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Research Article

An exhaustive investigation of Physicochemical and Antimicrobial activities of Vedi Annabedhi Chendhooram

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ABSTRACT

In India, the Siddha system of medicine, predominantly followed by the Tamil people, confines a diverse range of formulations, categorized into *Agamarunthu* (Internal medicines) and *Puramarunthu* (External medicines), derived from plant, metal-mineral and animal sources. Especially, the Siddha system places significant emphasis on higher order medicines originating from metals and minerals.*Vedi Annabedhi Chendhoora*m (VABC) is a traditional herbo-mineral formulation utilized for addressing a spectrum of health conditions, such as jaundice, anemia, dysentery, fever, dropsy, ascites and generalized anasarca. To elucidate the Physicochemical and antimicrobial activity of VABC. The Physicochemical analysis of VABC was performed according to Pharmacopoeial Laboratory for Indian Medicine (PLIM) Guideline for standardization and evaluation of Indian medicine (AYUSH systems). Antimicrobial activity of VABC performed by Determination of Minimum inhibitory concentration (MIC) using Resazurin MicrotitreAssay. The results of physicochemical analysis of VABC were done such as Loss on drying at 105^{o}C was 0.48±0.045%, Total ash was 87.1 ± 2.12, acid insoluble ash was 2.76±0.60%, alcohol soluble extractive was 2.53 ±0.65, water soluble extractive was 5.03 ± 1.18 and it was alkaline in nature with pH of 8.2. The results of antimicrobial assay revealed VABC exhibits notable antimicrobial activity against *Klebsiella pneumonoiae* and *Escherichia coli*, demonstrating a substantial effect. Additionally, it exerts a moderate inhibitory effect against *Pseudomonas aeruginosa* and *Staphylococcus aureus*. This preliminary study encompassing physicochemical analysis and assessment of the antimicrobial activity of VABC, serves as a crucial foundation for meeting the criteria necessary for subsequent preclinical and clinical research.

Keywords: Antimicrobial activity, Siddha, Physicochemical analysis, *Vedi Annabedhi Chendhoora*m.

1. INTRODUCTION

The Siddha system of medicine, predominantly followed by the Tamil people in India, encompasses a diverse range of formulations. These are categorized into *Agamarunthu* (Internal medicines) and *Puramarunthu* (External medicines), derived from plant, metal-mineral and animal sources. Notably, the Siddha system places significant emphasis on higher order medicines originating from metals and minerals. Two significant examples are *Annabedhi* and *Vediuppu*. *Chendhooram* is a specific internal medicine that involves the transformation of metallic substances, particularly arsenical compounds. This process results in the creation of fascinating red powders through methods such as burning, frying, incineration, insulating or grinding them with decoctions, juices, *seyaneers* etc.

Vedi Annabedhi Chendhooram (VABC) is a traditional herbo-mineral formulation utilized for addressing a spectrum of health conditions. These conditions include jaundice, anemia, dysentery, fever, dropsy, ascites and generalized anasarca. VABC shows the integration of traditional knowledge and practices in Siddha medicine for holistic healthcare. The existing demand for Siddha formulations has surged worldwide, driven by an increased response to the Siddha system of medicine. Consequently, essential factors such as safety, standardization and quality control have become paramount requirements for Siddha formulations. The test drug *Vedi Annabedhi Chendhooram* (VABC) was meticulously prepared at the Gunapadam (Pharmacology) lab of Sri Sairam Siddha Medical College in Chennai, adhering to the traditional method of incineration as outlined in classical text [1].

Following the preparation, a thorough physicochemical analysis was conducted to investigate the composition, structure, and properties of VABC. This analysis was including parameters such as particle size, solubility, pH, and elemental composition, providing valuable insights into the formulation's characteristics. Subsequently, we explored the antimicrobial activity of VABC against a range of microorganisms. This investigation was contributed to understanding the potential therapeutic applications of VABC in addressing microbial infections.

