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EVALUATION OF ANTIMICROBIAL ACTIVITY AND MICROBIAL LOAD OF THE POLYHERBAL SIDDHA FORMULATION SANGKAARAVAIRAVAN

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Abstract

Introduction: Sangkaara Vairavan (SV) is a polyherbal Siddha formulation composed of nine ingredients. Herbal medicines are often susceptible to microbial contamination, which can compromise their therapeutic benefits or even pose toxic effects.

Aim: To scientifically assess the efficacy of Sangkaara Vairavan (SV) by evaluating its microbial contamination and antimicrobial properties.

Study design

Place of study: The microbial load and anti-microbial activity were examined at the Regional Research Institute of Unani Medicine in Chennai, which is accredited by NABH and NABL.

Methodology: Sangkaara Vairavan was prepared traditionally according to the Standard Operating Procedure and formed into 130mg pills using ginger juice concentrate. Microbial load assessment was conducted using pour plate method following WHO standards with serial dilutions on selective media. Specific pathogenic bacteria were identified using biochemical tests. Antimicrobial activity was evaluated using agar well diffusion method against five clinical isolates. Hydroalcoholic extracts (50-150 mg/ml) were tested with standard antibiotic controls, measuring inhibition zones.

Results: No contamination detected: Enterobacteriaceae, E. coli, Salmonella spp., S. aureus, and P. aeruginosa were absent. Total bacterial and fungal counts remained within permissible limits. SV demonstrated notable antimicrobial activity across all tested strains, producing clear inhibitory zones that compared favourably to those of Ampicillin, Norfloxacin, and Amphotericin.

Conclusion:SV is both microbiologically safe (free from major pathogens and within limits for overall microbial load) and exhibits broad-spectrum antimicrobial efficacy. These findings support its therapeutic potential and warrant deeper pharmacological investigation.

Keywords: Antimicrobial activity, Microbial load, Sangkaaravairavan, Siddha formulation.

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INTRODUCTION

The Siddha medical system, which has a long tradition in ancient India is a unique and everlasting gift to society that has provided radiant health for many years. The siddha philosophy helps us understand the connection between the body, mind and soul and provides guidance for leading a healthier more natural way of life [1]. In the siddha system of medicine, the drug "Sangkaara Vairavan" (SV) is indicated for Iraippu, Kasam (Bronchial asthma) and sogi (Anasarca). SV is a polyherbal siddha formulation from the text Theraiyarvagadam [2]. The effectiveness, safety and quality of herbal medications and their formulations are still not well understood, despite the herbal drug industry's rapid development. The most prevalent microbial contaminants associated with medical plants are bacteria and fungi. These bacteria which include potentially dangerous infections can make it more difficult to use these plants properly and therapeutically [3]. Current hygiene criteria, which emphasize the least microbial presence or the full absence of hazardous germs, are expected to be maintained by herbal medicines, according to the European Pharmacopoeia [4] and The World Health Organization (WHO) [5]. Yet microbial contamination can result from post-harvest handling and inappropriate storage procedures, allowing pathogenic and saprophytic organisms to impact the herbal

products. The present study aims to evaluate microbial contamination and anti-microbial properties of the medicine SV.

Materials and Methods

Ingredients of Sangkaaravairavan

1. Saradai *(Trianthemadecandra)* - 1 palam (35gms)
2. Mukkavelai *(Tephrosia spinosa)*- 1 palam (35gms)
3. Nalvelai *(Gynandropis pentaphylla)* -1 palam (35gms)
4. Vattathiruppi *(Cissampelospereira)* -1 palam (35gms)
5. Chukku *(Zingiber officinale)* -1 palam (35gms)
6. Thippili *(Piper longum)* -1 palam (35gms)
7. Sithiramoolam *(Plumbago zeylanica)* -1/2 palam (17.5gms)
8. Injisaru(concentrate)*(Zingiber officinale)*-1200ml

Collection of Raw Material

The necessary raw materials were purchased from a reputable local raw material supplier. At Government Siddha Medical College in Chennai, Tamil Nadu, the Head of the department attested to the plant material's identity and authenticity (Certificate voucher number GSMC/GD/227-235).

