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PHYSICOCHEMICAL CHARACTERIZATION AND BIOLOGICAL ACTIVITY OF ABHRAK BHASMA: AN INVESTIGATION INTO ITS NATURE AS A CLASSICAL AYURVEDIC NANOMEDICINE

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ABSTRACT

The global demand for traditionally-rooted medical preparations, including Ayurvedic formulations, has witnessed a sustained upward trend. Abhrak bhasma, an Ayurvedic herbomineral preparation derived through iterative incineration of mica with decoctions of several medicinal plants, has long been employed in the management of conditions such as respiratory disorders, hemorrhagic diseases, urinary ailments, diabetes mellitus, anemia, dermatological complaints, and splenic dysfunctions.[12] In addition to these therapeutic applications, it is traditionally recognized for its rejuvenating and anti-infertility properties. However, scientific validation of such preparations in terms of their mechanism of action at the physiological and molecular level remains largely inadequate.3 The present investigation was designed to elucidate the probable chemical transformations that abhrak bhasma undergoes within the gastrointestinal (GI) tract. Spectrophotometric analysis conducted under simulated acidic and alkaline GI conditions demonstrated notable alterations in the absorbance profile, predominantly attributable to iron-containing compounds within the preparation. Infrared spectroscopy confirmed the dominance of metallic constituents with minimal organic or moisture moisture content. Assessment of antimicrobial properties revealed an absence of intrinsic antibacterial activity; however, partial inhibition of yeast cell proliferation was documented. Further mechanistic investigations are warranted to comprehensively characterize the bioactive constituents released during GI transit.

KEYWORDS: Ayurveda; Abhrak bhasma; Physicochemical modification; Gastrointestinal transformation; Nanomedicine; Antimicrobial activity.

1. INTRODUCTION

The utilization of traditional medicinal formulations in the Indian subcontinent spans millennia, and several of these preparations have demonstrated substantial clinical efficacy across a broad spectrum of ailments. This traditional medicine sector currently sustains a multibillion-dollar industry with continuous market expansion.[1] Bhasmas represent a unique category of Ayurvedic herbomineral preparations manufactured through the sequential processes of *Shodhana* (purification), *Bhavana* (levigation), and *Marana* (incineration), involving repeated calcination of metals or their mineral ores with herbal juices or decoctions.[2] A defining characteristic of these preparations is the nano-scale particle size of the final product, warranting their classification as classical nanomedicines.[2]

Abhrak bhasma (AB) is specifically prepared through the iterative incineration of muscovite mica with decoctions derived from approximately 72 plant species. Prior elemental analyses have identified iron (Fe), calcium (Ca), selenium (Se), magnesium (Mg), and potassium (K) as principal constituents, with a reported particle size distribution in the range of 29–88 nanometers.[3] The therapeutic grade of this preparation is contingent upon the number of incineration cycles (*puta*) performed; the *Sahastraputi* variant, which undergoes 1000 such cycles, is universally acknowledged as the highest quality formulation. Distinct grades of abhrak bhasma are indicated across a wide range of clinical conditions and constitute integral components of numerous *Rasayana* (rejuvenating) formulations.

Unlike allopathic pharmaceuticals, which are subject to rigorous preclinical and clinical evaluation prior to market authorization, traditional Ayurvedic preparations are rarely subjected to equivalent regulatory scrutiny.[4,5] Classical texts including *Charaka Samhita* and *Sushruta Samhita* provide detailed accounts of preparation methodology and therapeutic indications; however, they offer no insights into the molecular or physiological mechanisms underlying therapeutic action.[4,5]

Upon oral ingestion, any substance traverses multiple GI compartments characterized by distinct pH microenvironments.[6] The acidic milieu of the stomach constitutes the primary site of chemical transformation, followed by the progressively alkaline to neutral environment of the duodenum and distal intestinal segments. These pH-dependent transformations critically govern the bioavailability and pharmacological activity of orally administered substances. To date, no published data exist characterizing the GI-mediated chemical modifications of

abhrak bhasma. The present study was therefore conceived to investigate these probable transformations and to evaluate the antimicrobial potential of abhrak bhasma, with specific attention to its capacity to modulate the GI microenvironment.