2. MATERIALS AND METHODS

The formulation of *Vedi Annabedhi Chendhooram* (VABC) was carried out at the Gunapadam laboratory, located within Sri Sairam Siddha Medical College, Chennai, Tamil Nadu. The specific methods employed in the preparation process were derived from the book Siddha Vaithiya Pathartha Guna Vilakkam (Thathu-Jeeva Varkam), authored by Dr. C. Kannusami Pillai. The relevant details can be found on page no.43.

2.1. Preparation method of VABC:

The first two ingredients of VABC, Annabedhi and Vediuppu, were meticulously purified [2] individually. They were then combined with a sufficient quantity of lemon juice, which serves as the third ingredient of VABC, and thoroughly ground to achieve a waxy consistency. The ingredients of VABC are listed in Table1. Then the waxy content shaped into small circular cakes and allowed to undergo a drying process. Following this, the prepared cakes were placed within a mud vessel, covered with another mud vessel, and enveloped in seven layers of cloth cover known as "*Seelaiman*", ensuring a protective environment during the drying process.

To enhance the traditional method, the final step involved calcination, where the prepared mixture, encased in the mud vessels and cloth covers, was subjected to heat generated by burning 70 cow dung cakes. This careful and controlled process aimed to bring about specific transformative changes in the composition. Four *pudams* of the prepared material were subjected to this procedure. Finally, the resulting product was finely powdered, preserving the essence of the medicinal formulation.

2.2. Standardization Methods of Vedi Annabedhi Chendhooram:

2.2.1. Organoleptic Characters: [3]

The analytical specifications for Chendhooram were conducted in accordance with the protocol provided by the Pharmacopoeial Laboratory for Indian Medicine (PLIM), under the Department of AYUSH, Ministry of Health and Family Welfare, New Delhi. Following PLIM guidelines, the assessment of Analytical Parameter Characteristics (VABC) included the evaluation of organoleptic characters such color, odor, appearance, taste and solubility. [4, 5]

2.2.1.1.Accordance with Siddha aspect [6]

2.2.1.1.1. Color

Obviously *Chendhooram* is in dark red color. *Vedi Annabedhi Chendhooram* was also in the same color. It manifests the ideal hue of *Chendhooram*.

2.2.1.1.2. Taste

In adherence to Siddha principles, a meticulously prepared Chendhooram should be entirely devoid of taste. The presence of any taste in Chendhooram suggests a suboptimal preparation. A minute quantity of Chendhooram, placed on the tip of the tongue, should be tasteless, causing only mild irritation owing to its alkaline nature. The ultimate product, *Vedi Annabedhi Chendhooram*, underwent analysis following Siddha classical standardization methods. The findings indicated its suitability for further examination.

2.2.1.1.3. Finger print test

A small quantity of *Vedi Annabedhi Chendhooram*was taken and gently rubbed between the thumb and index finger (Figure 2). It permeated into the lines of the fingers, affirming the fine texture of *Chendhooram*.

2.2.1.1.4. Floating on Water test

A little quantity of VABC was delicately dispersed over the water within a glass bowel. Interestingly, the Chendhooram particles exhibited a unique characteristic by refraining from sinking into the water, instead,

they gracefully floated on the water's surface (Figure3). This phenomenon serves as a tangible indication of the remarkable lightness possessed by *Vedi Annabedhi Chendhooram*.

2.2.1.1.5. Luster test

The presence of any luminous particles within the *Chendhooram* signifies an inadequately prepared drug. To assess the quality of VABC, a sample was placed in a petri dish and scrutinized for any gleaming attributes under daylight using a magnifying glass. Importantly, no luster or shine was discerned in the *Chendhooram,* indicating a meticulous and proper preparation manner.

2.2.1.2. As per Modern technique[7]

2.2.1.2.1. Percentage of Loss on Drying

1g of VABC was accurately weighed in a tarred glass bottle. The sample was heated at 105°C for 6 hours in an oven till a constant weight. The Percentage moisture content of the sample was calculated with reference to the shade dried material.

Percentage loss in drying = Loss of weight of sample/ Wt. of the sample X 100

2.2.1.2.2. Determination of Total Ash

Weighed accurately 2g of VABC formulation was added in crucible at a temperature 600°C in a muffle furnace till carbon free ash was obtained. Percentage of total ash will be calculated with reference to the weight of air-dried drug.

