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Synthesis and Characterization of Swarnamakshika Bhasma

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Abstract : Swarnamakshika Bhasma is golden yellowish mineral which is found in many ancient Ayurvedic texts such as Sushruta samhita, Charaka samhita, Ashtanga sangreha etc. Its chemical composition is $CuFeS_2$ and this Bhasma found to contain Potassium (K), Magnesium (Mg), and Silicon (Si) and Aluminum (Al) in trace amounts. Swarnamakshika or $CuFeS_2$ is brassy-golden mineral with chemical composition of $CuFeS_2$ and FeS_2 that is widely used in Ayurveda & naturopathy to treat various disease conditions such as diabetes, skin diseases, anemia and cronic fever. The raw material needs to be processed by specified Ayurveda guidelines to make it more potent and therapeutically safe. Swarnamakshika needs to be processed by the following API guidelines in order to make it biologically safe and more potent. These processes include preliminary process of eliminating unwanted substances (Shodhana), Amritikarana & incineration (Marana) that are mandatory and play a crucial role in therapeutics. The spectroscopic characterization of prepared Swarnamakshika or $CuFeS_2$ nanoparticles was also done by using sophisticated instrumentation techniques such as X-Ray Diffraction (XRD), SEM, which represents that Swarnamakshika nanoparticles mainly consists of FeS_2, Fe_3O_4 and $CuFeS_2$ etc. The antimicrobial study indicates of Ayurvedic drug that prepared Swarnamakshika Bhasma was equally effective as the standard antimicrobial drug Streptomycin.

Keywords: Swarnamakshika; Bhasma; $CuFeS_2$; anemia; SEM; nanoparticles; XRD; Microbial studies.

Introduction

Ayurveda is a medicine system with historical roots in Indian subcontinent. The main and basic principle of Ayurveda is to prevent and treat illness and without having side effects rather than respond to indicators of disease. Rasashastra is drug production science of the Ayurveda known as Ayurvedic-pharmaceutics which explores with the processing of different metals and minerals by different methods and materials used. Swaranmakshik bhasma is an important formulation in ayurveda. Swarnamakshik is a mineral of maharasavarga having various uses in many diseases and has been use since long time in ayurveda. Many diseases like pandu (anemia), kustha (skin disease), apasmar (convulsions), anidra (Insomnia) etc. It is also a useful potent rasyana drug. Generally, this bhasma is prepared in two steps: shodhana and marana. This bhasma usually helpful as single constituent formulation or in multi integrated formulation[1-4]. The technical bhasma nanopaticle studies represents that the Swarnamakshika bhasma is manufactured according to the ancient process mentioned in the Ayurvedic texts. The synthesized swarnamakshik bhasma was characterized by ancient physical verification methods and by using other sophisticated techniques such as electron microscopy, GA-X-Ray diffraction and Antimicrobial techniques. Outcomes of this study could be used as standards for evaluating quality and reproducibility of the Swarnamakshika bhasma nanoparticles. Swarnamakshika is important part of the Maharasa groups of drugs according to ancient texts (Rasaratna Samuchaya)[5,6].

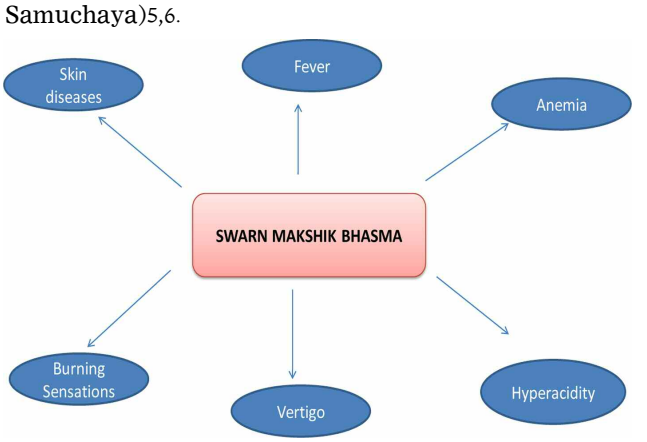

Fig. 1- Therapeutic benefits of Swarnmakshik Bhasma.

