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Anti-microbial effect of Herbo-mineral Siddha formulation *Saamuthra Chooranam*

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Abstract

Antimicrobial study is a process, which refers to the process of killing or inhibiting microbes, it is responsible for causing infections in human health. This is includes antiviral, antibacterial and antifungal effects. The antimicrobial activity of *Saamuthra Chooranam* is coded SAC, it has been used and analysed in this study. The aim of this study is to bring out the activity of SAC against 3 gram positive bacteria (*Staphylococcus aureus, Enterococcus faecalis*, and *Lactobacillus salivarius*,1 gram negative bacteria (*E. coli*) and 2 fungi (*Penicilliuim notatum* and *Candida albicans*).

The antifungal activity study wascarried by disc diffusion method. The activity of SAC studied about ethanol and aqueous extracts and it was recorded antimicrobial activity against specific gram positive, gram negative bacteria and fungi were interpreted. The highest antibacterial activities have recorded in ethanol of extract 100 against *Lactobacillus salivarius* (19mm). Activity of ethanol extract 100 of SAC against the gram-negative bacteria strain (*E. coli*) was higher (13mm) than the activity of aqueous extract 100 of SAC, which was 9mm. The antifungal activity of ethanol extract of SAC was found to be higher against *Candida albicans* (17mm) compared to *Penicillium notatum.* No response in aqueous extract of SAC.

Keywords: *Saamuthra Chooranam*, Antimicrobial activity, Disc diffusion method, Siddha medicine

1. Introduction

Microbial community occupies a huge space in human body. It includes both harmless and harmful microorganisms. Some microorganisms are responsible for a wide variety of manifestations in humans. Many Siddha herbs and minerals play a vital role in eliminating these microorganisms. So, there arises a need to bring out the activity of those herbs and minerals against microorganisms. Plants areproducea diverse range of bioactive molecules, making them a rich source of different types of medicinal compound, which have continued to play a dominant role in the maintenance of human health,Over 50% of all modern clinical drug sources are coming in natural product and it also plays an important role in the pharmaceutical industry. SAC is a combination of nine drugs of herbal- mineral formulations (Table no 1). The antimicrobial activities of this SAC against microorganisms including 3 gram positive, 1 gram negative bacteria and 2 fungi were recorded and their efficacy was interpreted.

2. Aim of the study

The aim of the study is to evaluate the antimicrobial effect of SAC in healing the manifestations caused by microorganisms including *Staphylococcus aureus, E. coli, Enterococcus faecalis, Lactobacillus salivarius, Candida albicans* and *Penicillium notatum.*

3. Materials and Methods

3.1. Collection and identification of plant materials

All the ingredients of SAC were collected from Raw drug house in Tirunelveli, India.

3.2. Purification and preparation of the Saamuthra Chooranam

All the collected ingredients of Saamuthra Chooranam were authenticated by the Department of Medicinal Botany in Government Siddha Medical College, Palayamkottai. Then all the ingredients were finely powdered into Chooranam form.

Table no. 1 Ingredients of Saamuthra Chooranam

S. No.	Ingredients	Botanical Name	Family	Part used
1.	*Inji*	*Zingiber officinale*	Zingiberaceae	Rhizome
2.	*Thippili*	*Piper longum*	Piperaceae	Flower bud
3.	*Vaaividangam*	*Embelica ribes*	Myrsinaceae	Seed
4.	*Kadukkai*	*Terminalia chebula*	Combretaceae	Pericarp
5.	*Perungayam*	*Ferula asafoetida*	Apiaceae	Resin
6.	*Omam*	*Carum coptium*	Apiaceae	Seed
7.	*Indhuppu*	Sodium chloride (Impure)	-	-
8.	*Evatcharam*	Potash carbonate (Impure)	-	-
9.	*Kalluppu*	Sodium chloride	-	-

3.3. Antibacterial Activity Procedure:

Dilution: 1mg in 1ml **Test Organism**: The test microorganisms used for antimicrobial analysis, microbial strains was purchased from Microbial Type Culture Collection and Gene Bank (***MTCC***) Chandigarh. The bacterial strain was maintained on Nutrient Agar (NA). In this research the antibacterial activity of **KC** were evaluated against gram positive bacteria such as *Staphylococcus aureus, Enterococcus faecalis, Lactobacillus salivarius* and gram-negative bacteria *E. coli.*

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 121^0C 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.

