), soluble, but a bit slower when compared to the previous category (IH) and 'not completely soluble in the mouth' (MS1, 3, 4 and 5), Iodine test showed three different categories of results namely 'complete colour change to blue' (BRS, IH, MS2, 6, 7, 8 and 9), 'Partially changed to blue colour' (MS3 and 5), 'Did not show any colour change' (MS1 and 4) (Figure 3).

The following observations were documented in powder microscopical analysis: BRS showed ovoid starch grains of 10-50 microns, with concentric striations and hilum with two radiating curved lines, IH showed ovoid starch grains measuring 15 to 50 microns with fissured hilum (Figures 4-6), MS2, 6, 7 and 9 showed spherical starch grains of sizes 2-18, 2-20, 2-20 and 5-18 microns respectively, with hilum in the center. MS8 showed oval starch grains of size 15-30 microns. MS1 and 4 did not show any starch grains instead flaky crystalline pieces were observed, MS3 and 5 showed a mixture of flaky crystalline pieces and starch grains (Table 2, Figure 7).

Table 1: Results of organoleptic parameters of BRS, IH, MS 1-9 samples.

Samples	Organoleptic evaluation			
	Texture	Color	Odor	Taste
BRS	Soft, fine powder.	White	odorless	Tasteless, dissolves completely in the mouth.
IH	Soft, fine powder.	White	odorless	Tasteless, dissolves in the mouth.
MS1	Granular powder.	Dull white	odorless	Tasteless, doesn't dissolve in the mouth, granular.
MS2	Very fine, soft powder.	Bright white	odorless	Tasteless, completely dissolves in mouth.
MS3	Soft, fine to touch.	White	odorless	Tasteless, some granular particles do not dissolve in the mouth (but not observed in touch).
MS4	Granular powder.	Dull white	odorless	Tasteless, doesn't dissolve in mouth
MS5	Soft, fine to touch.	White	odorless	Tasteless, some granular particles do not dissolve in the mouth (but not observed in touch).
MS6	Very fine, soft.	Bright white	odorless	Tasteless, completely dissolves in the mouth.
MS7	Very fine, soft.	Bright white	odorless	Tasteless, completely dissolves in the mouth.
MS8	Very fine, soft.	Bright white	odorless	Tasteless, completely dissolves in mouth.
MS9	Very fine, soft.	Bright white	odorless	Tasteless, completely dissolves in the mouth.

Table 2: Observation of starch grains from powder microscopy.

Samples	Size	Shape	Hilum
BRS	10-50 microns	Ovoid	Hilum towards the narrow end with two radiating curved lines from hilum.
IH	15-50 microns	Ovoid	Hilum towards the narrow end with fissured hilum.
MS1	*	*	*
MS2	2-18 microns	Spherical	Center
MS3	**	Spherical with uneven surface	Center
MS4	*	*	*
MS5	**	Spherical with uneven surface	Center
MS6	2-20 microns	Spherical	Center
MS7	2-20 microns	Spherical	Center
MS8	15-30 microns	Sac shaped	Near the tip
MS9	5-18 microns	Spherical	Center

*No starch grains observed, only crystalline flaky substance observed; **A mixture of starch grains and crystalline flaky substance observed.

Table 3: Observations from phytochemical analysis and Ash Tests of BRS, IH and MS1- 9 samples.

Phyto constituents	BRS	IH	MS1	MS2	MS3	MS4	MS5	MS6	MS7	MS8	MS9
Starch - Iodine test	+	+	-	+	+	-	+	+	+	+	+
Carbohydrate-Molisch's test	+	+	+ m	+	+	+	+	+	+	+	+
Sugar	+	+	+ m	+	+	+ m	+	+	+	+	+
Saponin	-	-	+	-	-	+	-	-	-	-	-
Triterpenoids	-	-	-	-	-	-	-	-	-	-	-
Steroids	+	+	+ m	+	+	+ m	+	+	+	+	+
Alkaloids	-	-	-	-	-	-	-	-	-	-	-
Protein	+	+	+	+	+	+	+	+	+	+	+
Amino acid	-	-	+	-	+ m	+	+ m	+ m	-	-	-
Flavonoids	-	-	-	-	-	-	-	-	-	-	-
Phenol	-	-	-	-	-	-	-	-	-	-	-
Tannin	-	-	-	-	-	-	-	-	-	-	-
Ash value, %	-	-	+	-	+	+	+	-	-	-	-

(+) - Presence, (-)- Absence, (+ m)- moderately present.

