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Evaluation of Microbial Contaminants and Aflatoxin Level in the Siddha Medicine Vengara Chunnam

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ABSTRACT:

Background: Vengara Chunnam is a mineral-based Siddha formulation known for its therapeutic applications in treating various urinary disorders, including renal calculi, anuria (absence of urine), oliguria (scanty micturition), albuminuria, and urethral strictures. Given its medical use, it is essential to assess the safety and quality of this formulation prior to its clinical application.

Aim: This study aims to evaluate the levels of microbial contaminants and aflatoxins present in the Siddha formulation Vengara Chunnam.

Methodology: The assessment of microbial contaminants and aflatoxin levels was conducted in accordance with WHO guidelines. The results were systematically recorded and analyzed.

Result: The findings revealed a total bacterial count of 2 × 10 cfu/g and a total fungal count of less than 3 cfu/g. Notably, Vengara Chunnam was found to be free from specific pathogens, including Salmonella spp., Enterobacteriaceae, Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa. The total aflatoxin level detected was 1 ppb.

Conclusion: The results indicate that Vengara Chunnam possesses low levels of microbial contaminants and aflatoxins, supporting its safety for clinical use.

Keywords: Microbial contaminants, Aflatoxin, Vengaram, Chunnam, Siddha

Introduction:

Traditional medicine is widely used to prevent and treat many diseases, as well as to boost energy and improve immunity [1]. Many people in developing countries continue to depend on traditional medicines for their primary healthcare [2]. Vengara chunnam comes under the Siddha system of medicine. Chunnam is a powdered form of medicine which can remain effectice for more than 500 yrs.It was found in the Siddha literature Noigaluku siddha parigaram part II.It is given for the management and treatment of urinary disorders like Renal Calculi[3].In this study, Vengara Chunnam was screened for Microbial contamination and assessment of aflatoxin level.As these can affect the safety and quality of the drug. Microbial load determination is the process of measuring the total number of microorganisms, primarily bacteria and fungi, in a sample.The results are often expressed in units like colony-forming units(CFUs) per unit of the sample. It ensures that the trial drug do not contain harmful levels of microbes. The Microbial contamination in medicine poses significant health risks to humans. A microbial load exceeding WHO norms can be harmful, as these microbes may produce toxic substances, which can threaten human health rather than aid in disease treatment. The presence of microbial contaminants in non-sterile pharmaceutical products can reduce or even inactivate their therapeutic effectiveness. Additionally, these contaminants may adversely affect patients taking the medication[2].Aflatoxins are a group of toxic substances that can cause cancer and mutations. They are generated by specific fungi, particularly species like Aspergillus flavus and Aspergillus parasiticus. As stated by the USDA (United States Department of Agriculture), these toxins are likely the most recognized and well-studied mycotoxins globally.[3].During 1960 in England Aflatoxin is identified as a causative agent of the mysterious Turkey X disease that causes excessive mortality in turkey poults[5].There are about 18 types of Aflatoxins identified so far in which Aflatoxin B1 is considered as most toxic.Exposure to high levels of aflatoxin can lead to acute hepatic necrosis, known as acute aflatoxicosis. This condition may result in long-term complications such as cirrhosis or liver cancer. Symptoms of acute liver failure include bleeding, fluid retention, digestive issues, changes in nutrient absorption and metabolism, as well as alterations in mental status, which can progress to coma[6].Chronic subclinical exposure does not produce symptoms as severe as acute aflatoxicosis. However, chronic exposure increases the risk of developing liver and gallbladder cancers, as aflatoxin metabolites may intercalate into DNA and alkylate the bases through their epoxide moiety[7].This process is believed to lead to mutations in the p53 gene, which plays a crucial role in halting cell cycle progression when DNA mutations are present and signaling apoptosis (programmed cell death). Certain locations within the gene appear to be more affected by these mutations than others. For instance, the third base of codon 249 in the p53 gene is

particularly susceptible to mutations induced by aflatoxin, compared to nearby bases[8].Aflatoxin B1, like other DNA-alkylating agents, can lead to immune suppression, and exposure to it is linked to increased viral loads in individuals who are HIV positive[9].

