

Standardization and antimicrobial studies of *Venkara neer*

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Abstract

Standardization of mineral formulation is essential in order to assess the quality of drugs. The present paper reports preparation and standardization of a mineral formulation which contains borax. This Siddha formulation is used for washing external wounds mentioned in the Siddha literature. The aim of this study to estimate quality of *Venkaraneer* by conducting physicochemical analysis, HPTLC, heavy metal analysis, test for specific pathogens and also antimicrobial activity. From this result the Siddha drug *Venkaraneer* has standard quality and have antimicrobial potency against bacterial pathogens.

Keywords: Standardization; *Venkara neer*; Mineral formulation; External wounds; Siddha.

1. Introduction

Venkaram commonly known as borax which is widely used in Siddha system of medicines for treatment of skin disease, uterine disorders, gastric ulcer, Dental disease, urinary tract infections, external wash for wound and burning micturation. The present study deals with standardization of Siddha mineral formulation *venkara Neer* a Siddha drug mentioned in the Siddha *Vaithiya Thirattu* which is used for washing in external wounds. Till now there is no clear documentation available on standardization. This is proved through the systematic standardization of the test drug by physicochemical and HPTLC finger printing aspects according to PLIM guidelines.

2. Materials and methods

2.1. Selection of the trial drug

For this present study, the mineral formulation "*Venkara Neer*" a drug preparation for washing External wounds has been chosen from classical *Siddha* literature – "*Siddha Vaithiya Thirattu*" written by Dr. K. N. Kuppusamy muthaliyar and Dr. K.S.Uthamarayan." Publication: *Department of Indian Medicine & Homoeopathy, Chennai, Govt. of Tamil Nadu*, page: No:305.

2.2. Identification and authentication of the drugs

The raw drugs will be collected from the raw drug shop, identification will be obtained from faculties Of Gunapadam department, Government Siddha medical college, Palayamkottai.

2.3. Purification of the Drugs

Fry the *venkaram* until the water dried up.

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2.4. Preparation of the drug

Preparation of *Venkara Neer*: Purified *venkaram* is powdered and mixed with boiled and cooled water as per the ratio mentioned in siddha literature.

2.4.1. Indications

Washing in External wounds.

These following studies were done at Noble Research Solutions, Perambur at Chennai.

2.4.2. Physicochemical Evaluation

The samples were analysed for the Physicochemical parameters like Description-Color, Odour, pH, Clarity test

2.4.3. Determination of pH

Required quantity of test sample were subjected to screening using pH meter.

2.4.4. Determination of Clarity test

Clarity testing was carried out to check the particulate matter in the sample VN. In this test transparent particles or white particles observed against the black background and the black or dark particles observed against the white background.

2.5. Thin Layer Chromatography (TLC)

2.5.1. Analysis

Test sample was subjected to thin layer chromatography (TLC) as per conventional one dimensional ascending method using silica gel 60F254, 7X6 cm (Merck) were cut with ordinary household scissors. Plate markings were made with soft pencil. Micro pipette was used to spot the sample for TLC applied sample volume 10-micro litter by using pipette at distance of 1 cm at 5 tracks. In the twin trough chamber with the specified solvent system after the run plates are dried and were observed using visible light Short-wave UV light 254nm and light long-wave UV light 365 nm.

2.6. High Performance Thin Layer Chromatography Analysis (HPTLC)

HPTLC method is a modern sophisticated and automated separation technique derived from TLC. Pre-coated HPTLC graded plates and auto sampler was used to achieve precision, sensitive, significant separation both qualitatively and quantitatively. High performance thin layer chromatography (HPTLC) is a valuable quality assessment tool for the evaluation of botanical materials efficiently and cost effectively. HPTLC method offers high degree of selectivity, sensitivity and rapidity combined with single-step sample preparation. Thus this method can be conveniently adopted for routine quality control analysis. It provides chromatographic fingerprint of phytochemicals which is suitable for confirming the identity and purity of phytotherapeutics.

2.6.1. Chromatogram Development

It was carried out in CAMAG Twin Trough chambers. Sample elution was carried out according to the adsorption capability of the component to be analysed. After elution, plates were taken out of the chamber and dried.

2.6.2. Scanning

Plates were scanned under UV at 366nm. The data obtained from scanning were brought into integration through CAMAG software. Chromatographic finger print was developed for the detection of phytoconstituents present in each sample and their respective R_f values were tabulated.

2.7. Sterility Test by Pour Plate Method

2.7.1. Objective

The pour plate techniques were adopted to determine the sterility of the product. Contaminated / un sterile sample (formulation) when come in contact with the nutrition rich medium it promotes the growth of the organism and after stipulated period of incubation the growth of the organism was identified by characteristic pattern of colonies. The colonies are referred to as Colony Forming Units (CFUs).