Table 4: Results of XRD analysis of MS 1, 3, 4 and 5.

Sample name	Ash content (%)	Minerals Identified by PXRD	Content of Minerals with respect to Ash (%)	Content of Minerals with respect to Sample (%)
MS 1	92.69	$CaCO_3MgCO_3$(Dolomite)	90	83.42
		$CaCO_3$ (Calcite)	5	4.63
		$Al_4Mg_2Si_5O_{18}$ (Cordierite)	4	3.71
		SiO_2 (Quartz)	1	0.93
MS 3	72.03	$Mg_3Si_4O_{10}(OH)_2$(Talc)	51	36.72
		SiO_2 (Quartz)	38	27.36
		$CaCO_3$ (Calcite)	11	7.92
MS 4	81.24	$CaCO_3$ (Calcite)	68	55.24
		$CaCO_3MgCO_3$(Dolomite)	23	18.68
		ZnS (Zinc sulphide)	10	8.94
MS 5	53.67	$Mg_3Si_4O_{10}(OH)_2$(Talc)	61	32.74
		SiO_2 (Quartz)	39	20.93

All the MS samples were subjected to ash value tests. The samples in which ash values were significantly high (MS samples 1, 3, 4 and 5) (Table 3) were selected for PXRD analysis. PXRD reported the major adulterants in the samples as dolomite, calcite, talc, quartz and zinc sulphide (Table 4 and Figure 8).

DISCUSSION

Adulteration is very common in the raw drug and food industry which is considered to be an intentional malpractice, where genuine materials are adulterated/admixed with those which look alike, smell alike, or taste alike or sometimes totally replaced for the purpose of commercial benefits. Now there is an active trend of adulterating the by-products from raw materials like starch, gum resins, or oils which is difficult indeed to validate or identify.[16]

The rhizomes of *Maranta arundinacea* is an excellent source of starch (>85%) which finds its use in the food industry as a thickener or stabilizer and herbal drug industry for its therapeutic value, is also recommended for people with gluten intolerance.[17,18] Studies have been conducted to assess the morphological, structural and functional properties of MS individually.[12,19] At present there is a scenario that most of the GMP pharmaceuticals rely on local vendors for the mass supply of the material at cheaper rates since the product price is cheap enough as compared to the original cost of the manufacturing expenses. For economic benefits, malpractices like adulterating MS with other edible starches or

even illegal whitening agents are very common. Many of the products are found to be marketed without the statutory Food Safety and Standards Authority of India (FSSAI) License. Hence this study aimed to assess the quality of MS available in the market which is being used at different levels right from pediatric food to industrial supply.

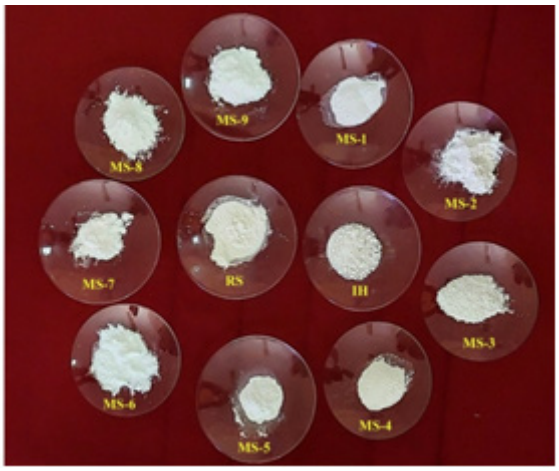

Figure 2: Samples of BRS, IH, MS 1-9.