2. MATERIALS AND METHODS

2.1 Procurement of Abhrak Bhasma

Sahastraputi abhrak bhasma was procured from Dhootapapeshwar Ltd., a recognized manufacturer of Ayurvedic pharmaceuticals (Batch No.: P150300110).

2.2 Spectrophotometric Analysis

Abhrak bhasma was suspended at concentrations of 3 mg/ml and 8 mg/ml in distilled water, varying molarities of HCl (0.01N, 0.1N, 0.5N, and 1N), and 1N NaOH. After vigorous mixing and equilibration for 30 minutes at ambient temperature, suspensions were centrifuged at 3000 rpm for 5 minutes. The resulting supernatants were carefully decanted and subjected to ultraviolet-visible spectrophotometric analysis using a BioTek EPOCH-Gen5 spectrophotometer across a wavelength range of 200–900 nm.

2.3 Infrared Spectroscopic Analysis

Fourier-transform infrared (FTIR) spectroscopy was performed using attenuated total reflectance (ATR) methodology with a BRUKER spectrometer. Direct powder of abhrak bhasma was applied onto a zinc selenide (ZnSe) crystal, and percent transmittance was recorded across the wavenumber range of 500–4000 cm^{-1}.

2.4 Evaluation of Antibacterial Activity

Antibacterial screening was performed using the disc diffusion method on LB-Agar plates, pre-verified for sterility at 37°C overnight. Four bacterial strains — *Escherichia coli*, *Staphylococcus* sp., *Micrococcus* sp., and *Bacillus subtilis* — were used as test organisms. Sterile filter paper discs (6 mm diameter) were loaded with 10 µl of each test solution in duplicate. Ampicillin (100 mg/ml) served as the positive control; solvent preparations without abhrak bhasma were used as negative controls. Plates were incubated overnight at 37°C and evaluated for zones of inhibition.

2.5 Evaluation of Antifungal Activity

Baker's yeast was used as a fungal model system. A stock suspension of 10 mg/ml was prepared in yeast growth medium (0.1% yeast extract + 1% dextrose). Separate experimental sets were prepared with: (i) 1 mg abhrak bhasma powder directly; (ii) acid-extracted abhrak bhasma solution; and (iii) acid solution alone. Untreated yeast culture served as the control.

Absorbance at 570 nm was measured at the start and after 20 hours of incubation at room temperature to calculate percent growth inhibition.

3. RESULTS AND DISCUSSION

3.1 pH-Dependent Spectrophotometric Modifications

Upon suspension in 1N HCl followed by centrifugation, the clear supernatant of abhrak bhasma demonstrated significantly elevated absorbance across the wavelength ranges of 220–280 nm and 300–400 nm, with a characteristic absorption peak at 330 nm. The amplitude of this peak exhibited a direct proportional relationship with both the concentration of abhrak bhasma and the molarity of hydrochloric acid employed, indicating concentration- and pH-dependent ionic release from the preparation.

Subsequent neutralization of the acidified solution with 1N NaOH resulted in a leftward spectral shift of the 330 nm peak toward shorter wavelengths, while the 220–280 nm absorbance range remained largely unaffected. To identify the specific constituent responsible for the observed spectral profile, individual salt solutions of the principal elemental components of abhrak bhasma — calcium (Ca), silicon (Si), potassium (K), phosphorus (P), and iron (Fe) — were similarly processed in 1N HCl.[3] Of these, only the iron salt generated an absorbance peak within the 300–400 nm range comparable to that of acidified abhrak bhasma, though with additional peaks in the 400–500 nm range absent in the bhasma preparation. Notably, no other tested element produced peaks in either the 220–280 nm or 300–400 nm ranges, thereby implicating iron-based compounds — potentially iron oxides — as the primary chromophoric constituents undergoing GI-simulated modifications.[7]

