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Antimicrobial potency and phytochemical analysis of Siddha formulation '*Seedhopaladhi Choornam*'

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Abstract

Siddha is an ancient system of medicine. According to this system, the human body is the replica of the universe. This system has developed a rich and unique treasure of drug knowledge in which use of metals, minerals and herbals are very much advocated .The main objective of this article is to explain the antimicrobial activity and phytochemical analysis of Siddha polyherbal formulation *'Seedhopaladhi Choornam'* (SPC). The invitro antimicrobial study of this formulation has been done through Agar disk diffusion method against the bacteriae *Staphylococcus aureus*, *Streptococcus mutans*, *Klebsiella pneumoniae*, and *E. coli*. It showed positive result to all the microorganisms and the drug revealed marked potency against *Klebsiella pneumoniae* strains. The presence of tannins has been found out in the phytochemical analysis.

Key words

Seedhopaladhi Choornam, Siddha Medicine, *Elettaria cardamomum*, Phytochemicals, Antimicrobial potency.

Introduction

Seedhopaladhi Choornam is a siddha formulation cited in the traditional Tamil literature book *Sigicha Rathina Deepam* written by Kannusaami Pillai. In this book, he has quoted that this formulation would be used for tuberculosis, haemorrhagic disorders, vomiting, ageusia, indigestion. Tuberculosis is one of the most prevalent diseases in India. It is caused by Mycobacterium tuberculosis. The 2019 report says that the estimated incidence of TB in India is 27 lakh out of which, about 8 lakh cases are still out of the ambit of notification. The antimicrobial study of this

formulation showed positive result against *Staphylococcus aureus, Streptococcus mutans, Klebsiella pneumoniae,* and *E. coli* with a marked potency against *Klebsiella pneumoniae.*

Materials and methods

Collection of drugs

The drugs *Saccharum officinarum* (cane candy), *Bambusa arundinacea, Piper longum, Elettaria cardamomum* (seeds alone), *Cinnamomum verum* were collected from the Traditional Raw Drug Store, Nagercoil, Kanyakumari district.

Purification

Saccharum officinarum – Used directly

Bambusa arundinacea – Used directly

Piper longum – Dry roasted

Elettaria cardamomum – Dry roasted

Cinnamomum verum – Dry roaste

Ingredients

Table 01.List of the ingredients for the preparation *Seedhopaladhi Choornam*

S.No	Botanical name & Tamil name	Family	Parts used	Taste (Suvai)	Quantity
01	*Saccharum officinarum (Karkandu)*	Poaceae	Extract (cane candy)	*Inippu* (sweet)	80g
02	*Bambusa arundinacea (Mungiluppu)*	Poaceae	Salt extract	*Uppu* (salt)	40g
03	*Piper longum (Thipilli)*	Piperaceae	Inflorescence	*Thuvarppu* (Astringent)	20g
04	*Elettaria cardamomum (Elarusi)*	Zingiberaceae	Seed	*Kaarppu* (Pungent)	10g
05	*Cinnamomum verum (Elavanga pattai)*	Lauraceae	Bark	*Innipu* (sweet), *Kaarppu* (pungent)	5g

Preparation

The purified drugs are grinded separately and mixed well. The grinded particles are sieved using the method *Vastharakayam* (Traditional sieving method using clean thin cotton cloth).

Antimicrobial analysis procedure

Dilution

0.1g in 1ml

Test Organism

The test microorganisms used for antimicrobial analysis, *Staphylococcus aureus, Streptococcus mutans, Klebsiella pneumoniae,* and *E. coli* were purchased from Microbial Type Culture Collection and Gene Bank (MTCC) Chandigarh. The bacterial strains were maintained on Nutrient Agar (NA).

Nutrient Broth Preparation

Pure culture from the plate were inoculated into Nutrient Agar plate and sub cultured at 37ºC for 24 h. Inoculum was prepared by aseptically adding the fresh culture into 2 ml of sterile 0.145 mol/L saline tube and the cell density was adjusted to 0.5 McFarland turbidity standard to yield a bacterial suspension of 1.5×108 cfu/ml. Standardized inoculum Used for Antimicrobial test.

Antimicrobial 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 1210C 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. *Staphylococcus aureus, Streptococcus mutans, Klebsiella pneumoniae,* and *E. coli*. Finally, The Sample or Sample loaded Disc was then placed on the surface of Muller-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 millimeters. 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; Mathabe 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). The zone of inhibition for each microorganism is given in the following figures.

Phytochemical analysis

Qualitative analysis

Alkaloids

Mayer's test (Ansari, 2006)

The extract was evaporated in a test tube. To the residue dilute HCL was added, shaken well and filtered. To the 2-3 ml of filtrate Mayer's reagent was added. Formation of yellow precipitate showed the presence of alkaloids.

Flavonoids

Shinoda Test (Kokate, 1994)

To the extract, 5 ml of 95% ethanol and few drops of concentrated hydrochloric acid was added. To this solution 0.5 gm of magnesium turnings were added. Pink colouration indicated the presence of flavonoids.

