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Qualitative and quantitative analysis of purified hatched and non-hatched country fowl's eggshell powder – A comparative study

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Abstract

Siddha system of medicine has a hoary past. It is one of the indigenous medical system. It has been long playing its vital role in serving in public health in various part of India and in recent times all over the world. It consists of lots of herbs, metals, minerals and animal origin to treat diseases by their vast activities. Eggshell is a animal origin drug which is used as a main and one of the ingredients in many Siddha preparations to treat various diseases. *Anda parpam* is a drug which has the eggshell as its main ingredient. There are many different preparations of *Anda parpam* which was mentioned by Siddhars. Some of them use hatched eggshell powder for making some specific preparations. So a comparative study is need to validate the components of purified Country fowl's eggshell before and after hatching. This work explains the difference between the hatched and non hatched eggshells through Physico-chemical analysis, Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) and Fourier Transform Infra-red Spectroscopy (FTIR). This study also forms the base for the pharmaceutical analysis of hatched and non-hatched hen eggshell powder which will be followed by safety and efficacy studies later.

Keywords: ICP-OES, FTIR, Physico-chemical analysis, hatched and non-hatched eggshell.

Introduction

Eggshell is a white hard fragile calcareous substance composed of carbonate of lime, phosphate of lime and traces of sulfur and iron. The eggshell makes upto 9-12 percent of an egg's total weight. In Siddha system, eggshells of the birds such as hen, parrot, crow, peacock, kite and

quail were used in the preparation of medicines. However hen eggshell is used widely in many preparations. In some Siddha texts Siddhar's emphasized using only hatched eggshell for preparing medicines. Siddha preparations like *singi vanga parpam*, *karuvanga sunnam*, *anda sunnam*, *anda parpam* etc., it has been emphasized to use hatched eggshells.

Materials and Methods

Preparation of samples:

Country fowl's non - hatched eggshell and hatched eggshell was collected, measured and kept separately in vessels. 50gms of non hatched eggshells and 50gms of hatched eggshells was placed separately in a vessel containing water

which was dissolved with 17gms of *Appalakaram* (Sodium carbonate) and then boiled separately. Then the shell membranes were removed and washed with normal water. And then the eggshells were dried in sunlight and powdered separately. Purified country fowl's non hatched eggshell powder was kept in a container named as A and purified hatched eggshell powder was kept in a container named as B.



Fig.1: Unpurified non hatched eggshell



Fig. 2 : Unpurified hatched eggshell



Fig.3: Purified non hatched eggshell



Fig.4: Purified hatched eggshell

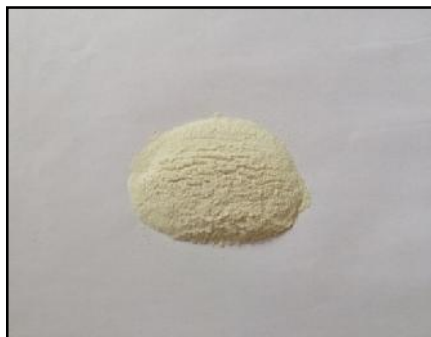


Fig.5: Purified non hatched eggshell powder (Sample-A)

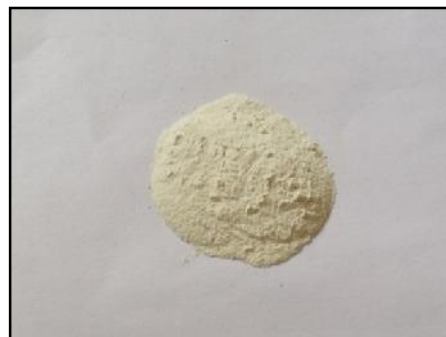


Fig.6: Purified hatched eggshell powder (Sample - B)

Determination of Moisture content (Loss on Drying):

10 gm of test drug samples A and B were accurately weighed in evaporating dish separately. The samples were dried at 105°C for 5 hours and then weighed. Drying and weighing of the test drugs continued at one hour interval until difference between two successive weighing corresponds to not more than 0.25 percent.

Determination of Total Ash:

3 gm of test drug samples A and B were accurately weighed and incinerated in a tared platinum or silica dish at a temperature not exceeding 450°C until free from carbon, cool and weigh. Calculate the percentage of ash with reference to the air-dried drug.