3. Methodology

Test sample was inoculated in sterile petri dish to which about 15 mL of molten agar 45°C were added. Agar and sample were mixed thoroughly by tilting and swirling the dish. Agar was allowed to completely gel without disturbing it (about 10 minutes). Plates were then inverted and incubated at 37° C for 24-48 hours and further extended for 72 hrs. for fungal growth observation. Grown colonies of organism was then counted and calculated for CFU.

3.1. Test for Specific Pathogen

3.1.1. Methodology

Test sample was directly inoculated in to the specific pathogen medium (EMB, DCC, Mannitol, Cetrimide) by pour plate method. The plates were incubated at 37°C for 24 - 72h for observation. Presence of specific pathogen is identified by their characteristic colour with respect to pattern of colony formation in each differential media.

Table 1 Detail of Specific Medium and their abbreviation

Organism	Abbreviation	Medium
<i>E-coli</i>	EC	EMB Agar
<i>Salmonella</i>	SA	Deoxycholate agar
<i>Staphylococcus aureus</i>	ST	Mannitol salt agar
<i>Pseudomonas aeruginosa</i>	PS	Cetrimide Agar

3.2. Analysis of Pesticide Residue Organochlorine, Organophosphorus,

3.2.1. Organocarbamates, Pyrethroids

Extraction

Test sample were extracted with acetone and followed by homogenization for brief period. Further filtration was allowed and subsequent addition of acetone to the test mixture. Heating of test sample was performed using a rotary evaporator at a temperature not exceeding 40°C until the solvent has almost completely evaporated. To the residue add a few millilitres of toluene and heat again until the acetone is completely removed. Resultant residue was dissolved using toluene and filtered through membrane filter.

3.3. Heavy metal analysis by atomic absorption spectrometry(aas)

Standard: Hg, As, Pb and Cd – Sigma

Atomic Absorption Spectrometry (AAS) is a very common and reliable technique for detecting metals and metalloids in environmental samples. The total heavy metal content of the sample was performed by Atomic Absorption Spectrometry (AAS) Model AA 240 Series. In order to determination the heavy metals such as mercury, arsenic, lead and cadmium concentrations in the test item.

Sample Digestion

Test sample was digested with 1mol/L HCl for determination of arsenic and mercury. Similarly, for the determination of lead and cadmium the sample were digested with 1mol/L of HNO₃.

Standard preparation

As & Hg- 100 ppm sample in 1mol/L HCl

Cd &Pb- 100 ppm sample in 1mol/L HNO₃

3.4. Anti microbial activity

3.4.1. Agar Disc Diffusion Test

The antibacterial screening of the *Venkara neer* (VN) was carried out by determining the zone of inhibition using agar disc diffusion method (Bauer, 1996). The sample were tested against pathogenic bacteria (*Streptococcus sps*, *Staphylococcus sps* and *Pseudomonas sps*).

3.4.2. Bacterial Inoculums Preparation

Inoculum of (*Streptococcus sps*, *Staphylococcus sps* and *Pseudomonas sps*) were prepared individually in a respective broth and kept for incubation at suitable temperature.

3.4.3. Antibacterial Test

The medium was prepared by dissolving 38 g of Muller Hinton Agar Medium (Hi Media) in 1000 ml of distilled water. The dissolved medium was autoclaved at 15 Lbs pressure at 121°C for 15 min (pH 7.3). The autoclaved medium was cooled, mixed well and poured petriplates (25 ml/plate) the plates were swabbed with Pathogenic Bacteria culture viz. analysis analysis *Streptococcus sps*, *Staphylococcus sps* and *Pseudomonas sps*. Finally, about 25µL, 50µL, 75µL sample was loaded onto the disc then placed on the surface of Mullar-Hinton medium and the plates were kept for incubation at 37°C for 24 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimetres. The size of the zone of inhibition (including disc) was measured in millimeters. The absence of zone inhibition was interpreted as the absence of activity (Kohner *et al.*, 1994; Mathabeet *et al.*, 2006). The activities are expressed as resistant, if the zone of inhibition was less than 7 mm, intermediate (8-10 mm) and sensitive if more than 11 mm (Assam *et al.*, 2010).