Voluminous data is available on the application of powder microscopy to analytical chemistry for the identification of adulterants in food samples including various plant starches. An integrated approach is still lacking, and certain fields are untouched for finding a solution for the malpractices in present day food market industry. Macroscopically or in naked eye examinations, all the products labelled and marketed as MS appear similar as white fine powder to its variant forms like dull white to bright and is difficult to confirm the authenticity in aspects of organoleptic indices. Microscopically, details of individual starch granules could be deliberated by considering the size, shape, arrangement of rings and the location of hilum (nucleus). As an established finding, MS granules usually have the size range from 7-75 microns, with concentric striations and the positioned hilum at the narrow end.[20]

Figure 3: Iodine test of BRS, IH and MS1 to 9.

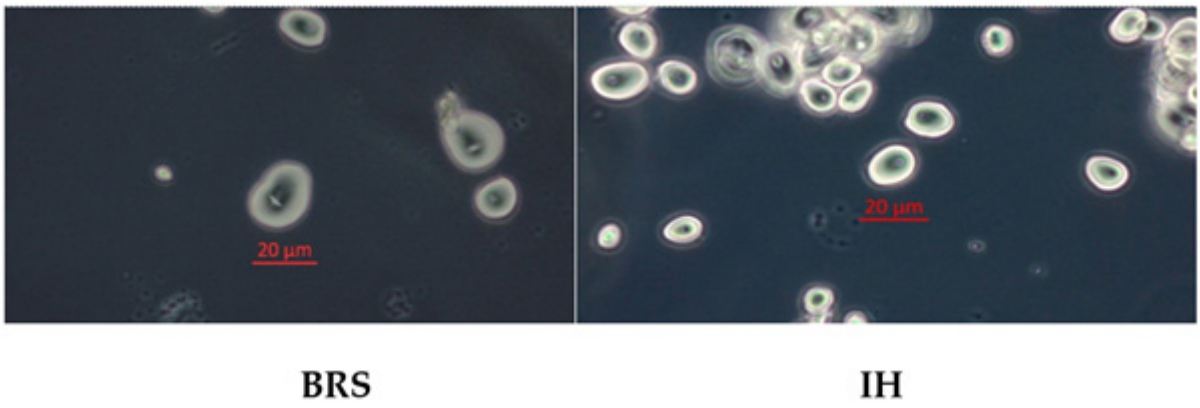

Figure 4: Starch grains of BRS and IH samples viewed under polarizer showing hilum and concentric striations.

