

**PHARMACEUTICO-ANALYTICAL STUDY OF KAPARDIKA
BHASMA AND DNA SEQUENCING OF KARPARDIKA****Nitin Kumar^{1*}, Manbi Sharma² and Tanuja Bharti³**

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ABSTRACT

Rasashastra is a science of ayurvedic pharmaceuticals which deals with the drugs of mineral origin and here Kapardika (*Cypraea moneta* Linn.) is the choice of drug having various therapeutic uses in Ayurvedic practice. The shodhana and marana are two basic concepts of Rasashastra. Almost all drugs are advised to be processed with specific shodhana and marana methods before their internal uses, remove the harmful effects and to convert it into absorbable form. By the application of shodhan and marana methods as per classics, we have purified and made bhasma of karpardika. The present study was conducted to assess the physico-chemical analysis of Kapardika Bhasma and its DNA profiling with special reference to its calcium content and comparative screening with

human calcium.

INTRODUCTION

Ayurveda is a well-documented traditional system of Indian Medicine. Rasa Shastra, an offshoot of Ayurveda popular from medieval period, mostly deals with therapeutic utilization of metals and minerals.^[1,2] Rasa Shastra is the unique knowledge system which provides unmatched database of economical, effective and wide range of medicine. It utilizes almost all forms of matter available on earth. In this regard there is a segment which deals with

products of marine origin under the heading Sudha Varga. Among those Varatika (also under Saadharna rasa varga), which is identified as the external shell of sea animal *Cyprea moneta* linn., is having upper hand and is popular among traditional practitioners. Again, this is used widely in the form of Bhasma, which is the exclusively unique dosage form of Ayurveda, these acts in small doses and can cure chronic ailments. In traditional system of medicine the Kapardika (*Cypraea moneta*), have been used as medicine to cure various ailments mainly related with stomach and in the treatment of dyspepsia, jaundice, enlarged spleen, liver, asthma, cough and also reported to be externally used as caustic in various forms of ointments.^[3,4] Kapardika is also included in Sadharan Rasa.^[5,6] Kapardika is the name given to small convolute glossy shells of variegated colours of oblong, oval shape varying in size.^[7] Cowries shells were used in many area of medicines i.e. dyspepsia, jaundice, enlarged spleens and liver, asthma and cough.^[8] Since DNA is more steady and does not change occasionally and with age of the plant. DNA based fingerprinting methods have more prominent part in the confirmation of botanicals which are restoratively essential. DNA markers are dependable for data on hereditary polymorphism as the hereditary creation is one of a kind for every animal categories independent of plant part utilized and is not influenced by age, physiological condition and in addition natural components.

The present audit essentially concentrates on validation of Ayurvedic home grown solutions by DNA based fingerprinting strategies to avert deliberate and accidental contaminated or substitution of focused Ayurvedic restorative herbs. DNA Fingerprinting DNA fingerprints are a standardized identification like examples produced by enhancement of chromosomal DNA of a person which can recognize the uniqueness of this person from another. Also called DNA writing, hereditary fingerprinting, DNA profiling and DNA writing. DNA fingerprinting in plants can be connected to various applications and uses. DNA based marker examination has been demonstrated as an imperative apparatus in home grown medication institutionalization. DNA is the fundamental part of every single living cell. The justification of hunting down medications from marine condition originate from the way that marine plants and creatures have adjusted to a wide range of marine situations and these animals are always under huge choice weight counting space rivalry, predation, surface fouling, and multiplication. The consideration of discovering drug from the ocean had begun from the 1970s. For example, around 300 licenses on the bioactive marine common item have been issued near 1969 and 1999. Up until this point, more than 10,000 mixes have been detached from marine organisms. Only 10% of more than 25,000 plants have been researched for

organic action. The marine condition may contain more than 80% of world's plant and creature species. Lately, numerous bioactive mixes have been removed from different marine creatures like tunicates, wibes, delicate corals, bryozoans, ocean slugs and marine organisms. The marine condition covers a wide warm, weight and supplement extents and it has broad photic and non-photoc zones. This broad inconstancy has encouraged broad detail at all phylogenetic levels, from microorganism to warm blooded creatures. In spite of the way that the biodiversity in the marine condition for surpasses that of the earthly condition, examine into the utilization of marine regular items as the pharmaceutical operator is still in its earliest stages.