4. Results

4.1. Physico chemical evaluation

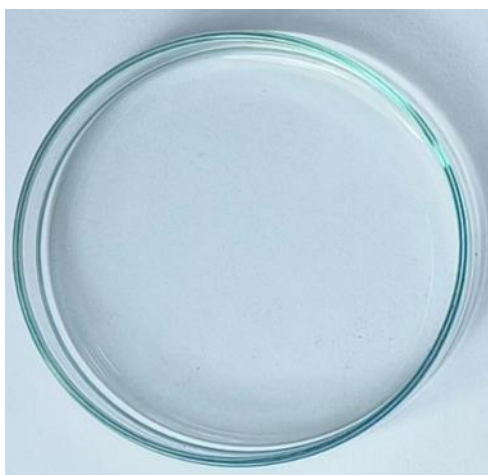
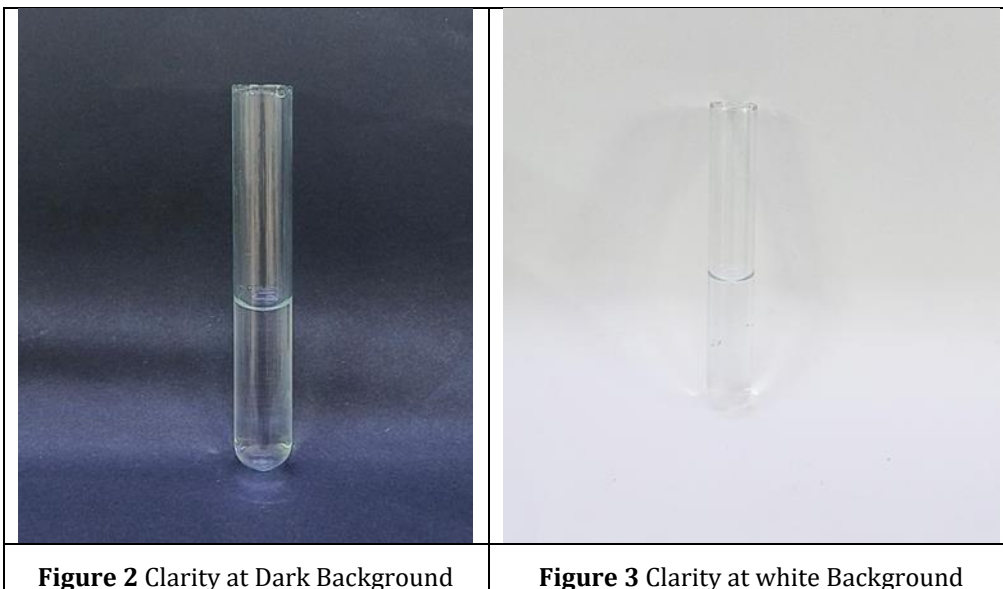


Figure 1 *Venkara Neer*

Table 2 Physicochemical Parameters

State	Liquit
Odour	No Characteristic
Touch	Non greasy
Flow Property	Free Flowing
Appearance/ Colour	Colorless / Transparent
Ph	8.5

4.2. Clarity test result



From the observation it was found that there are no particulate matters found in the sample VN and hence it here by described as clear solution with no visible particles.