Determination of water soluble Ash:

Boil the ash for 5 minutes with 25 ml of water; collect insoluble matter in a Gooch crucible, or on an ashless filter paper, wash with hot water, and ignite for 15 minutes at a temperature not exceeding 450°C. Subtract the weight of the insoluble matter from the weight of the ash; the difference in weight represents the water-soluble ash. Calculate the percentage of water-soluble ash with reference to the air-dried drug.

Determination of Acid Insoluble Ash:

Boil the ash obtained for 5 minutes with 25 ml of dilute hydrochloric acid; collect the insoluble matter in a Gooch crucible, or on an ash less filter paper, wash with hot water and ignite to constant weight. Calculate the percentage of acid-insoluble ash with reference to the air dried drug.

Determination of Water-soluble Extractive:

Macerate 5 g of the air-dried drug, coarsely powdered, with 100 ml of chloroform - water of the specified strength in a closed flask for twenty-

four hours, shaking frequently during six hours and allow to stand for eighteen hours. Filter rapidly, taking precautions against loss of solvent, evaporate 25 ml of the filtrate to dryness in a tared flat bottomed shallow dish, and dry at 105°C, to constant weight and weigh. Calculate the percentage of alcohol-soluble extractive with reference to the air-dried drug.

Inductively Coupled Plasma Optic Emission Spectrometry (ICP-OES):

The analysis was carried out using Perkin Elmer Optima 5300 dv ICP – OES instrument. It offers the performance required to maximize productivity. It ensures accuracy, improves method development, and consistently delivers the correct answer. The system is ideal for laboratories with moderate to heavy loads of difficult samples.

Fourier Transform Infrared Spectroscopy (FT-IR):

The analysis was carried out using IRTracer-100. The IRTracer-100 offers high sensitivity with a 60,000:1 S/N ratio. This sensitivity combined with the Lab Solutions IR Contaminant Analysis Macro enables easier, quicker and more accurate analysis of small samples. It can be customized by the user, with a range of accessories and user friendly software options to meet the needs of a specific application. FTIR is an effective analytical instrument for detecting functional groups.

Results

Physicochemical analysis

Table 1: Organoleptic characters of sample A (Non-hatched eggshell powder) and sample B (Hatched eggshell powder)

S. No	Parameters		Sample A (Non hatched eggshell powder)	Sample B (Hatched eggshell powder)
1.	Ash value	Water soluble ash	8.60±0.050	4.70±0.048
		Acid insoluble ash	7.10±0.020	2.80±0.043
2.	Water soluble extractive value		8.70±0.510	10.20±0.500
3.	Loss on drying at 105°C		1.20±0.540	8.23±0.500
4.	Color		White	White
5.	pH		8.80	8.99

ICP-OES Analysis: (Perkin Elmer Optima 5300 DV ICP-OES)

Table 2: ICP-OES analysis of sample A (Non - hatched eggshell powder) and sample B (Hatched eggshell powder)

S. No	Elements	Symbol	Wavelength (nm)	Concentration	
				Sample A (non hatched eggshell powder)	sample B (hatched eggshell powder)
1.	Aluminium	Al	396.152	BDL	BDL
2.	Arsenic	As	188.979	BDL	BDL
3.	Carbon	C	193.030	01.230 mg/L	03.230 mg/L
4.	Calcium	Ca	315.807	672.180 mg/L	602.100 mg/L
5.	Cadmium	Cd	228.802	BDL	BDL
6.	Chlorine	Cl	725.670	BDL	BDL
7.	Copper	Cu	327.393	BDL	BDL
8.	Iron	Fe	238.204	00.376 mg/L	00.016 mg/L
9.	Mercury	Hg	253.652	BDL	BDL
10.	Potassium	K	766.491	00.821 mg/L	00.001 mg/L
11.	Magnesium	Mg	285.213	01.210 mg/L	01.010 mg/L
12.	Manganese	Mn	257.610	BDL	BDL
13.	Sodium	Na	589.592	01.320 mg/L	01.000 mg/L
14.	Lead	Pb	220.353	BDL	BDL
15.	Phosphorus	P	213.617	56.341 mg/L	186.341 mg/L
16.	Sulfur	S	180.731	01.254 mg/L	0.254 mg/L

BDL - Below Detection Limit

Fourier Transform Infra-red Spectroscopy of Sample A (Non-hatched eggshell powder)