Glycosides

Keller-Killiani Test (Ansari, 2006)

To 2 ml of the extract, glacial acetic acid, one drop 5% FeCl3 and conc. H2SO4 was added. Reddish brown colour appeared at junction of two liquid layers and upper layer turned bluish green indicating the presence of glycosides.

Phenol

Ferric chloride test (Mukherjee, 2002)

The extract was diluted to 5 ml with distilled water. To that a few drops of neutral 5% ferric chloride solution was added. A dark green colour indicates the presences of phenolic compounds.

Tannins

Lead Acetate Test (Mukherjee, 2002)

On addition of 5% lead acetate solution to the extract white precipitate appeared.

Terpenoids (Horbone, 1984)

To the 5ml test solution 2ml chloroform was added with few drops of conc. Sulphuric acid (3ml) at the side of the test tube. An interface with a reddish brown coloration is formed if terpenoids constituent is present.

Steroids

Salkowski Test: (IP, 1996)

To 2 ml of extract, 2 ml of chloroform and 2 ml of conc. H2SO4 was added. The solution was shaken well. As a result chloroform layer turned red and acid layer showed greenish yellow fluorescence.

Saponins

Foam Test (Ansari, 2006)

Sample extract was added with distilled water and shaken vigorously. Observe the stable foam formation.

Quinones

1ml sample was mixed with 1ml of concentrated sulphuric acid and stand for few minutes. Observe the formation red colour indicates the presence of quinines in the sample.

Anthocyanin

2ml of 2N Hcl was mixed with 2ml of test sample. Observe the pink red colour turns into blue violet colour indicates the presence of anthocyanin in sample.

Quantitative analysis

Quantitative Estimation of Tannins (Robert, E.B. 1971. Agro.J.63, p.511)

1ml of the extract was mixed with 5ml of vanillin hydrochloride reagent (mix equal volumes of 8% HCL in methanol and 4% vanillin in methanol). The mixed was allowed to stand for 20mins and measure the absorbance at 500nm. The standard graph was plotted for working standard catechin solution (0 to 250µg/µl).

Results

Table 02.Zone of inhibition produced in the test Bacterial Strain Name

Samples	Strains					
	Staphylococcus aureus (G+)	Streptococcus mutans (G+)	Bacillus subtilis (G+)	E. coli (G-)	Klebsiella pneumoniae (G-)	Proteus vulgaris (G-)
SP. Aq 25 µl	8	NZ	9	NZ	7	NZ
SP. Aq 50 µl	9	7	10	7	9	NZ
SP. Aq 75 µl	10	10	11	9	11	9
SP. Aq 100 µl	12	11	14	10	13	11
PC	16	13	20	12	14	17
NC	NZ	NZ	NZ	NZ	NZ	NZ

Note: NZ –No zone, mm-millimetre, G+ve - Gram positive, G-ve - Gram negative organism, PC- Positive Control, NC- Negative Control.

Figure 01.Graphical representation of Antimicrobial analysis of *Seedhopaladhi Choornam*