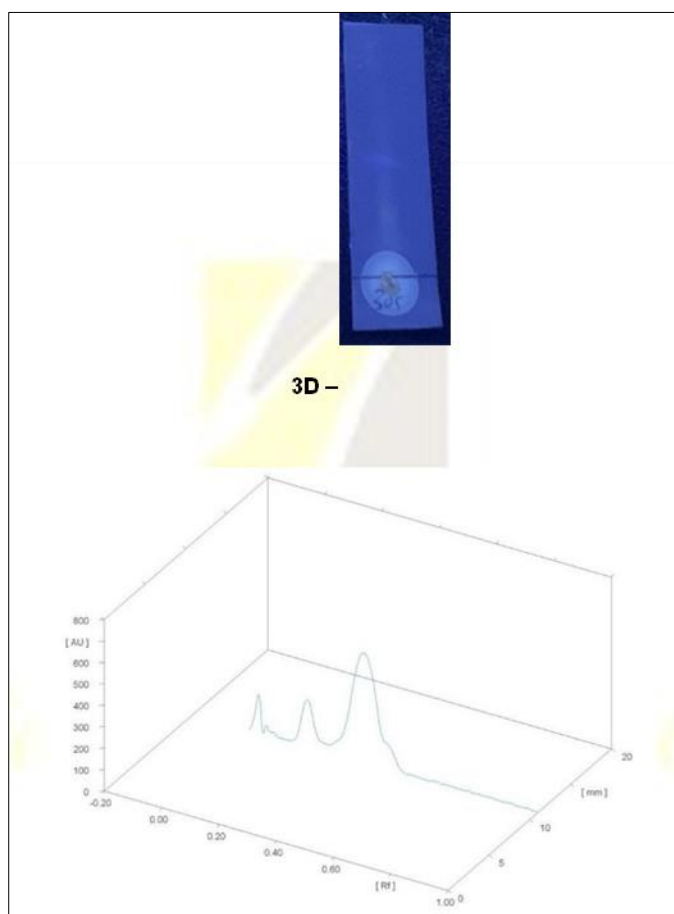


Figure 4 TLC visualisation of VN at 366 nm 3D- chromatogram

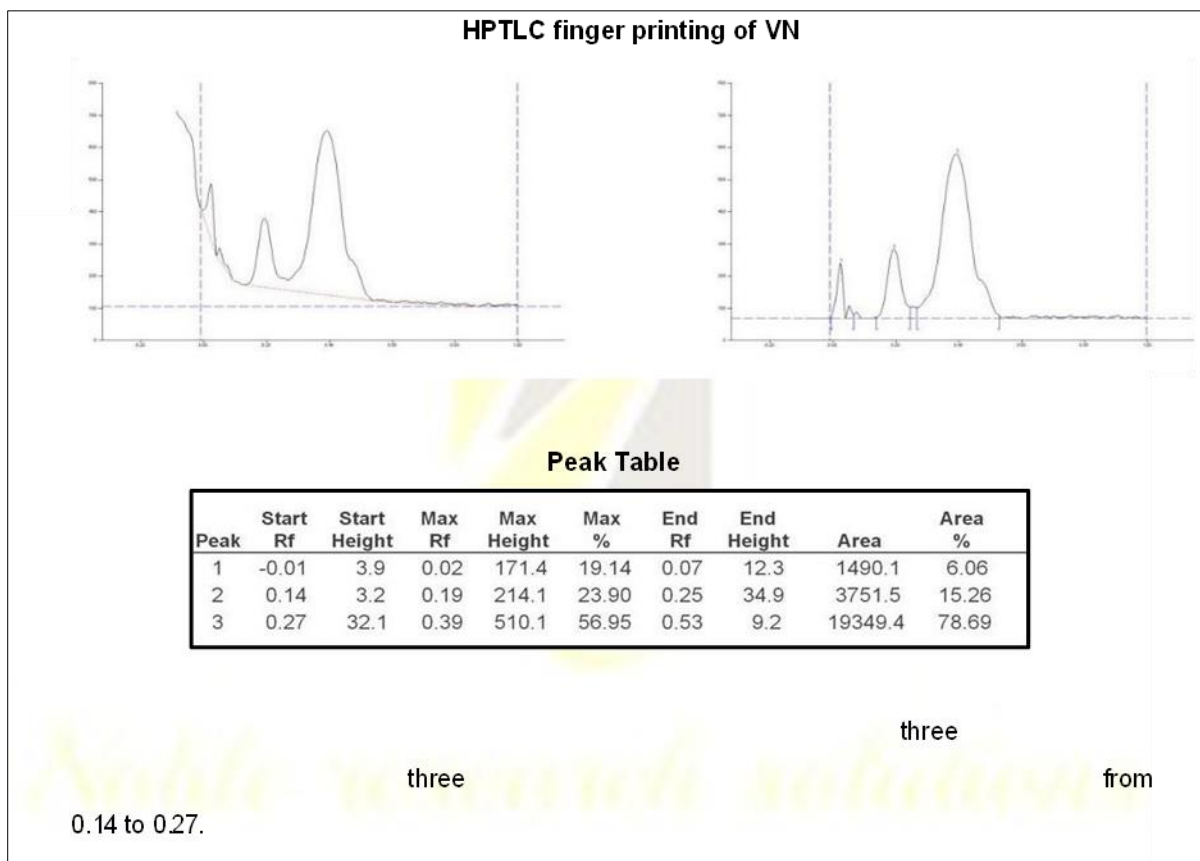


Figure 5 HPTLC Finger printing and Peak Table

HPTLC finger printing analysis of the sample reveals the presence of three prominent peaks corresponds to the presence of three components present with in it. Rf value of the peaks ranges from 0.14 to 0.27.