Preparation

The purified raw drugs (1-8) were ground into powder using a mortar and pestle. The powder was sieved with white cloth, mixed with ginger juice to form karkampatham, shaped into 130mg (kundrialavu) pills, dried in the shade, and sealed in an airtight container. The prepared trial drug was sent to Regional Research Institute of Unani Medicine in Chennai, accredited by NABH and NABL, to examine microbial load and anti-microbial activity.

Microbial Load Determination

The Unani Pharmacopoeia (2016) and WHO Standards (2007) were followed in testing the microbial quality, which involved the separation and identification of harmful microbes among commercial and homemade herbal products [6]. Tests were done to determine the number of isolated bacteria and fungus might develop aerobically in 1g of material. The material had been thoroughly mixed with water to blend it. 1gm of the material was added to 9ml of peptone broth. Serial dilutions were then made to get the desired concentration. Every microbiological analysis was done three times. In summary, the pour plate method was used to evaluate viability via serial dilutions on Casein, also known assoyabean digest agar and Sabouraud dextrose agar for fungus identification and bacterial counts, respectively. Before seeding and incubating at 37°C for 24 to 48hours for bacterial screening and at 25 ℃for 48 to 72hours for fungus screening, all dehydrated mediums were prepared in accordance with the manufacturer's instructions. By multiplying the average number of colonies by the dilution factor at the end of the incubation period, the number of colony-forming units per gram (CFU/g) was determined. The CFU/g of the sample that was obtained was compared to WHO criteria for aerobic bacteria; samples were deemed inadequate or unsatisfactory if the bacterial growth in 1 g of herbal medicine was larger than 105 CFU [7].

Identification of Bacteria

To isolate and identify the bacteria, the samples were diluted in either water or tween 80 based on their solubility and then forcefully mixed to homogenize them. Following the suggested time and temperature, the 1ml aliquots were moved to 9ml of peptone broth and cultivated. Every microbiological analysis was done three times. MacConkey agar, Deoxycholate citrate agar, Cetrimide agar, EMB agar, and Mannitol salt agar culture mediums were used to examine Salmonella spp., Escherichia coli, Pseudomonas aeruginosa, and Staphylococcus aureus, respectively. Biochemical tests (oxidase, gas, and catalase production), colony morphology, and Gramme staining were used to characterize the pathogenic bacterial isolates at the end of the incubation period.

Antimicrobial Study

Chemicals and Glassware

Merck chemicals contributed to the analytical and laboratory grade solution and substances used in the investigation, while Hi-media supplied the study's media. Throughout the study, glassware composed of borosil glass was utilized. Glassware, growth media, and other accessories were sterilized in an autoclave at 121℃for 15 mins at a pressure of 15 pounds per square inch.

Sterilization

Formaldehyde and potassium permanganate fumigation was used 24hrs each month to sterilize the testing microbiology facility. The inoculum hood was sanitized with 95% ethanol and exposed to UV light for 20 minutes before each use. The digital weighing balance was also cleaned with 95% ethanol and gloves were used during the experiment for maintaining aseptic condition.

Collection of test microorganism

Clinical laboratories in and around Chennai, few cultures were procured to evaluate the microbial studies. The organisms used were Staphylococcus aureus (ATCC-29213), Bacillus cereus (clinical cochin university) (Gram positive), Escherichia coli (ATCC-25922), Klebsiella pneumonia (ATCC-700603) (Gram negative), and Candida albicans (Clinical Cochin University). All the organisms were confirmed using specific biochemical tests [8].

Medium preparation

A uniform suspension of the organisms was prepared by inoculating a loopful of each culture in 10 ml of nutrient broth and incubating at 37℃for 6 to 8 hours.

Table.1 Composition Mueller Hinton Agar (M-H Agar) Sigma-70191:

Ingredients	Grams/Litre
Beef infusion solids	4.0
Starch	1.5
Casein hydrolysate	17.5
Agar	15.0
Final Ph 7.4±0.5	0.2 at 37℃

38 g of the media was suspended in 1000 ml of distilled water. It was boiled until the medium completely dissolved and then sterilized by autoclaving at 121℃for 15 minutes.