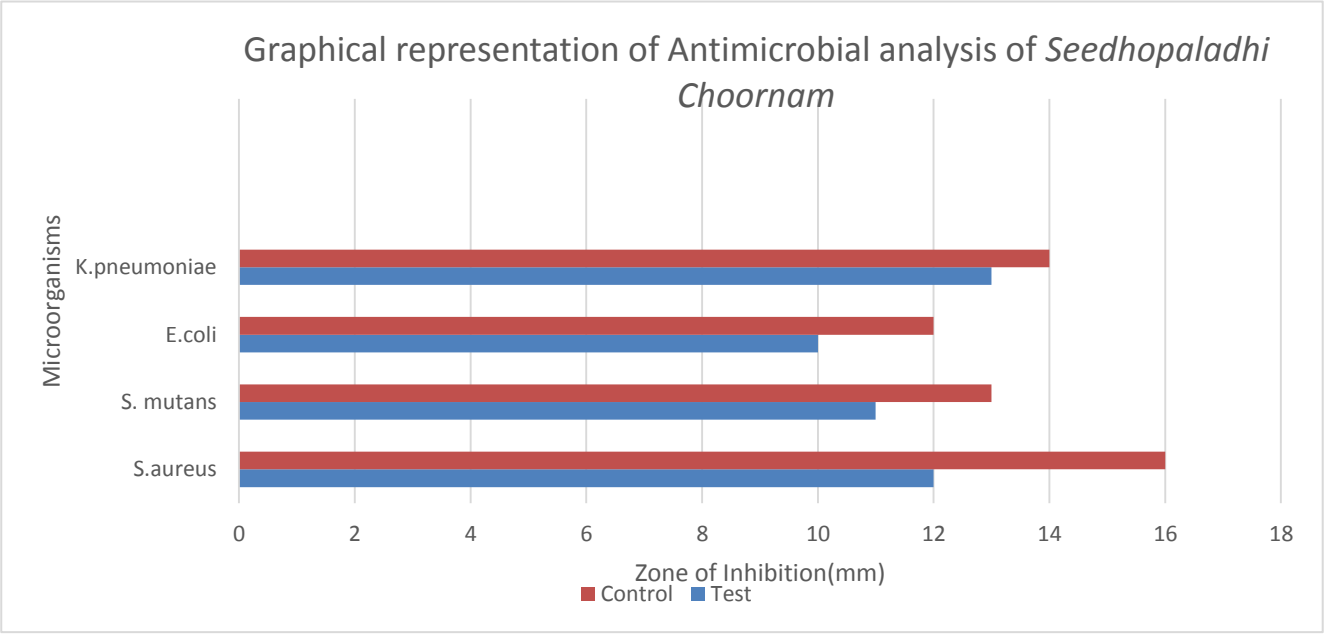
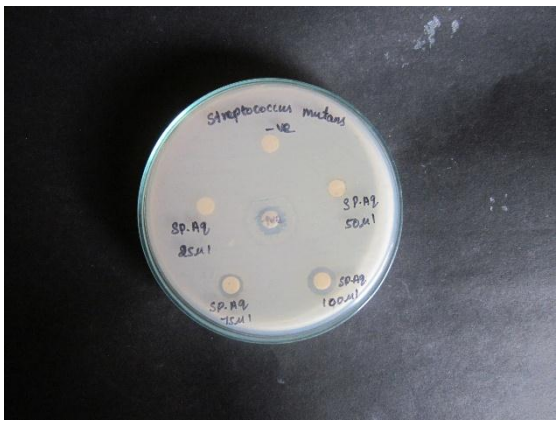
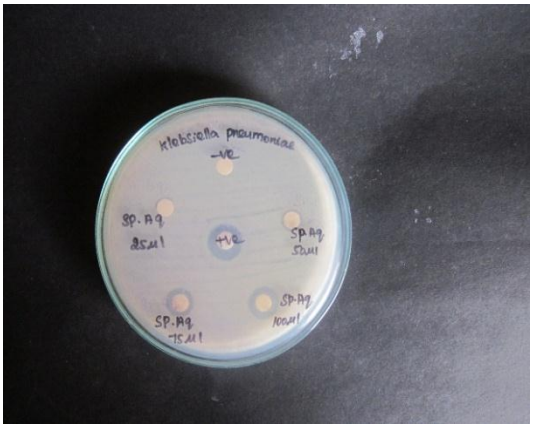


Figure 02.Zone of inhibition of test drug against tested organisms

Zone of inhibition for Staphylococcus aureus (G+) 	Zone of inhibition for Streptococcus mutans (G+) 
Zone of inhibition for Klebsiella pneumoniae (G-) 	Zone of inhibition for E. coli(G-) 

Staphylococcus aureus

Staphylococcus aureus is an important pyogenic organism and lesions are localized in nature in contrast to streptococcal lesions which are spreading in nature. It causes cutaneous infections, deep infections, toxic shock syndrome, nosocomial infections and food poisoning.

Streptococcus mutans

*Streptococcus mutan*s is known to be associated with bacteremia and infective endocarditis. *Streptococcus mutans* is implicated in the pathogenesis of certain cardiovascular diseases and is the most prevalent bacterial species detected in extirpated heart valve tissues, as well as in atheromatous plaques.

Escherichia coli

Escherichia coli is a gram-negative organism. It may cause wound infection, peritonitis, cholecystitis and neonatal meningitis. It is an important cause of neonatal meningitis.

Klebsiella pneumoniae

Klebsiella pneumoniae causes a wide range of infections including pneumonia, UTI, bacteremia and liver abscesses. The most common condition caused by *Klebsiella* bacteria is pneumonia, typically in the form of bronchopneumonia and also bronchitis. Individuals with *Klebsiella pneumoniae* tend to cough up characteristic sputum as well as having fever, nausea, tachycardia and vomiting. It has toxin very similar to the heat-stable toxin of E. coli. The disease is characterized by a massive mucoid inflammatory exudate of lobar or lobular distribution involving one or more lobes of the lung. Necrosis and abscess formation are more frequent than in pneumococcal pneumonia.

Quantitative analysis of phytochemicals

Table 03.Qualitative analysis of *Seedhopaladhi Choornam*

According to the phytochemical study, tannins are present in *Seedhopaladhi Choornam.*

Table 04.Quantitative results of *Seedhopaladhi Choornam*

Test	Aqueous
Tannins µg/ml	25 ± 0.2

Tannins may be employed medicinally in antidiarrheal, haemostatic, antihemorrhoidal compounds. The anti-inflammatory effects of tannin help control all indications of gastritis, esophagitis, enteritis, and irritating bowel disorders. Tannins not only heal burns and stop bleeding, but they also stop infection while they continue to heal the wound internally. Tannins can also be effective in protecting the kidneys. They can cause regression of tumours that are already present in the tissues. They have been also reported to have anti-viral, antibacterial, and antiparasitic effects.

Conclusion

The diseases affecting liver is nowadays becoming a major challenge to this society. The effect of Klebsiella pneumonia in liver is drastic. *Seedhopaladhi Choornam* is mainly indicated for liver related symptoms in the classical siddha literature. Hence it would be much effective against

Klebsiella induced liver disorders. Further studies are to be done in order to prove the efficacy of the test drug.

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