4.3. Sterility test by pour plate method

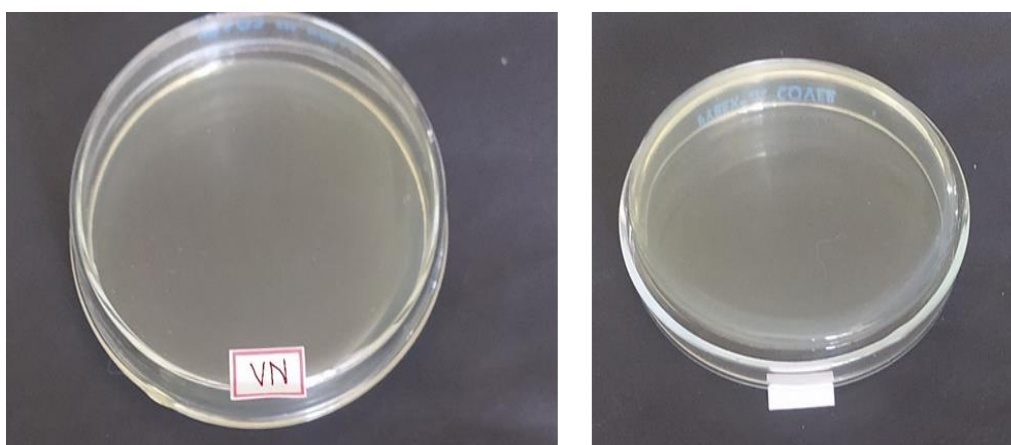


Figure 6 St test pour plate of *venkara neer*

4.3.1. Observation

No growth was observed after incubation period reveals the absence of specific pathogen

No growth / colonies was observed in any of the plates inoculates with the test sample.

Table 3 Sterility Test Report

Test	Result	Specification	As per AYUSH/WHO
Total Bacterial Count	Absent	NMT 10 ⁵ CFU/g	As per AYUSH specification
Total Fungal Count	Absent	NMT 10 ³ CFU/g	

4.3.2. Test for Specific Pathogen

Observation

No growth was observed after incubation period. Reveals the absence of specific pathogen

Result

No growth / colonies were observed in any of the plates inoculated with the test sample.

Table 4 Specific Pathogen Report

Organism	Specification	Result	Method
<i>E-coli</i>	Absent	Absent	As per AYUSH specification
<i>Salmonella</i>	Absent	Absent	
<i>Staphylococcus Aureus</i>	Absent	Absent	
<i>Pseudomonas Aeruginosa</i>	Absent	Absent	