Total Ash = Weight of Ash/ Wt. of the Crude drug taken X 100

2.2.1.2.3. Determination of Acid Insoluble Ash

Ash above obtained, was boiled for 5min with 25ml of 1M Hydrochloric acid and filtered using an ash less filter paper. Insoluble matter retained on filter paper was washed with hot water and filter paper was burnt to a constant weight in a muffle furnace. The percentage of acid insoluble ash was calculated with reference to the air-dried drug.

Acid insoluble Ash = Weight of Ash/Wt. of the Crude drug taken X 100

2.2.1.2.4. Determination of water-soluble ash

Total ash 1g was boiled for 5min with 25ml water and insoluble matter collected on an ash less filter paper was washed with hot water and ignited for 15 min at a temperature not exceeding 450°C in a muffle furnace. The amount of soluble ash is determined by drying the filtrate.

Water Soluble Ash = Weight of Ash/Wt. of the Crude drug taken X 100

2.2.1.2.5. Determination of Water-Soluble Extractive

5gm of air-dried drug, coarsely powered VABC was macerated with 100ml of distilled water in a closed flask for twenty-four hours, shaking frequently. The Solution was filtered and 25 ml of filtrated was evaporated in a tarred flat bottom shallow dish, further dried at 100°C and weighted. The percentage of water-soluble extractive was calculated with reference to the air-dried drugs.

Water soluble extract = Weight of Extract/ Wt. of the Sample taken X 100

2.2.1.2.6. Determination of Alcohol Soluble Extractive

1gm of air-dried drug coarsely powdered VABC was macerated with 20 ml alcohol in closed flask for 24 hrs. With frequent shaking, it was filtered rapidly taking precaution against loss of alcohol. 10ml of filtrate was then evaporated in a tarred flat bottom shallow dish, dried at 100°C and weighed. The percentage of alcohol soluble extractive was calculated with reference to air dried drug.

Alcohol soluble extract = Weight of Extract/ Wt. of the Sample taken X 100

2.2.1.2.7. pH determination

Required quantity of VABC was admixed with distilled water and the subjected to screening using pH meter. The pH of VABC was 8.2. Physiochemical examination of the VABC sample, with values representing the average of three determinations, presented as the mean ± standard error of the mean (SEM).

2.3. Anti-Microbial Assay [8]

Test was carried out in a 96 well Plates under aseptic conditions. A sterile 96 well plate was labeled. Volume of sample in DMSO comprises of 1000µg was pipetted into the first well of the plate and transferred to subsequent wells by half of its weight until 8th Well. To all other wells 50µl of nutrient broth was added and serially diluted it. To each well 10µl of resazurin indicator solution was added. 10µl of bacterial suspension was added to each well. Each plate was wrapped loosely with cling film to ensure that bacteria did not become dehydrated. The plate was incubated at 37 °C for 24-48 hr. The color change was then assessed visually. Any color changes from purple to pink or colorless were recorded as positive. The lowest concentration at which color change occurred

was taken as the MIC value (Figure 4). Standard drug Chloramphenicol (10µg) was used as a positive reference standard to determine the sensitivity of the bacterial species tested.

3. RESULTS

3.1. Physicochemical Evaluation of VABC

The physicochemical assessment of the VABC drug yielded the following results: a loss on drying value of 0.48 ± 0.045%, a total ash value of 87.1 ± 2.12%, with acid insoluble ash at 2.76±0.60%. The alcohol soluble extractive value was determined to be 2.53 ± 0.65%, while the water-soluble extractive value was found to be 5.03 ± 1.18%. These findings have been organized in Table 4 for reference.

3.2. Anti-microbial profiling of VABC

It was observed from the results of the present investigation that the sample reveals convincing anti-microbial activity among all tested organisms. It was observed that the sample reveals significant activity against the pathogens such as *Klebshiella Pneumonia* (MIC 250µg) and *Escherichia coli* (MIC 250µg), Moderate activity against the pathogens *Psedumonas aeruginosa* (MIC500µg) and *Staphylococcus aureus* (MIC 500µg). The least activity was observed against *Bacillus subtilis* and *Salmonella typhi* with MIC value of 1000µg.