Aim:

The objective of this study is to assess the microbial contamination and aflatoxin level in the Siddha formulation Vengara Chunnam. This evaluation was conducted in accordance with the guidelines set forth by AYUSH and WHO to ensure that the drug is both safe and effective for use.

Methodology:

The determination of microbial load and assessment of aflatoxin levels were conducted at the Regional Research Institute of Unani Medicine (RRIUM). This was carried out in accordance with the standard testing procedures outlined in the "Protocol for Testing Ayurvedic, Siddha, and Unani Medicines," issued by the Government of India, Department of AYUSH, Ministry of Health and Family Welfare, and the Pharmacopeial Laboratory for Indian Medicine.

1.Drug authentication

The essential raw materials for the drug were sourced from a reputable supplier. The key ingredients included Vengaram (Borax) and the latex from the Brahma Thandu plant (Argemone Mexicana). The components of the drug were verified by botanists and the department.of Gunapadam, Government Siddha Medical College, Arumbakkam, Chennai-106.

2.Sample preparation

Vengaram, was taken as one large piece weighing 10 palams or in smaller bits, should be placed in a jam jar or a porcelain/glass jar. It is then coated with brahma thandu paal around one thread thickness all around the Vengaram for five consecutive mornings. After the coating is applied, it should be exposed to direct sunlight for approximately ten days, or until the coating is completely dry and can be easily peeled off. Finally,Vengaram is powdered and stored.

3.1. Microbial Load Determination:

The microbial quality, including the isolation and identification of pathogenic bacteria from commercial and homemade herbal medicines, was tested according to the regulations of the Unani Pharmacopoeia (2016) and WHO standards (2007). The tests were used to quantify the number of bacteria and fungi isolated that are able to grow aerobically in 1 g of sample. The sample were homogenized by mixing vigorously with water. One gram of sample was transferred to 9 mL of peptone broth. Then, serial dilutions were made to achieve an appropriate concentration. All microbial analyses were carried out in triplicate. Briefly, serial dilutions were made, and viability was assessed using the pour plate method on Casein soyabean digest agar and Sabouraud dextrose agar for bacterial counts and fungal identification, respectively. All dehydrated media were prepared according to the manufacturer's instructions and seeded and incubated at 37 °C for 24 to 48 hours for bacterial screening and at 25 °C for 48 to 72 hours for fungal screening. At the end of the incubation period, the number of colony-forming units per gram (CFU/g) was calculated by multiplying the average number of colonies by the dilution factor. The obtained CFU/g of sample was compared with WHO standards. Samples that presented bacterial growth greater than 105 CFU in 1 g of herbal medicine were considered unsatisfactory or inadequate according to WHO guidelines for aerobic bacteria.

3.2.Identifiation of Bacteria:

For bacterial isolation and identification, the samples were diluted in water or Tween 80, according to the solubility and homogenized by vigorously mixing. The 1-mL aliquots were transferred to 9 mL of peptone broth and cultured at the recommended time and temperature. All microbial analyses were carried out in triplicate. For investigating Escherichia coli, Salmonella spp., Pseudomonas aeruginosa and Staphylococcus aureus EMB agar, MacConkey agar, Deoxycholate citrate agar, Cetrimide agar and Mannitol salt agar culture media were used, respectively. At the end of the incubation period, pathogenic bacterial isolates were preliminarily characterized by colony morphology, Gram staining, and biochemical tests (oxidase, gas, and catalase production).