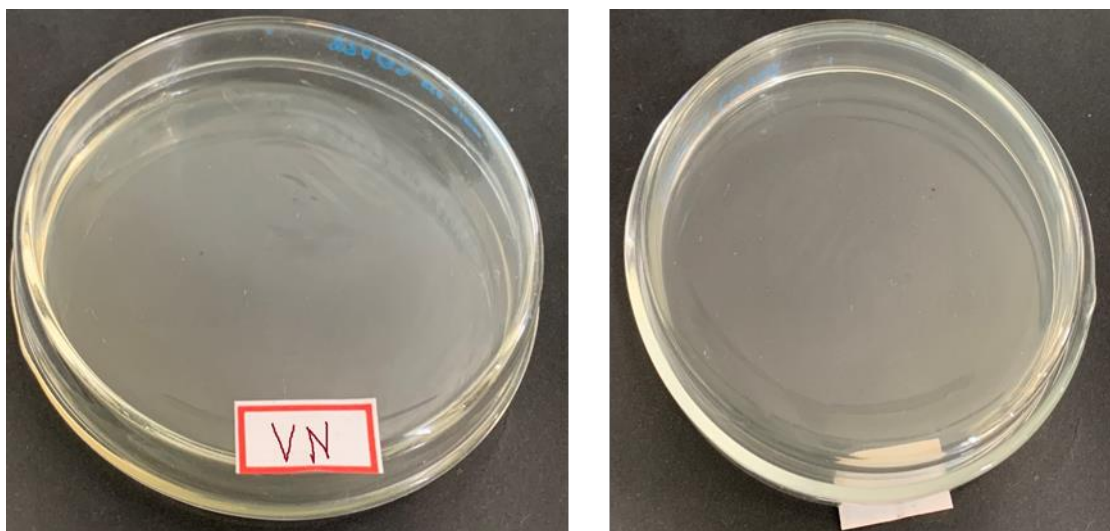


Figure 7 Culture plate with Pseudomonas Aeruginosa (PS) specific medium

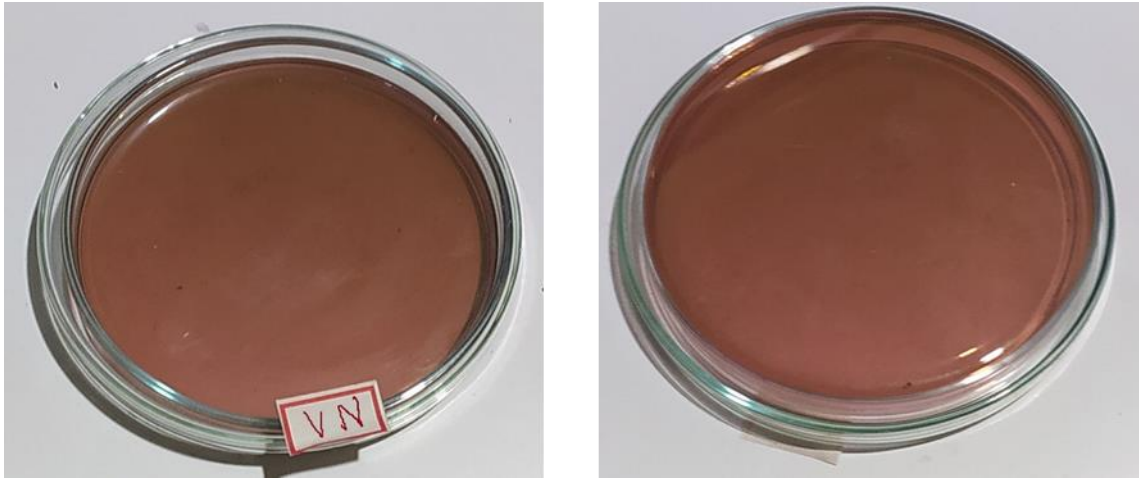


Figure 8 Culture plate with E-coli (EC) specific medium

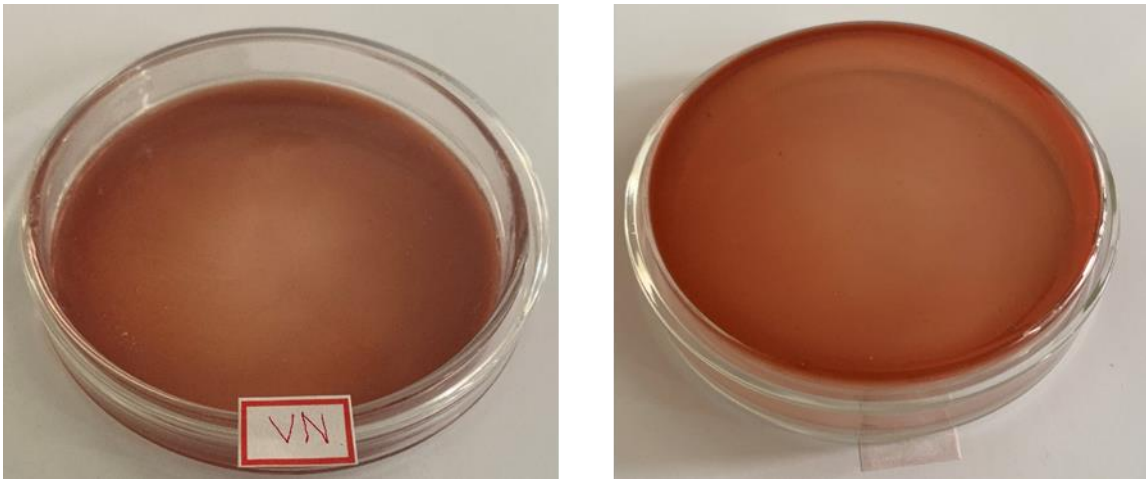


Figure 9 Culture plate with Salmonella (SA) specific medium

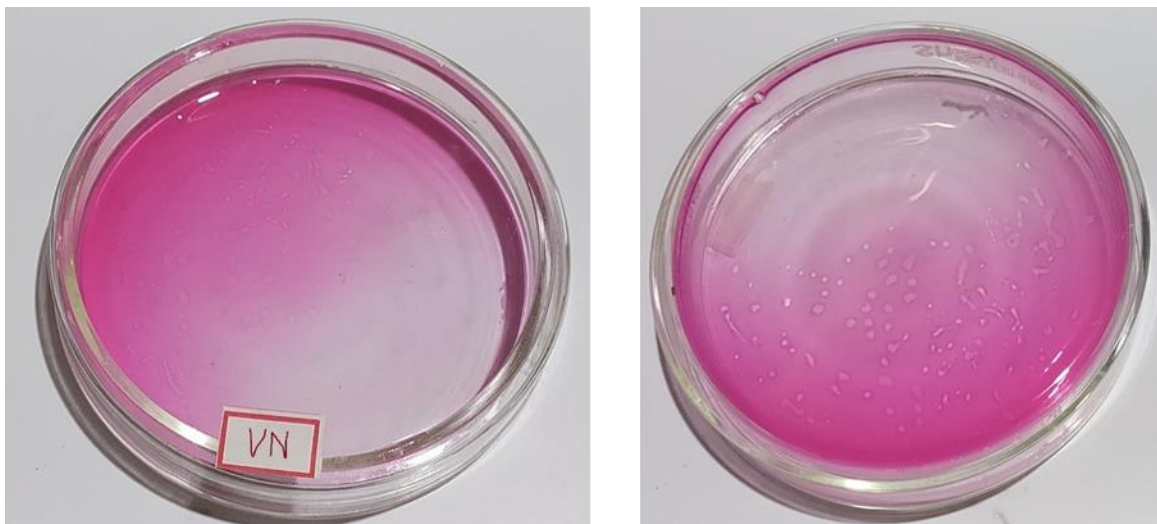


Figure 10 Culture plate with Staphylococcus Aureus (ST) specific medium

Table 5 Test Result Analysis of the Sample VN

Pesticide Residue	Sample VN	AYUSH Limit (mg/kg)
I.Organo Chlorine Pesticides		
Alpha BHC	BQL	0.1mg/kg
Beta BHC	BQL	0.1mg/kg
Gamma BHC	BQL	0.1mg/kg
Delta BHC	BQL	0.1mg/kg
DDT	BQL	1mg/kg
Endosulphan	BQL	3mg/kg
II.Organo Phosphorus Pesticides		
Malathion	BQL	1mg/kg
Chlorpyriphos	BQL	0.2 mg/kg
Dichlorovos	BQL	1mg/kg
III. Organo carbamates		
Carbofuran	BQL	0.1mg/kg
III.Pyrethroid		
Cypermethrin	BQL	1mg/kg

BQL- Below Quantification Limit

The results showed that there were no traces of pesticides residues such as Organo chlorine, Organo phosphorus, Organo carbamates and pyrethroids in the sample provided for analysis.