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The novelty of this experimental study to describes the low cost, low toxic and low particle distribution of Swarnamakshik. This is the important observations, to our knowledge, that has characterized surface passivated pyrite nanoparticles for Anemia, jaundice, children's fever. This study would definitely innovative in building confidence in use of such herbo mineral products for medication by batch manufacturing, efficacy, and safety.

Experimental Details

Materials

Raw Swarnamakshika was taken in dry and sterilized mechanical kharal and pounded well to prepare its fine powder. The powdered Raw material was kept in another well dried iron pan and subjected to pre observed temperature at between 750oC – 900oC. The subjected iron pan is then closed with an top iron cap to avoid loss of Swarnamakshika due to dusting. This purification process was continued for 32 hours after complete cessation of sulphur fumes and till the mixture become totally fire red. The fresh plant extract & purified Swarnamakshik mixed paste was then transferred to pan covered with a sealed lid for final bhasma product preparation.

Experimental Methods

The quality control & assurance of traditionally synthesized nanomedicine can be aimed only through Good Raw material purchase, regulatory control, good manufacturing practices (GMP), research process development and physicochemical characterization with reference to stable & authentic nano drug formulation. The nanotized and pure bhasma was analyzed using sophisticated instrumentation techniques such as X-ray diffractometry (XRD), Electron microscopy (i.e TEM or SEM) and antimicrobial or antibacterial activity.

Analytical Characterization

The X-ray diffraction (XRD) images were recorded through Bruker-D8 GA diffractometer mode at the angle of incidence of 0.2o. The X-ray spectra of Swarnamakshika bhasma were recorded in the 2θ range of 21o-80o with a step size of 0.01o for mixed phase's identification. In the continuation the morphological examination of the Swarnamakshika Bhasma an Ayurvedic drug was studied with the help of electron microscope instrument JSM-5600.

Antibacterial activity

The antibacterial activity of Swarnamakshika bhasma was performed by agar well diffusion method. The microbialculture plates were prepared by pouring 20ml sterilized Muller-Hinton Agar (MHA) into pre-sterilized petridishes. The inoculums suspension of all four pathogens (0.1ml) was spread equally over the agar or reagent medium using the sterile glass wires in plated. Wells in the agar plates were made by using sterile cork-borer. Different concentrations (125, 100, 75, 50 mg/ml) of Swarnamakshika bhasma were separately added into well plates. The plates were incubated at 38°C for 24 hours. The mean or average diameter of zone of inhibition (in mm) was recorded. The standard antibiotics drug streptomycin (10mcg) was used as positive control and blank MHA media plate was used as a control7.

Results and discussion

X- ray diffraction

The X- Ray diffraction pattern of Swarnamakshik bhasma or $CuFeS_2$ nanoparticles at final stage of its preparation is exhibited in Fig. 2. The presence of the acute diffraction peak shows the intensely crystalline nature of Swarnamakshik bhasma. The Swarnmakshik bhasma shows diffraction peak majorly at 2θ = 34.50o, 36.10, 41.36o, 49.16o, 54.32o, 62.5o and 64.58o in finally prepared Swarnamakshik bhasma (Fig. 2) which indicates the presence of $CuFeS_2$, Fe_3O_4, CuS_2, FeS_2& Cu_2O8. The traditionally synthesized Swarnamakshik bhasma also compared with the diffractogram of marketed Swarnamakshik bhasma sample which almost shows the similar crystalline geometry. The X-Ray diffraction pattern in final swarnamakshik bhasma shows that iron oxide (Fe_3O_4) is majorly present in the form of FeS_2, Fe_3O_4 and $CuFeS_2$ during heating in Bhasmikaran at high temperature (650oC) chalcopyrite & Iron oxide convert into its most suitable crystalline state. In this Swarnamakshik bhasma, $CuFeS_2$ is mixture of two states of Cu & Iron.9