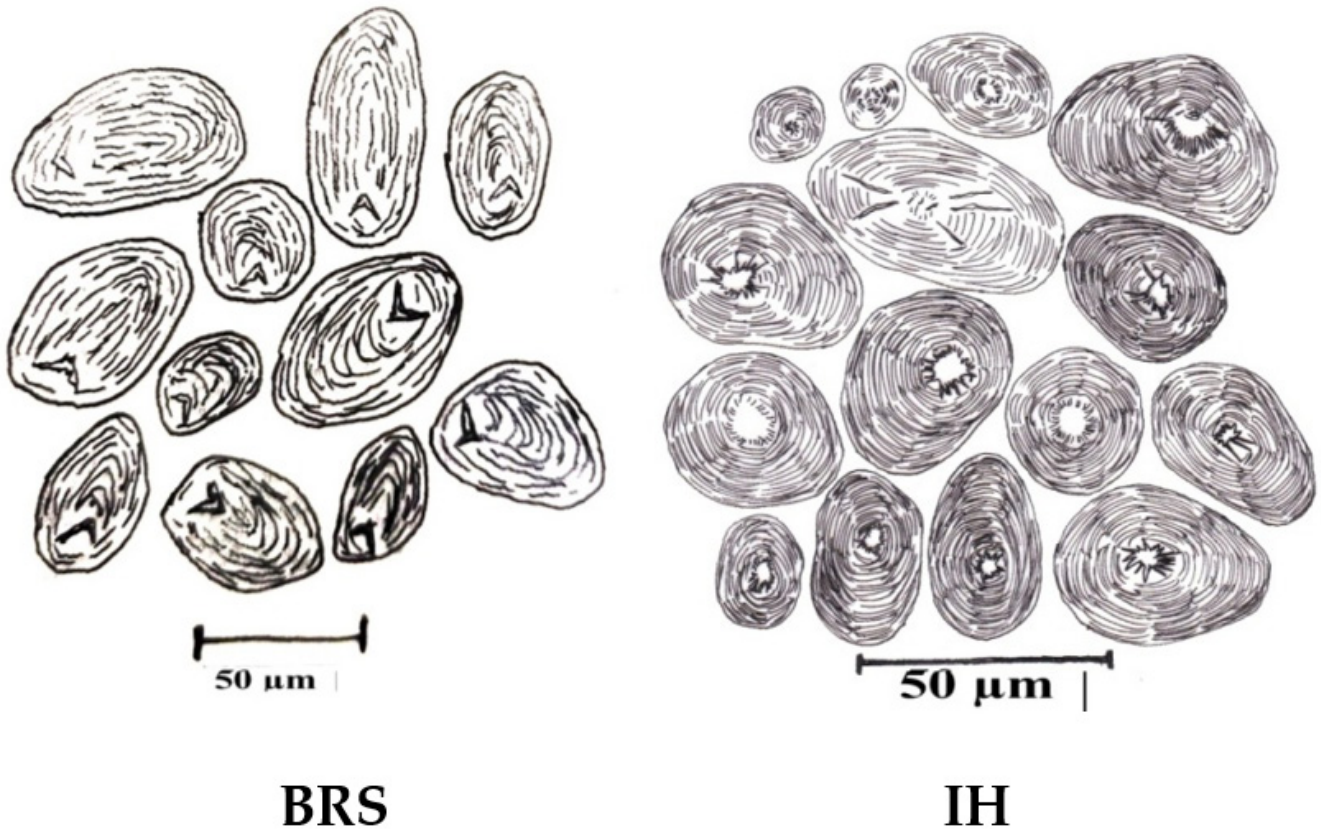

Figure 5: Powder microscopic illustration of starch grainsof BRS and IH.