4.3.3. Heavy metal analysis by aas

Table 6 Heavy metal analysis results table

Name of the Heavy Metal	Absorption Max λ max	Result Analysis	Maximum Limit
Lead	217.0 nm	3.889	10 ppm
Arsenic	193.7 nm	BDL	3 ppm
Cadmium	228.8 nm	BDL	0.3 ppm
Mercury	253.7 nm	BDL	1 ppm

BDL- Below Detection Limit

4.3.4. Report and Inference

Results of the present investigation have clearly shows that the sample has no traces of heavy metal such as Arsenic, Cadmium and Mercury were as the sample evident the presence of Lead at 3.889 ppm as listed in the table.

4.3.5. Anti-bacterial potential of Venkaraneer(VN)

Table 7 Antibacterial potency of venkara neer

Sample Code	Zone of inhibition (mm in diameter)				
	25µL	50µL	75µL	Positive control	Negative control
Bacterial strain					
Streptococcus sps(G+)	23	24	27	12	Nil
Staphylococcus sps (G+)	NIL	14	15	12	Nil
Pseudomonas sps (G-)	10	16	17	12	Nil

Keywords: PC Positive control (Streptomycin), NC Negative control, "NIL" No Zone, mm (Millimetre), G+ (Gram Positive Organism), G- (Gram Negative Organism),