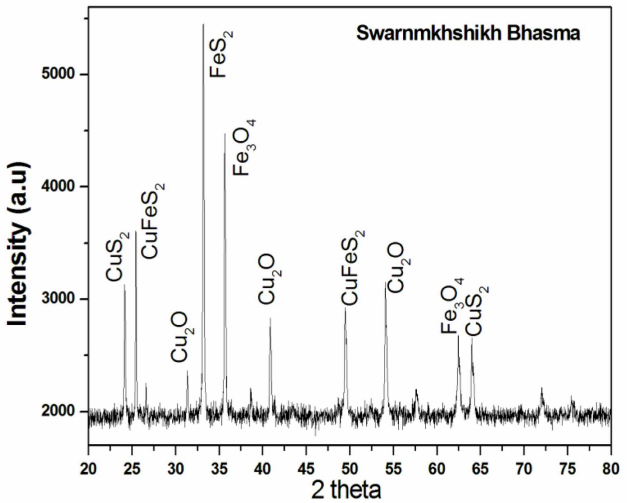

Fig. 2 X-Ray Diffraction image of Swarnmakshik bhasma.

Table 1 Summary of the Rietveld refinement analysis for the Swarnamakshik Bhasma (Chalcopyrite).

Sample	Lattic Const 'Å'	Space Group \ Phase	X-Ray peak profile function	Size Distribution function	Numerical fit residuals		Gaussian Variance 'Z'	GOF	χ^2	R_B	R_F	Part. size
					R_{wp}	R_{ex}						
Swarnamakshik	a=b= 5. 235 c= 10.428	(122) Tetragonal	Pseudo Voit	Gaussian	21.56	9.1	1.07	1.1	1.15	3.12		

Scanning electron microscopy

Scanning Electron Micrograph of Swarnamakshika Bhasma sample is shown in fig 3. The fig 3 represents outer surface morphology of Swarnamakshika bhasma sample at final stages of its nanocrystal preparation. The SEM study revealed regular, smooth, and uniform arrangement of cluster of granules in finally prepared nanoparticles of bhasma which was not observed in raw swarnamakshik or copper pyrite. It is clearly observed that the surface area was looking too smooth after visheshshodhan & other procedures. In the presence of different plant extract Swarnamakshik Bhasma formation, the particle size is small as revealed by SEM studies. The aggregation of the Swarnamakshik bhasma particle is due to kiln drying by high temperatures and the Swarnamakshik nanoparticles are bound by certain forces visible in SEM image. The surface energy of surface passivated Swarnamakshik Bhasma nanoparticles would be dominating the shape of the crystal and therefore the spherical crystallites are observed in closely packed system10.

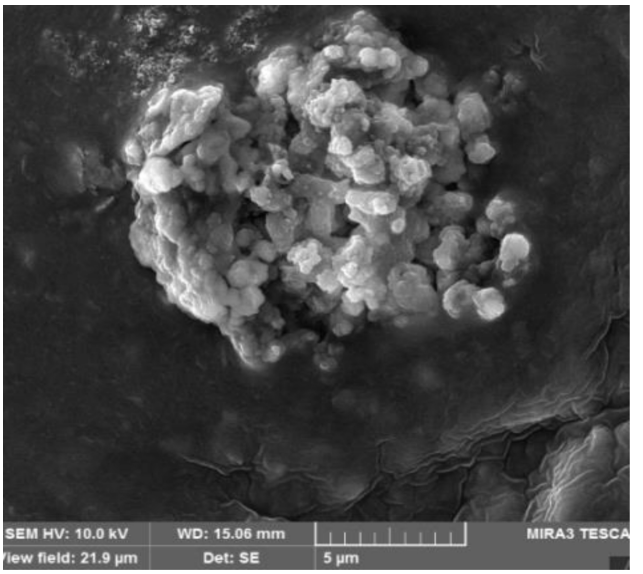

Fig.-3 Scanning Electron Microscopy image of Swarnmakshik bhasma.