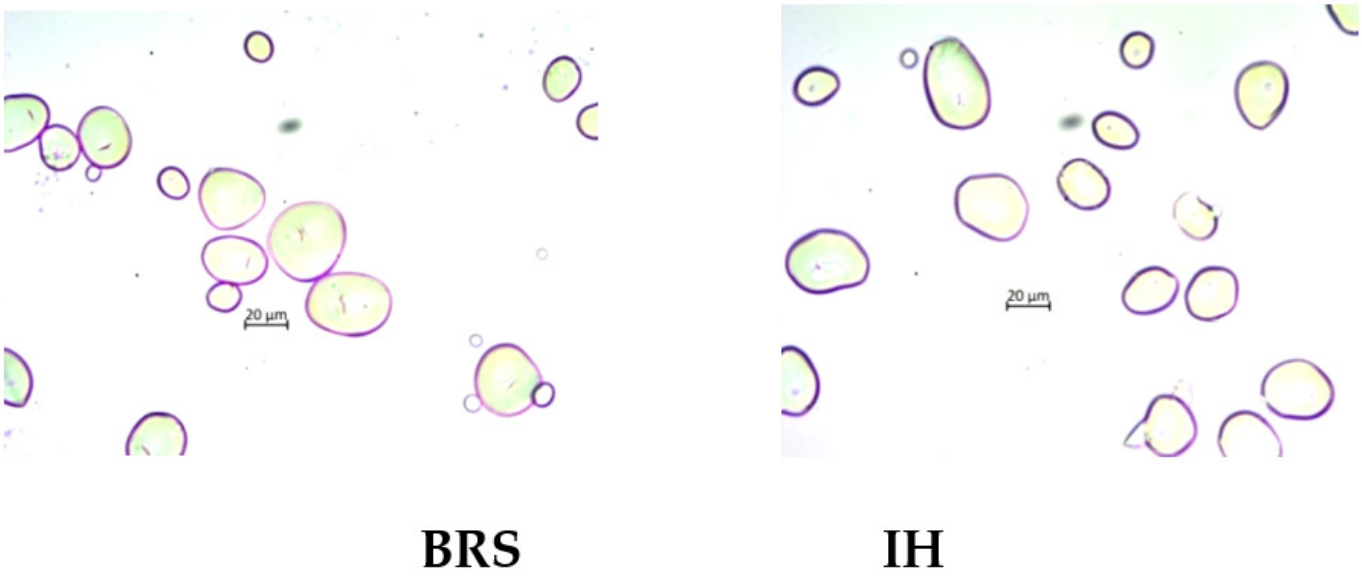

Figure 6: Powder microscopy of starch samples of BRS and IH.