4.Aflatoxin test using afla-test fluorometer:

Aflatoxins are a group of naturally occurring toxins produced by Aspergillus flavus and Aspergillus parasiticus, two common mold species. AflaTest is a quantitative method for the detection of aflatoxin in B1, B2, G1, G2, M1, and M2. One gram of Vengara chunnam and 0.4 g of sodium chloride was mixed with methanol: 2% Tween 20 or phosphate buffer (60:40 v/v). Vortex the mixture of extract on high speed 3 minutes. Filter the extract through fluted filter paper. Add 10 ml of filtered extract in measuring cylinder, in that 20 mL purified water was added and vortex on high for 1 minute. Then filter the diluted extract through a pre-wet glass microfiber filter (1.5µm). Pass 10 mL of diluted extract through AflaTest WB column. Apply pressure to get 1-2 drops per second. Wash the column with 10 mL 2% Tween 20. Wash column with 10 mL purified water twice. Elute AflaTest WB columns by passing 1 mL HPLC-grade methanol (100%) through column, apply pressure to get 1 drop per second. Collect eluate in sterile VICAM

cuvette. In that add 1.0 mL of AflaTest Developer and mix well, then immediately place in fluorometer (VICAM fluorometer-sereies 4EX). Fluorometer will read concentration after 60 seconds.

Results:

The results of this study demonstrated that microbial load as listed in Table 1 and aflatoxin level as debicted in Table 2.

Table 1: Microbial Load Determination

S.NO	Parameters	Results
1	Total Bacterial Count (TBC)	2×103 cfu/g
2	Total Fungal Count(TFC)	Less than 3 cfu/g
3	Enterobacteriacea	Absent
4	Escherichia coli	Absent
5	Salmonella Spp	Absent
6	Staphylococcus aureus	Absent
7	Pseudomonas aeruginosa	Absent

Table 2: Aflatoxin Level assessment:

S.NO	Parameters	Method/Reference	Results
1	Total Aflatoxin B1B2G1G2	Vicam Aflatest Fluorometer	1 ppb

Discussion:

The study sample Vengara chunnam was prepared and the microbial load and aflatoxin level were assessed.The results were observed and tabulated above.According to the determination of microbial load, the Total Bacterial Count(TBC) found in Vengara chunnam was 2×103 cfu/g .The Total Fungal Count(TFC) was less than 3 cfu/g.The bacteria like Enterobacteriaceae,Escherichia coli,Salmonella spp,Staphylococcus aureus,Pseudomonas aeruginosa were absent. The Aflatoxin level was assessed by Aflatest fluorometer.It reveals the total aflatoxin level B1B2G1G2 are present in 1 ppb.Safety studies of Siddha drugs provided a scientific justification for their traditional use, and proved that they are safe and efficacious. The presence of microbial contamination in a drug beyond permissible limits can lead to serious side effects affecting the brain, kidneys, developing fetus, vascular system, and immune system[11]. Similarly, aflatoxins pose a significant threat to human health due to their association with serious adverse effects, including hepatotoxicity, carcinogenicity, and immune suppression. As a result, the World Health Organization (WHO) has established permissible limits for their concentration in certain drugs. While the complete absence of these contaminants is ideal, if they are detected, their concentration must remain within the established limits; exceeding these limits will prohibit the drug's use in disease management.Standardizing Siddha medicine through scientifically validated techniques may help build confidence in its therapeutic potential and enhance its global acceptance.

Conclusion:

The study focused on two key parameters: microbial load determination and aflatoxin level assessment, to evaluate the safety and toxicity of Vengara Chunnam. The results indicated that the microbial counts (bacteria, yeast, and mold) were below permissible limits, suggesting that they do not pose any toxicity risk. Aflatoxins (B1, B2, G1, and G2) are known to cause severe side effects, including hepatotoxicity and carcinogenicity. The absence of these harmful substances in the test drug confirmed that it is safe and free from significant toxic effects. Additionally, Vengara Chunnam did not show any pesticide residue contamination. Overall, the findings demonstrated that all safety parameters assessed for Vengara Chunnam fell within acceptable limits, indicating that the drug can be used safely.

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