Antimicrobial Activity of Swarnamakshik Bhasma The Antibacterial activity of SwarnamakshikBhasma was checked by agar well diffusion method on microorganism E-coli, Pseudomonas aeruginosa, Streptococcus pyogenes, and Salmonella typhi11. The Swarnammakshik bhasma was diluted to obtain the solution at the desired experimental concentration. DMSO-20% was used as diluents to get desired concentration of drugs to test upon different standard bacterial strains. Three solutions of 50 mg/ml, 75 mg/ml, 100 mg/ml and 125 mg/ml were prepared by mixing DMSO -20% and Swarnamakshika Bhasma. In this diffusion method, Mueller-Hinton agar media was prepared and the test micro-organisms were aseptically inoculated onto the surface of the media using a sterile swab.

The ayurvedic drug results were compared to the commercially available antibacterial drug Streptomycinin fig.4. The study indicates that Swarnamakshika Bhasma exhibited good antimicrobial activity against E-coli, Streptococcus pyogenes, Salmonella typhi, and Pseudomonas aeruginosa. At low concentration, Swarnamakshika Bhasma inhibited the growth of E-coli and Salmonella typhibut was not effective against Streptococcus pyogenes& Pseudomonas aeruginosa. In this study, the best combination of Aurvedic drug to inhibit the growth of bacteria was found to be a100mg/ml concentration of Swarnamakshika Bhasma. The observed data of the antimicrobial study of

nanocrystals indicate that prepared Swarnamakshika bhasma was equally effective as the standard antimicrobial drug Streptomycin12.

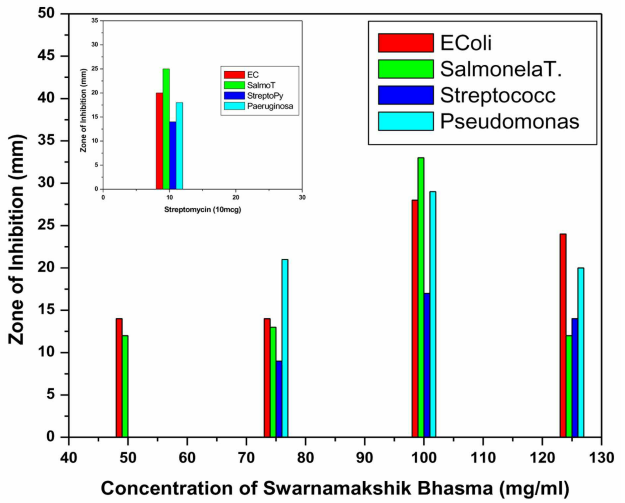

Fig.- 4Antimicrobial activity of Swarnmakshik bhasma.

Conclusion

The X-Ray differaction spectra of processed bhasma product shows that iron based nanocrystals is mainly present in form of FeS_2, $CuFeS_2$ and Fe_3O_4. The XRD & SEM-EDAX analysis indicates that elements other than iron are added or incorporated in the swarnamakhshik bhasma during various preocessing steps. The electron microgram (SEM) results clearly represents the change in morphological structure with down fall in particle size range for the final sm bhasma product. Its observed that the techniques employed for calcination and purification of Swarnmakhshik significantly reduces the size of particles in Swarnamakshik bhasma. Significant bioactivity & antimicrobial activity was observed in Swarnamakshik bhasma where the maximum zone of inhibition up to was observed which was better than streptomycin or standard drug. Due to the existence of Fe_3O_4, FeS_2& $CuFeS_2$ nanoparticles, further scientific exploration of Swarnamakshik Bhasma is necessary for the potential applications in the management of cancer and other diseases.

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