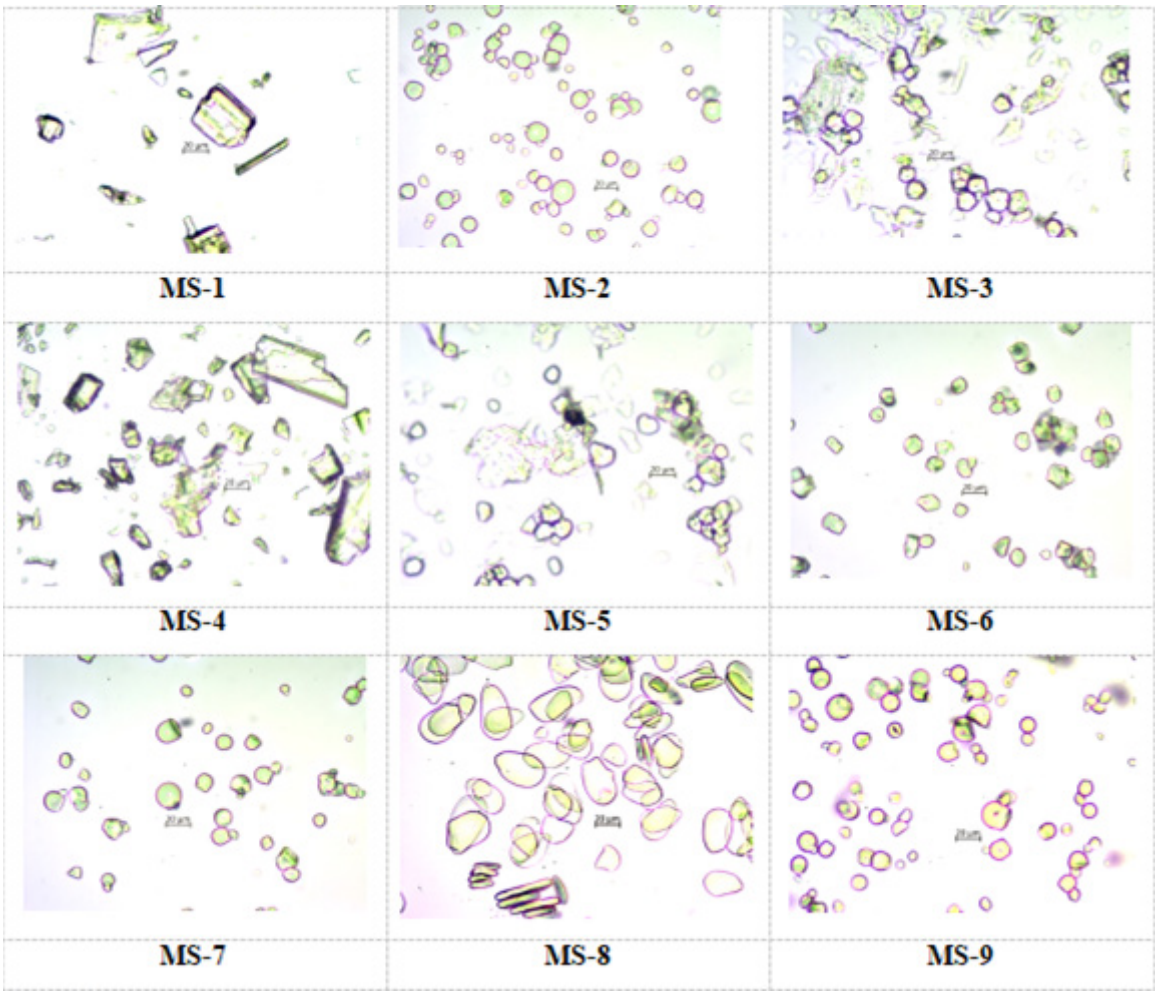

Figure 7: Powder microscopy of starch samples MS1 to 9.

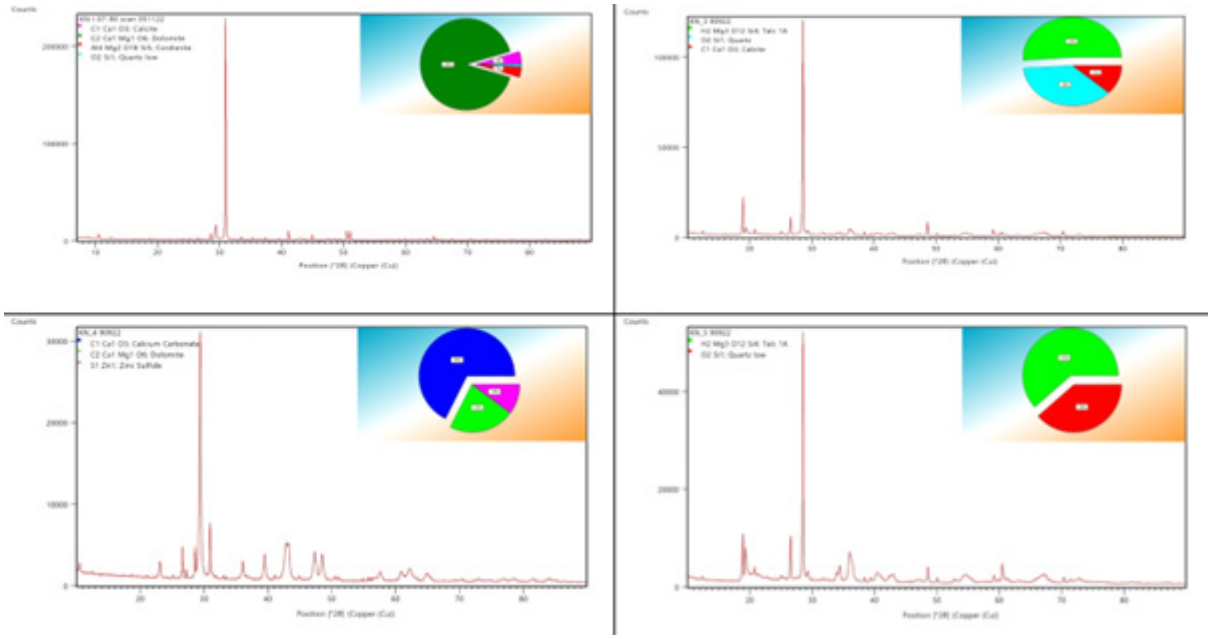

Figure 8: XRD reports of MS 1, 3, 4 and 5. *The major adulterants areTalc and calcite, followed by quartz and dolomite.