This might be because of the absence of ethno medical history and the troubles required in the accumulation of marine organisms. However, with the advancement of new jumping strategies, remote worked machines and so on, it is conceivable to gather marine specimens and amid the previous decade, more than 4200 novel mixes have been detached from shallow waters to 900-m profundities of the sea. Several marine living beings are sessile and delicate bodied, at that point, the inquiry will emerge. The response to this examination is the guard system of the marine life form. The synthetic mixes (like auxiliary metabolites) which created or gotten from a miniaturized scale life form. By the marine verdure is exceptionally strong and naturally dynamic. The power of bioactive from marine life is predominantly because of the serious biology weight and from the more grounded and/or predators. Examinations in their compound biology have uncovered that the auxiliary metabolites not just assume different parts in the digestion of the maker additionally in their procedures in the given condition. The examination on marine synthetic mixes created by various creatures; demonstrated the systems for their utilization for human advantage.

Ayurvedic pharmaceuticals assumed a critical part in keeping up wellbeing and treating the sickness worldwide since antiquated circumstances. Because of recharged premium and prevalence of Ayurveda and homegrown solution as of late, the worldwide market exchange is worth about \$ 60 billion annually¹ and a yearly turnover of Ayurvedic meds in the universal market is 3500 crores. Genuineness identifies with demonstrating the material is valid and compares to right character. As commercialization and expanded request wellbeing, quality and affirmation of Ayurvedic natural pharmaceuticals is greatest lacuna. Therapeutic plant material are regularly substituted and additionally debased either incidentally or deliberately with herbs from firmly related species, which are morphologically undefined, or

by materials from random plants. Since DNA is more steady and does not change occasionally and with age of the plant. DNA based fingerprinting methods have more prominent part in the confirmation of botanicals which are restoratively essential. DNA markers are dependable for data on hereditary polymorphism as the hereditary creation is one of a kind for every animal categories independent of plant part utilized and is not influenced by age, physiological condition and in addition natural components.

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MATERIALS AND METHODS

Kapardika (Cowrie shells) were procured from authenticate source of Local market. Lemons required for the Shodhana of Kapardika are prepared as per Ayurvedic text.

Kapardika shodhana

Swedana covers the maximum area of preference. Dolayantra is said to be most convenient and repeatedly used instrument for swedana. And the time mentioned was 3 hours in most of the contexts. About 1000gm of raw varatika were subjected to shodhana process. Raw varatika were heated with lemon juice and water(1:6) for about 3 hours. After heating process these purified varatika were removed, cooled and dried.

Marana of suddha kapardika

The lemon juice treated varatika were directly placed in crucibles which were subjected to heating by puta mentod for about 550°C for 8 hrs. For this heating process i.e. Gajaputa, temperature was gradually increased to 550°C. When the temperature reaches 550°C, the temperature was maintained constant for about 3hrs and after 2hrs the temperature was decreased gradually to self-cool. After this process, the varatika becomes brittle. These brittle varatika where collected and powdered with the help of mortar and pestle. The powdered varatika is given bhavana (trituration) with of aloe vera juice for 3hrs. Then Cakrikas (pellets) were prepared and dried. After drying these pellets were subjected to second puta for 825°C. After cooling white coloured Varatika Bhasma was obtained. Here bhavana refers to the

process of grinding the mineral drugs in the liquids like juices or decoction of herbs, cow's milk, urines or any such specified liquids. The quantity of liquid should be sufficient to immerse the mineral powder. The grinding was continued, until liquid added dried up and semisolid consistency was achieved.

Sharava samputa nirmana

Encircled sustained temperature can be obtained. And also can prevent the contamination of materials kept inside. We can also expect the oxidation of materials. It may be altering the preparation time of bhasma by reducing the diffusion of released gases, thus building up the pressure inside the apparatus. The obvious reason for forming this provision is to avoid direct loss of material in the completely burnt fuel. After following all the parameters of bhasm pariksha we conducted the analytical study, experimental study.

DNA sequencing

DNA profiling of Kapardika with reference to calcium and comparative study with human bone calcium was tedious process. Data for human calcium profiling was generated from the gene bank and comparative studies were carried out.

RESULTS AND DISCUSSIONS

The present study was conducted to assess the physico-chemical analysis of Kapardika Bhasma and its DNA profiling with special reference to its calcium content and comparative screening with human calcium. The formulation is prepared according to classical text and all the other standardization aspect has been considered.

For the study raw materials like Grahya Kapardika collected from market by observing grahya laxanas. Herbal drugs like Nimbuka, ghrit kumara collected from natural habitat and all the collected raw materials get authenticated from experts in the subjects of Rasashastra & Dravyaguna. Qualitative and quantitative analyses were carried in Research laboratory of DBU PGSOAR, Herbal Health Research Cluster. DNA screening studies were carried out from Kolkata.