Table 2. Sabouraud dextrose agar (SDA)

Dextrose (glucose)	40 g
Peptone (or mycological peptone)	10 g
Agar	15 g

Final pH of 6.9 ± 0.5	0.2 at 25℃

Suspend 65 g of the powder in 1000 ml of distilled water. Mix well. Heat to boil shaking frequently until completely dissolved. Sterilize in autoclave at 121℃for 15 minutes.

Sample Preparation

10 g of Sangkaaravairavan were dissolved in50 ml of hydroalcohol (1:1) and the sample was kept in a shaking incubator for 24 to 48 hours. The solution was collected after the complete solubility of the formulation through Whatman no. 1 filter paper. The filtered extract was evaporated in a hot air oven at 40℃for one day. The final extract was weighed and dissolved using the same solvent at 50,100, &150 mg/ml concentration used for the further assay.

Antimicrobial Activity of Formulation

The Sangkaaravairavan were tested for their antimicrobial activity using the Agar well diffusion method. All cultures for the study were sourced from Clinical Laboratories in and around Chennai. The medium, prepared using Muller Hinton Agar and Sobouraud Dextrose Agar (HiMedia), was autoclaved. Autoclaved media was mixed and poured into 100mm petri plates (25-30ml/plate). Bacterial strains cultured for 24 hours were transferred to the medium. Agar wells (10 mm diameter) were filled with 100µl of diluted formulations at various concentrations (50, 100, & 150 mg/ml). Controls used were Ampicillin (10mcg), Norfloxacin (10mcg), and Amphotericin (20mcg). Plates were incubated at 37℃ for bacteria and 30℃ for fungus for 12 to 24 hours. After incubation, the efficacy of the formulation was assessed by the zone of inhibition. Each experiment was conducted twice [9].

Results

The microbial load was analyzed by Pour plate method for investigating *Escherichia coli, Salmonella spp., Pseudomonas aeruginosa and Staphylococcus aureus* and the result is given below in Table 1. For antimicrobial activity, a total of five microbial cultures in which two-gram positive organisms namely *S. aureus & B. cereus* two-gram negative organisms namely *E. coli, K. pneumonia* and fungal strain *C. albicans* were screened. A significant growth of inhibition was shown against all the tested organisms indicating the profound potency of the Sangkaaravairavan and the results were presented in Table 2 &figure 1.

Table 3. Antimicrobial load of *Sangkaara Vairavan*

S. No	Parameters	Results	Remarks
1	Total Bacterial Count (TBC)	Less than 24 cfu/g	
2	Total Fungal Count (TFC)	Less than 3 cfu/g	
3	Enterobacteriacea	Absent	Within permissible limits
4	*Escherichia coli*	Absent	
5	*Salmonella Spp*	Absent	
6	*Staphylococcus aureus*	Absent	
7	*Pseudomonas aeruginosa*	Absent	

Table 4. Antimicrobial activity of Sangkaara Vairavan

Test samples	CONC (mg/ml)	*S A*	*B C*	*KP*	*EC*	*CA*
		(Zone of inhibition mm in dm)				
Sangkaara vairavan	50	16	10	-	25	14
	100	20	12	15	27	17
	150	21	15	19	31	22
	SC	-	-	-	-	-
	Std	19	-	18	27	14
Standard (Std)	Amp: Ampicillin 10mcg (Gram positive organisms); Nx: Norfloxacin10mcg (Gram negative organisms) & Ap: Amphotericin 20mcg (Fungi)					
Organisms Abbreviation	*SA: Staphylococcus aureus* (ATCC-29213); *BC Bacillus cereus* (Clinical Cochin University); *KP: Klebsiella pneumonia* (ATCC-700603);*EC: Escherichia coli* (ATCC 25922)*& CA: Candida albicans* (Clinical Cochin University)					
SC	Solvent control					