Finally, The Sample or Sample loaded Disc was 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 millimeters. The size of the zone of inhibition (including disc) was measured in millimetres.

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)

3.4. Antifungal Assay by Disc Diffusion Method (Bauer *et al*., 1966)

Test Organism: *Penicillium notatum* and *Candida albicans*

Cleaning and sterilization: Glass-wares used were cleaned with cleaning solution and sterilised in hot air oven to 180° C for 3 hours. All nutrient media were sterilized by autoclave (121° C, 15psi for15-20 mins).

Preparation of test drug samples: 1gram of test drug was diluted in 1ml of distilled water and methanol respectively. The percolation time was 5- 7 days. The sample thus prepared in methanol was stored in room temperature and aqueous extract in 4° C to avoid the fermentation of the sample. Then the extracts were subjected to anti-microbial assay.

Anti-fungal assay: Antibiotic susceptibility tests were determined by agar disc diffusion (Kirby–Bauer) method. Fungi strainswere swabbed using sterile cotton swabs in SDA agar plate. Up to 40 µl of each concentration of the extract were respectively introduced in the sterile discs using sterile pipettes.

The disc was then placed on the surface of SDA medium and the compound was allowed to diffuse for 5 minutes and the plates were kept for incubation at 22°C for 48 hours. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimetres.

4. Results and Discussion

Table no 2. Anti-Microbial effect *Saamuthra Chooranam*

Sample Code	Strains Name					
	***Staphylococcus aureus* (G+) MTCC 916**	***Enterococcus faecalis* (G+) MTCC 439**	***Lactobacillus salivarius* (G+) MTCC 1026**	***E. coli*(G-) MTCC 1671**	***Candida albicans* (F) MTCC 277**	***Penicillium notatum*(F) MTCC 2647**
SAC.E. 50	**11**	**13**	**16**	**10**	**15**	**-**
SAC.E. 100	**13**	**16**	**19**	**13**	**17**	**10**
SAC. Aq.50	**8**	**7**	**10**	**7**	**13**	**-**
SAC . Aq.100	**11**	**9**	**14**	**9**	**15**	**-**
PC	**17**	**19**	**18**	**18**	**18**	**35**
NC	**-**	**-**	**-**	**-**	**-**	**-**

PC (Bacterria) - Positive control (Streptomycin- S 25), N - Negative (plain disc)
PC (Fungi) - Positive control (fluconazole), - - No Zone, Mm - Millimetre G+ - Gram Positive, G- - Gram Negative