4.4. Anti-bacterial potential of (Venkaranéer VN)

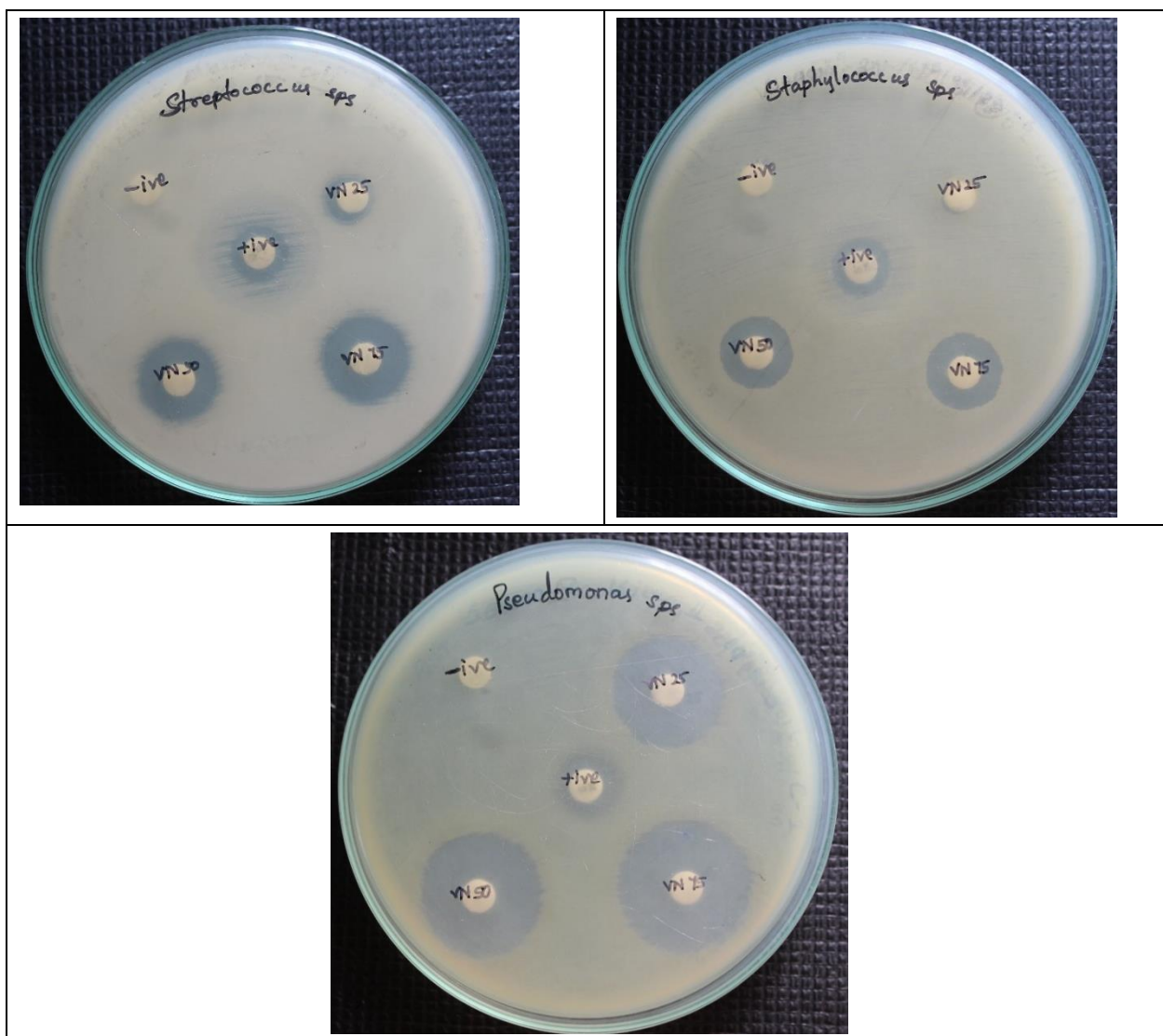


Figure 11 Antibacterial potency of venkara neer

5. Discussion

Standardization of the drugs is more essential to derive the efficacy, potency of the drug. The standardization of *Venkara Neer* (VN) was achieved through various procedures like analysing the physicochemical characters. The physical parameters like state, odour, touch, flow Property and appearance/colour revealed that it was liquid, characteristic odour, non greasy to touch, free flowing, colourless and transparent. So the drug VN is good in nature and safe to use.

- The pH value of VN was which indicates that the drug is basic in nature.
- In the clarity test observation it was found that there are no particulate matters found in the sample VN and hence it here by described as clear solution with no visible particles.
- The results of HPTLC finger printing analysis of the sample VN reveals the presence of 3 prominent peaks corresponds to presence of three components present with in it. Rf value of the peaks ranges from 0.02 to 0.27 with percentage area of 0.85% to 90.09%.
- The result of the Sterility test shows no growth was seen in any of the plates inoculates with the test sample VN. The revealed that the drug VN is free from the viable microorganisms and the absence of total bacterial and fungal colonies which indicates that the drug VN have good quality and safer drug.
- The result of the Specific pathogen was observed that there was no growth in any of the plates inoculated with the test sample VN which confirms that there are no viable aerobic microorganisms present in the sample.
- The results showed that there were no pesticide traces such as Organo chlorine, Organo phosphorus, Organo carbamates and Pyrethroids in the VN for analysis. This result suggests that VN have good quality.
- Results of the present investigation have clearly shows that the sample has no traces of heavy metal such as Arsenic, Cadmium and Mercury were as the sample evident the presence of Lead at 3.889 ppm below the maximum limit as listed in the table.
- The result represent the sample venkaara neer potentially inhibit the growth of all above organisms.

So the drug VN Is non-toxic and there is no contamination and does not possess carcinogenic property. As a result, *VENKARA NEER* was proved for its safety over the defined standardization method. Op

6. Conclusion

All the parameters and result of this study provides quality standards for *venkaraneer*.this can be utilized for the overall quality check over its preparation and formulation and also the drug *Venkaraneer* has antimicrobial potency against bacterial pathogens.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

Statement of ethical approval

Ethical approval was Approved.

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