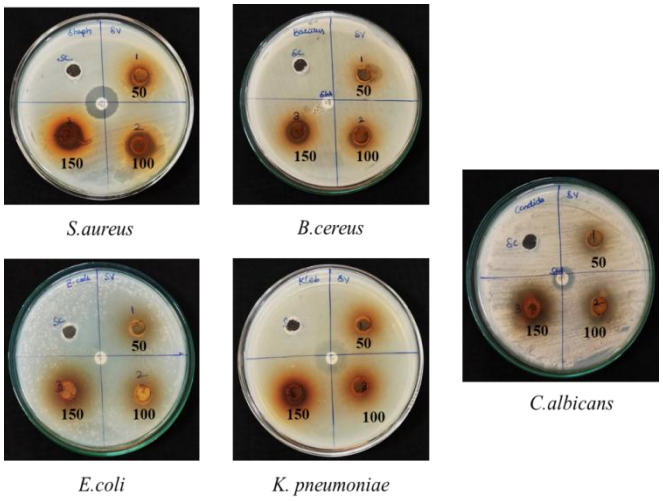

Figure 1. Antimicrobial Activity of Sangkaara Vairavan

Discussion

Microbial contamination typically happens when plant materials are improperly dried or stored, which ultimately leads to the plant contents degrading. Additionally, microbial contamination can make plant material poisonous by changing the chemicals present in the plant material or by microorganisms to produce harmful substances. Microbial quality tests should be conducted on plant materials and products as needed. During quality analysis, it is essential to prevent external conditions from affecting microorganisms

under investigation. Microbial load was assessed using the Pour plate method, which indicated that the total bacterial count (TBC) was less than 24 cfu/g and the total fungal count (TFC) was less than 3 cfu/g; both results are well within acceptable limits. No presence of Enterobacteriaceae, Escherichia coli, Salmonella spp., Staphylococcus aureus, or Pseudomonas aeruginosa was detected. This study examined the antimicrobial activity of Sangkaaravairavan extract against several clinically relevant microorganisms, including two Gram-positive bacteria, two Gram-negative bacteria, and one fungal strain. The findings show that antimicrobial activity increased with concentration, with the largest zone of inhibition observed at 150 mg/ml. E. coli showed the greatest sensitivity to the extract, with a 31 mm inhibition zone at 150 mg/ml—exceeding that of standard Norfloxacin (27 mm). Candida albicans showed strong inhibition (22 mm), indicating antifungal activity comparable to Amphotericin (14 mm). Staphylococcus aureus and Bacillus cereus exhibited moderate zones of 21 mm and 15 mm, with S. aureus slightly more sensitive than Ampicillin (19 mm). K. pneumoniae was less sensitive (19 mm) but still showed dose-responsive inhibition. No activity was observed in the solvent control, indicating that the antimicrobial effect was associated with the bioactive compounds in the Sangkaara Vairavan extract. These results indicate that the extract has broad-spectrum antimicrobial properties and may serve as a source of natural antimicrobial agents.

Conclusion

The findings of this study demonstrate that Sangkaara Vairavan is free from microbial contamination, thereby supporting its safety profile. The microbial screening data provides a valuable benchmark for assessing the quality of this drug. Furthermore, the results indicate that the Sangkaara Vairavan extract displays notable antimicrobial activity against a wide range of microorganisms, including Gram-positive and Gram-negative bacteria as well as fungi. The antimicrobial effect was concentration-dependent, with maximum efficacy observed at 150 mg/ml. Importantly, the extract exhibited substantial inhibitory effects on Escherichia coli and Candida albicans, in some instances outperforming standard pharmaceuticals. These observations indicate that Sangkaara Vairavan may be a potential natural source for developing alternative antimicrobial agents. However, comprehensive investigations including compound isolation, determination of minimum inhibitory concentrations (MIC), and thorough toxicity assessments are necessary to conclusively establish its therapeutic potential and safety profile.

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Inform Consent

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It is not applicable.

Conflicts of Interest

Authors have declared that no conflicts of interest

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