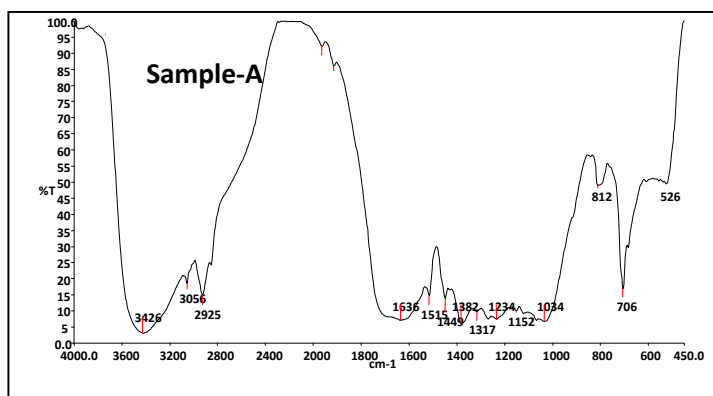


Fig: 7 Fourier Transform Infra-red Spectroscopy of Sample A (Non-hatched eggshell powder)

Functional groups present in Sample A (Non – hatched eggshell powder)

Table 3: Functional groups present in sample A (Non – hatched eggshell powder)

S. No	Frequency (cm ⁻¹)	Bond	Intensity	Functional group
1.	3426	O-H Stretch	Strong, Broad	Alcohols
2.	3056	N-H Stretch	Strong, Broad	Amine salt
3.	2925	C-H Stretch	Medium	Alkanes
4.	1636	C=C Stretch	Medium	Alkenes
5.	1515	N-O Stretch	Strong	Nitro compounds
6.	1449	C-H Bend	Medium	Alkane
7.	1382	C-H Bend	Medium	Alkane
8.	1317	S=O Stretch	Strong	Sulfone
9.	1234	C-O Stretch	Strong	Alkyl aryl ether
10.	1152	C-O Stretch	Strong	Alcohols
11.	1034	S=O Stretch	Strong	Sulfoxide
12.	812	C=C Bend	Medium	Alkene
13.	706	C=C Bend	Strong	Alkene

Fourier Transform Infra-red Spectroscopy of Sample B (Hatched eggshell powder) Sample-B

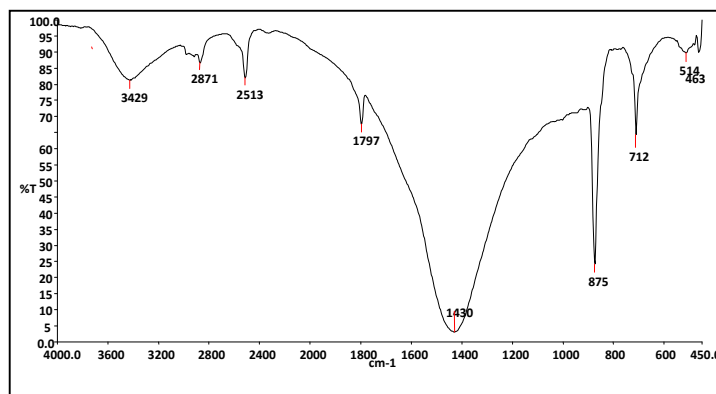


Fig:8 Fourier Transform Infra-red Spectroscopy of Sample B (Hatched hen eggshell powder)

Functional groups present in Sample B (Hatched eggshell powder):

Table 4: Functional groups present in sample B (Hatched eggshell powder)

S.No	Frequency (cm ⁻¹)	Bond	Intensity	Functional group
1.	3429	O-H Stretch	Strong, Broad	Alcohol
2.	2871	N-H Stretch	Strong, Broad	Amine salt
3.	2513	O-H Stretch	Strong, Broad	Carboxylic acids
4.	1797	C=O Stretch	Strong	Acid halide
5.	1430	O-H Bend	Medium	Carboxylic acid
6.	875	C-Cl Bend	Strong	Halo compound
7.	712	C=C Bend	Strong	Alkene
8.	514	C-I Stretch	Strong	Halo compound

Discussion

In this study, non-hatched eggshells and hatched eggshells were purified and analyzed according to the standard procedures.