4. DISCUSSION

During the organoleptic evaluation of *Vedi Annabedhi Chendhooram* (VABC), presented a deep red color without any discernible odor or taste. The tactile examination highlighted qualities such as smoothness, softness and fineness. Siddha classical analytical parameters held significant importance in the assessment. The sprinkle test performed on the water's surface demonstrated the weightlessness of VABC, while the furrow filling test showcased the micro-fineness of the drug as it permeated the creases of fingers. Notably, the total ash value of VABC was determined to be 87.1±2.1%, indicating a lower presence of organic matter in the formulation [9] and a high amount of minerals present in the sample. This denotes purification and preparation process underscore the pivotal role, it plays in augmenting the concentration of minerals and certain organic compounds within the drug. The observed low value of acid-insoluble ash signifies that the preparation process excluded any siliceous matter, suggesting meticulous procedures were employed to maintain the purity and quality of the final product. The Loss on Drying at 105°C revealed minimal moisture content of only 0.48±0.045%, a crucial factor preventing a reduction in efficacy and averting degeneration. Consequently, the shelf life for *Chendhooram* type medicines, as per Siddha literature, has been extended up to 75 years, attesting to their enduring potency and stability.

Within physicochemical analysis, the pH value acts as a pivotal indicator of the formulation's alkalinity or acidity, significantly influencing drug absorption. The measured pH of *Vedi Annabedhi Chendhooram* (VABC) stood at 8.2, characterizing it as a weak base. Weak bases, owing to their limited ionization in a basic medium, exhibit enhanced absorption in the intestine, particularly with the pH range of 7.50 to 8. This alkaline nature strongly implies efficient and rapid absorption within the intestinal environment.[10]

An elevated ash value in a drug may signal potential concerns, including contamination, substitution adulteration or lapses in the preparation process. It is noteworthy that the acid-insoluble ash in *Vedi Annabedhi Chendhooram* (VABC) was found to be 0%, indicating that the drug underwent preparation without external contamination that could jeopardize absorption [11]. These findings emphasize the critical significance of upholding stringent quality standards in the formulation of Siddha medicines.

The results from the antimicrobial assay revealed that the *Vedi Annabedhi Chendhooram* (VABC) exhibits notable antimicrobial activity, particularly against *Klebsiella pneumoniae* and *Escherichia coli*. Additionally, a moderate effect was observed against *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

5. CONCLUSION

In this study, the preparation and analysis of *Vedi Annabedhi Chendhooram* (VABC) adhered to standard procedures, establishing this report as a reliable reference for future standardization of VABC. The powder properties of the samples exhibited favorable characteristics for absorption and flow ability. Organoleptic evaluations indicated that the purification and preparation processes were conducted under hygienic conditions, with the samples displaying an alkaline nature.

The data obtained from the present investigation clearly indicates that the formulation VABC not only complies with regulatory standards but also demonstrates significant antimicrobial activity against the tested microbes. These findings underscore the quality, purity and efficacy of VABC, providing a pathway for researchers to explore further research and potential therapeutic applications.

Conflict of interest

We declare that we have no conflict of interest.

Authors contributions

S. Chithra conducted the analysis and structured the framework, preparing the initial draft of the manuscript. N. Kabilan contributed valuable insights, refined, and corrected the manuscript. S. Visweswaran meticulously edited and finalized the review. All authors thoroughly reviewed the final manuscript.