Findings from this study which analysed nine samples from different vendors across Chennai, Tamil Nadu demonstrated none of the samples were genuine. The samples, MS2, 6, 7, 8 and 9 which yielded a positive iodine test for the presence of starch could not be identified as MS, as none of the typical features were observed in it. As the starch granules of *Maranta* are simple, ovoid in shape measuring 7 to 75 microns having small tuberosities with concentric striations, the hilum is usually located at the broader end.[20]

To justify the findings and to identify the adulterant material, ash value test was done in all the samples and those with significant ash values were further subjected to PXRD analysis for tracing the mineral adulterants. The ash values of MS1, 3, 4 and 5 samples were determined as 92.69, 72.03, 81.24 and 53.67 % respectively and identified as adulterated with minerals samples. The MS1 sample was identified and calculated to contain dolomite (83.42%), calcite (4.63%), cordierite (3.71%) and quartz (0.93%). MS3 was identified and determined to contain talc (36.72%), quartz (27.36%) and calcite (7.92%). MS 4 sample was analyzed and calculated to have calcite (55.24%), dolomite (18.68%) and zinc sulphide (8.94%). MS 5 was found to contain talc (32.74%) and quartz (20.93%). The phytochemical tests revealed positive response in carbohydrate, sugar, steroid and protein by all samples. While sample MS 1 and MS 4 did not answer for starch but answered for sugar (mildly) and saponin. The samples MS1, 3, 4 and 6 answered for amino acids indicating its adulteration in these five samples. The ash values of MS 1, 3, 4 and 5 were indicated to be 7.31, 27.97, 18.76 and 46.33%, respectively of organic matter which include sugar, carbohydrate, steroid, protein, amino acid, etc. With respect to ash content values, the quality of the MS samples BRS, HIS, MS2, 6, 7, 8 and 9 were found to be unadulterated and MS1, 3, 4 and 5 are adulterated and can be graded as MS5>MS3>MS4>MS1.

Mineral substances like dolomite ($CaMg(CO_3)_2$), cordierite ($Al_4Mg_2Si_5O_{18}$), quartz (SiO_2), Zinc Sulphide (ZnS), talc and calcite which are spotted in significant percentage in the samples are considered as established food adulterants. Dolomite lime stone powder because of its similarity to starch in color and texture is commonly admixed with it and is indeed difficult to detect or differentiate by visual inspection alone. Health implications of long-term usage of dolomite adultered foods are rather alarming. Many studies reported the presence of potential toxic metals like arsenic, mercury, lead in dolomite which may harm the functions of nervous system and gastrointestinal system.[21] Diseases of the skin and blood may intervene on long term exposure. There are studies that prove the damaging effects of micro fine powder of dolomite in liver and laryngeal cells.[22] Low-cost talc is also been used frequently in food industry as an adulterant for wheat flour and Kudzu starch and this is purposed to increase the mass and delicate nature of the powder besides imparting a whitening effect with good lustre and antisticking properties. Severe effects of long-term usage of talc may include the high risk of cancer.[23] European Union (EU) and U.S. Food and Drug Administration (FDA) restrict the usage of talc as food additives considering its deleterious effects in health.[24]

CONCLUSION

Considering the mandate of adulterant identification in food substances particularly those given as infant nutrition, here we followed the method that is highly reliable. Apart from microscopic identification of the authenticity of the material, the mineral substances used in adultering the MS were quantitatively determined with accuracy. This case study exposes the extensive adulteration of marketed MS in the South Indian market, particularly Chennai.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

MS: Maranta Starch; **BRS:** Botanical Reference Standard; **IH:** In-house preparation; **XRD:** X-ray Diffraction.

SUMMARY

The study is all about testing the authenticity of commonly available marketed samples of MS by pharmacognostical identification and X-ray diffraction analysis. Nine market samples were tested for macro-microscopical analysis, preliminary phytochemical analysis, ash values, etc. The results showed none of the marketed samples were authentic and contained inorganic matter which was confirmed to be dolomite, calcite, talc, quartz and zinc sulphide.

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