Organoleptic characters:

Colour of Sample A (Non-hatched eggshell powder) and Sample B (Hatched Eggshell powder) is white. pH of Sample A is 8.80 and Sample B is 8.99, both are alkaline. Water soluble ash value and Acid insoluble ash value for Sample A is 8.60 ± 0.050 , 7.10 ± 0.020 respectively. Water soluble ash value and Acid insoluble ash value for Sample B is 4.70 ± 0.048 , 2.80 ± 0.043 respectively. Water soluble extractive value for Sample A is 8.70 ± 0.510 . Water soluble extractive value for Sample B is 10.20 ± 0.500 . The values of Loss on drying at 105°C for Sample A is 1.20 ± 0.540 . The values of Loss on drying at 105°C for Sample B is 8.23 ± 0.500 .

ICP-OES analysis: ICP-OES analysis for Sample A (Non-hatched eggshell powder) indicates the presence of elements with their concentration such as Calcium (Ca) - 672.180 mg/L, Phosphorus (P) - 56.341 mg/L, Sodium (Na) - 01.320 mg/L, Sulfur (S) - 01.254 mg/L, Carbon (C) - 01.230 mg/L, Magnesium (Mg) - 01.210 mg/L, Potassium (K) - 00.821 mg/L and Iron (Fe) - 00.376 mg/L.

ICP-OES analysis for Sample B (Hatched Eggshell powder) indicates the presence of elements with their concentration such as Calcium (Ca) - 602.100 mg/L, Phosphorus (P) - 186.341 mg/L, Carbon (C) - 03.230 mg/L, Magnesium (Mg) - 01.010 mg/L, Sodium (Na) - 01.000 mg/L, Sulfur (S) - 00.254 mg/L, Iron (Fe) - 00.016 mg/L and Potassium (K) - 00.001 mg/L. The heavy metals such as Mercury (Hg), Copper (Cu), Lead (Pb), Cadmium (Cd), Aluminium (Al), Manganese (Mn), Arsenic (As) and the element Chlorine (Cl) is below detection limit in both Sample A and Sample B. From this study it is observed that the element Phosphorus (P) and Carbon (C) is increased in Sample B when compared to the Sample A. The concentration of

the element such as calcium (Ca), Sulfur (S), Potassium (K), Iron (Fe), Sodium (Na) and Magnesium (Mg) is decreased in Sample B when compared to the Sample A.

FTIR analysis: In the FT-IR Spectra analysis, the Non-hatched eggshells powder (sample-A) exhibits the peak value at the wave number of 3426, 3056, 2925, 1636, 1515, 1449, 1382, 1317, 1234, 1152, 1034, 812, 706 having O-H Stretch, N-H Stretch, C-H Stretch, C=C Stretch, N-O Stretch, C-H Bend, C-H Bend, S=O Stretch, C-O Stretch, C-O Stretch, S=O Stretch, C=C Bend, C=C Bend. This indicates the presence of some organic functional groups such as Alcohols, Amine salt, Alkanes, Alkenes, Nitro compounds, Sulfone, Alkyl aryl ether, Sulfoxide. In the FT-IR Spectra analysis, the Hatched eggshells powder (sample-B) exhibits the peak value at the wave number of 3429, 2871, 2513, 1797, 1430, 875, 712, 514 having O-H Stretch, N-H Stretch, O-H Stretch, C=O Stretch, O-H Bend, C-Cl Bend, C=C Bend, C-I Stretch. This indicates the presence of some organic functional groups such as Alcohol, Amine salt, Carboxylic acids, Acid halide, Halo compound, Alkene. The functional groups present in both the samples are Alcohol, Amine salt and Alkene.

Conclusion

In this study, Non-hatched eggshells and Hatched Eggshells were purified and analyzed according to the standard procedures. There is a slight variation in physicochemical properties between Sample A (Non-hatched eggshells powder) and Sample B (Hatched Eggshells powder). In ICP-OES analysis there is a quantitative difference between Sample A and Sample B. pH of Sample A and Sample B indicates that both the Samples are alkaline in Nature. However pH of Sample B is more alkaline when compared to sample A. It is clear that there is slight variation in functional groups between Non-hatched eggshells powder and hatched eggshells powder. From this study it is clear that there is qualitative and quantitative difference between non-hatched eggshells powder and hatched eggshells powder. Hence, there is a reason behind the usage of

hatched eggshells in specific preparations. So it is necessary to use Hatched eggshells wherever it is mentioned in the preparation of specific Siddha medicines in Siddha literatures. This study forms the base for the pharmaceutical analysis of hatched and non hatched eggshells powder which will be followed by safety and efficacy studies later.

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