3.2 Infrared Spectroscopic Characterization

FTIR analysis of abhrak bhasma powder revealed an absence of absorption peaks in the 1200–4000 cm^{-1} region, confirming negligible moisture content and the absence of significant organic constituents. This is consistent with the thorough calcination-driven elimination of organic matter during the *Marana* process. Prominent absorption bands were identified at 968.82 and 683.78 cm^{-1}; however, definitive molecular assignment of these peaks was beyond the resolution of the current analytical approach. Overall, the ATR-FTIR profile confirmed a predominantly metallic composition, corroborating findings from earlier analytical characterizations of similar bhasma preparations.[89]

3.3 Antibacterial Evaluation

Direct application of abhrak bhasma suspended in distilled water failed to produce zones of inhibition against any of the four bacterial strains tested. When the acid-processed abhrak

bhasma extract was evaluated, bacterial growth suppression was observed; however, equivalent inhibition was also produced by the HCl control solution, precluding the attribution of antimicrobial activity specifically to abhrak bhasma components. The study thus establishes that abhrak bhasma does not intrinsically possess direct antibacterial properties under standard physiological conditions.

3.4 Antifungal Evaluation

In the control group, yeast cell density increased by over 200% over the 20-hour incubation period, confirming robust fungal proliferation. Direct addition of abhrak bhasma powder to the culture medium restricted cell density to 56% of the initial value, reflecting partial antifungal activity. The acid extract of abhrak bhasma demonstrated markedly superior efficacy, limiting yeast growth to only 4.9% of baseline density. The acid-only control permitted 10% growth, suggesting that while the acidic medium contributed to antifungal activity, a specific component within the acid-extracted abhrak bhasma conferred additional inhibitory effects.

3.5 Mechanistic Interpretation

The growing consumption of traditional medicinal preparations globally underscores the urgent need for rigorous scientific validation.[13] Abhrak bhasma has long been associated with benefits in conditions including respiratory disorders, diabetes, anemia, and reproductive debility.[7] The present findings suggest that upon ingestion, the iron-containing constituents of abhrak bhasma undergo progressive chemical transformation across the pH gradient of the GI tract — transitioning from soluble ionic forms in the acidic stomach to modified species in the neutral-to-alkaline intestinal environment. This pH-governed speciation may be central to the bioavailability and downstream pharmacological activity of the preparation.[6]

The demonstrated antifungal activity of abhrak bhasma suggests a capacity to modulate GI fungal ecology, potentially reducing pathological fermentation and putrefaction of intestinal contents. Prior studies have established that nano-scale iron oxides — similar to those likely present in abhrak bhasma — possess heavy metal chelating capacity, which may constitute a significant mechanism of biological action.[10] Furthermore, the nano-scale dimensions of abhrak bhasma constituents facilitate mucosal absorption and potential traversal of blood-tissue barriers, providing a pharmacokinetic basis for its systemic therapeutic effects.[13] The broader GI microbiota is additionally subject to modulation by the chemical environment created by abhrak bhasma, with potential implications for overall GI homeostasis and systemic physiological balance.[11,12,14]

4. CONCLUSION

The present investigation provides mechanistic insights into the behavior of abhrak bhasma within the GI tract, identifying iron-based compounds as probable key effectors undergoing pH-dependent chemical transformations. The preparation demonstrated selective antifungal activity while lacking intrinsic antibacterial properties. These findings support the hypothesis that abhrak bhasma exerts its therapeutic effects through GI-mediated chemical transformation, modulation of fungal microbiota, and systemic distribution of bioavailable nano-scale constituents.[27] Further investigations employing advanced analytical approaches — including inductively coupled plasma mass spectrometry (ICP-MS), X-ray diffraction (XRD), and in vivo bioavailability modeling — are recommended to fully characterize the active constituents and mechanistic pathways of this classical Ayurvedic preparation.

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