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Table:1 Ingredients of VABC

Tamil Name	English Name/ Chemical Name	Nature of the Drug	Images	Quantity
Annabedhi	Green virtriol / Ferrous Sulphate	Inorganic Salt		35gm
Vediuppu	Saltpeter / Potassium Nitrate	Naturally occurring mineral source of Nitrogen		7gm
Elumitchai	Lemon / Citrus Limon	Plant-Fruit		Sufficient quantity

Table: 2 Confirmatory Specification for Chendhooram

S. No	Parameter	Observation for VABC
1.	Finger print test	**Confirms the fineness of the sample.**
2.	Float on Water	**Floats on the water surface, confirms the lightness of VABC**
3.	Tasteless	**Tasteless quality ensures the fulfillment of incineration process.**
4.	Luster	**Lusterless Confirms the quality of the C*hendhooram*.**
5.	Appearance	**Dark red colour signifies proficient incineration technique.**

Table: 3 Solubility Profile

S.No	Solvent Used	Solubility / Dispensability
1	Chloroform	Insoluble
2	Ethanol	Soluble
3	Water	Soluble
4	Ethyl acetate	Insoluble
	DMSO	Soluble

Table: 4 Physicochemical analysis

S.No	Parameter	Mean (n=3)SD
1	Loss on Drying at 105 °C (%)	0.48 ± 0.045
2	Total Ash (%)	87.1 ± 2.12

3	Acid insoluble Ash (%)	2.76 ±0.60%.
4	Alcohol Soluble Extractive (%)	2.53 ± 0.65
5	Water soluble Extractive (%)	5.03 ± 1.18

Table: 5 Report on MIC (Minimum inhibitory concentration) value of the Sample – VABC

S.No	Name of the Organism	MIC Value (µg)
1	*Bacillus subtilis*	1000 µg
2	*Klebshiella pneumonia*	250 µg
3	*Escherichia coli*	250 µg
4	*Pseudomonas aeruginosa*	500 µg
5	*Salmonella typhi*	1000 µg
6	*Staphylococcus aureus*	500 µg

Figure 1 Prepartion process of Vedi Annabedhi Chendhooram.

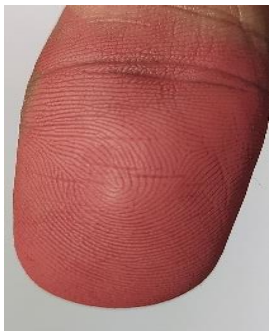

Figure 2
Fineness – Finger ridge deposit Analysis

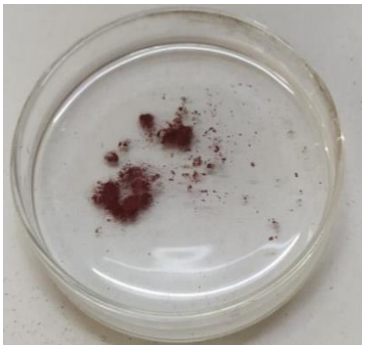

Figure 3
Float on Water Test

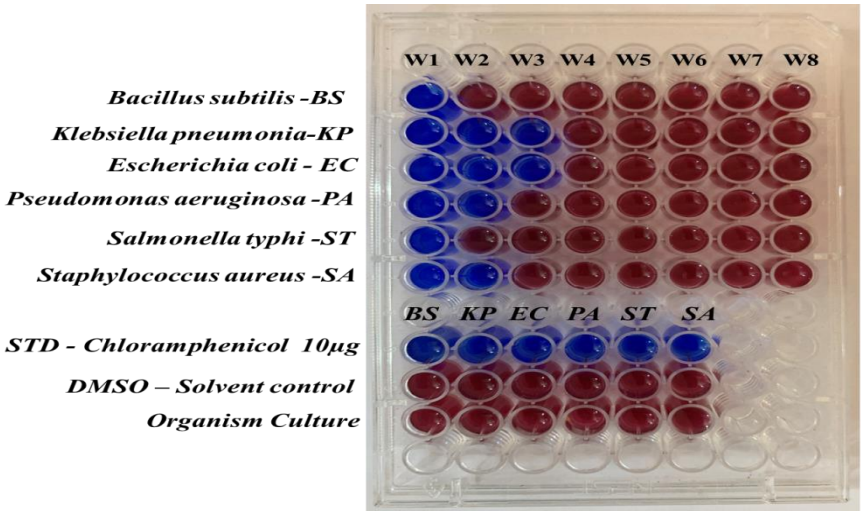

Figure 4 96 Well plate- Anti-Microbial Spectrum Image of Test Drug

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