Figure .1. *Staphylococcus aureus*

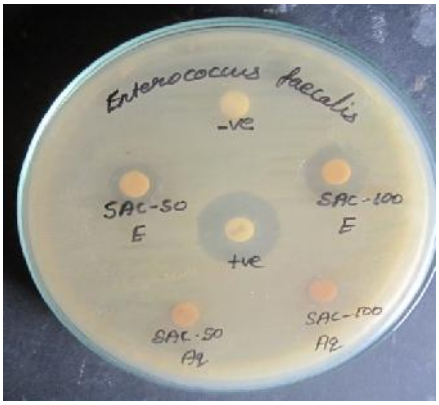

Figure . 2. *Enterococcus faecalis*

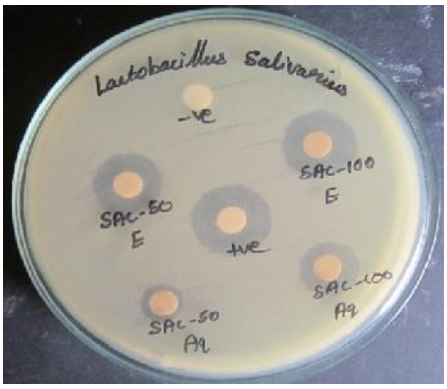

Figure .3. *Lactobacillus salivarius*

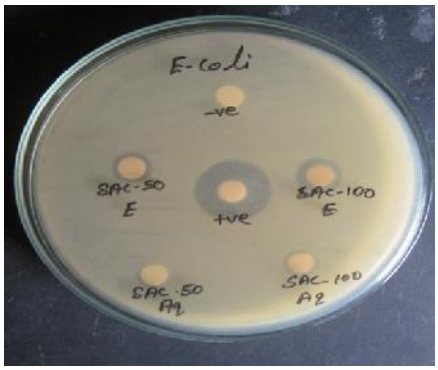

Figure . 4. *E. coli*

Figure .5. *Penicillium notatum*

Figure . 6. *Candida albicans*

4.1. Anti - bacterial activity of *Saamuthara Chooranam* on bacterial strains

In ethanol extract

As per table no: 2 & Fig; 1- 4, the ethanol extract of SAC 100 µl in bacterial strains shows more inhibition in the order of followed by *Lactobacillus salivarus* (19 mm) *Enterococcus faecalis* (16 mm), both *Staphylococcus aureus* (13mm) and *E. coli* (10 mm) respectively.

As per table no: 2 & Fig; 1-4, the ethanol extract of SAC50 µlin bacterial strains shows more inhibition in the order of followed by *Lactobacillus salivarus* (16 mm) *Enterococcus faecalis* (13 mm) and *Staphylococcus aureus*(11mm) respectively. *E. coli* (10 mm) possess least activity.

In aqueous extract

As per table no: 2 & Fig; 1- 4, the aqueous extract of SAC 100µl in bacterial strains shows more inhibition in the order of followed by *Lactobacillus salivarus* (14 mm) and *Staphylococcus aureus* (11 mm) respectively. Both *Enterococcus faecalis* (9 mm) and *E. coli* (9 mm) possess least activity.

As per table no: 2& Fig; 1- 4, the the aqueous extract of SAC 50µl in bacterialstrains shows lesser inhibition in *Lactobacillus salivarus* (10 mm). *Staphylococcus aureus* (8 mm) *Enterococcus faecalis*(7 mm) and *E. coli* (7 mm) possess least activity respectively.

The ethanol extracts of the SAC isshowed significant activity against all the tested bacteria when compared to aqueous extract. The highest antibacterial activity was recorded SAC E 100 µl in *Lactobacillus salivarus* (19 mm) which it was compared with positive control Streptomycin (18mm).

4. 2. Anti fungal activity of *Saamuthara Chooranam*

In ethanol extract

As per table no 2 & Fig no 5 and 6, the ethanol extracts of SAC 100µl in fungal strains possess more inhibition in *Candida albicans* (17 mm) followed by *Penicillium notatum*(10mm) showed minimal inhibition.

As per table no 2 & Fig no 5 and 6, the ethanol extracts of SAC 50µl in fungal strains possess more inhibition in *Candida albicans*(15 mm). *Penicillium notatum* did not show no inhibition.

In aqueous extract

As per table no 2&Fig 5 and 6, the aqueous extracts of SAC 100 µl in fungal strains possess more inhibition in *Candida albicans* (15 mm). *Penicillium notatum* did not show inhibition.

As per table no 2& Fig 5 and 6, the aqueous extracts of SAC 50 µl in fungal strains possess more inhibition in *Candida albicans* (13 mm). *Penicillium notatum* did not show no inhibition.

The same antifungal activity was recorded at SAC E 100 µl in fungal strains against *Candida albicans* (17 mm) which compared with positive control drugfluconazole (18mm).

So, concluded about this study was clear that the microbial activity of SAC was highly sensitive against the gram positive bacteria *Lactobacillus salivarus* and the fungi *Candida albicans*.

5. Acknowledgements

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