For the preparation of Kapardika Bhasma the steps followed are shodhana of Kapardika .For this firstly the whole Kapardika was cut in to small pieces to ensure proper involvement of each part of Kapardika with nimbu swarasa.Kapardika pieces were tied into pottali and suspended in dola yantra for swedana.

Dola yantra was filled with fresh nimbu swarasa. After the swedana few changes were noticed in the Shudha Kapardika like change in luster and surface become bit rough. After this shodhita Kapardika were placed in to sharava and kapad miiti were done. After proper drying of sharava these were allowed for puta.

Gaja puta was prepared as per the classical reference of agni sanskara. Upon self-cooling of sharava, Kapardika bhasma was again powdered well with the kharal yantra. After bhasma pareeksha Ghrita, kumara swarsa bhavana was carried out. Chakrika were prepared and sharava samputa was done and puta was given. Two puta were carried out to prepare bhasma. Peak temperature was noticed I first puta was 500⁰C and 2nd puta 830⁰C resp.

Kapardika, a mineral drug of animal origin is used in Ayurvedic therapeutics in many diseases. It is the key ingredient in drugs prescribed in gastrointestinal symptoms. The various stages of formulation techniques like shodhana (which involves roasting, with addition of herbal juices and continuous stirring) and marana [which heating], the particle size reduces significantly, which may facilitate absorption and assimilation of the drug into the body system. The micro to nano size of the formulations was confirmed by the analytical techniques, which could be specified as the criterion for the final product conforming to all the traditional parameters under bhasma pariksha (examination of properly prepared bhasma). The results of the present study revealed that Kapardika bhasma is an efficient herbometallic preparation. Attempts have been made to comparatively evaluate metallic Ayurvedic formulations using sophisticated analytical tools such as AAS, XRD and SEM.

The physico-chemical analysis was carried to evaluate the qualitative and quantitative data. Organoleptic changes are observed and noted in each step during the preparation of Kapardika Bhasma. Qualitative analyses like LOD, Ash value, carried for Kapardika Bhasma. Quantitative analysis of Grahya Kapardika show the % age of Ca and even after Kapardika Bhasma preparation %age of Calcium was documented. DNA profiling was also done for the Kapardika Sample with special reference to calcium. The study was intended to find out the relation between human calcium and calcium present in the marine originated drugs used in Ayurveda.

Dna study

DNA SEQUENCING (CALCIUM) OF HUMAN AND KAPARDIKA NABHI GIVEN BELOW

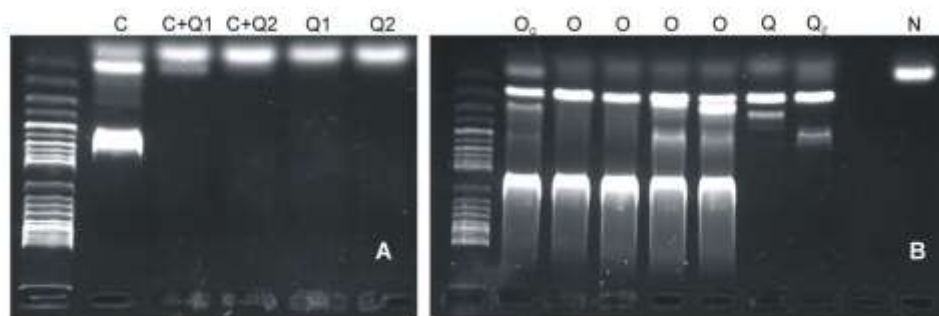


Image: Showing PCR results from extraction procedure of Kapardika nabhi

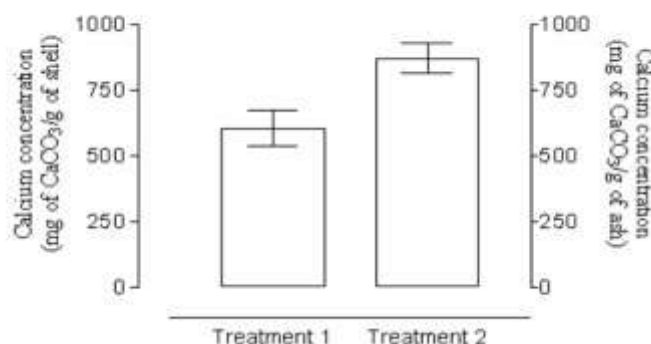


Image: Showing Calcium content in Cowrie as mg of CaCO₃/g (treatment 1) and mg of CaCO₃/g of ash (treatment2) exposed to different treatments.

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