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ANTIMICROBIAL ACTIVITY OF SANGAN ILAI KUDINEER AGAINST SELECTED HUMAN PATHOGENS

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ABSTRACT

This study was carried out with an objective to investigate the antibacterial and antifungal activities of Siddha formulation *Sangan ilai kudineer (Azima tetracantha*). The aim of the study isto determine the antimicrobial and fungal activity and to evaluvate the zone of inhibition of extracts on somebacterialand fungal strains. In the present study the microbial activity of Methanol and Aqueous extract of Siddha formulation *Sangan ilai kudineer* (SKC). The studies were carried out in 50µl and 100µl concentration in each extracts against MTCC strains. The anti microbial activities of extracts of *Azima tetracantha* leaves were tested against two Gram positive bacteria- *Staphylococcus aureus and Bacillus subtilis*; three Gram negative bacteria- *Klebseilla pneumonia, E.coli and Pseudomonas aeruginosa*; and two fungal strains –*Aspergillus flavus and Aspergillus niger*. Siddha formulation of *Sangan ilai kudineer* was indicated for the treatment of Neuro muscular and chronic degenerative diseases.

KEYWORDS: Anti –bacterial, Anti-fungal, *Sangan ilai kudineer, Azimatertracantha.*

I. INTRODUCTION

Siddha system of medicine was one of the ancient Indian systems of medicines containing large number of medicines. The uniqueness of Siddha system was evident by its continuous service to the humanity for many years in combating diseases and also in maintaining its physical, mental health. In Siddha system, drugs were safe and available for long period

compared to modern medical system. The Siddha system of medicine includes herbs, minerals, metallic salts and animal products. The Siddha formulation of mono herbal therapy was widely used nowadays because its low cost effective and high efficacy in nature. Currently, microbial infections had become an important vital role to cure many clinical conditions. In this article to screen the anti bacterial and antifungal potential of the Siddha formulation *Sangan ilai kudineer* mentioned in *Gunapadam Mooligai Vaguppu*- pg. no 415[6] in the treatment of Neuro muscular and chronic degenerative diseases.

Leaves of Azima tetracantha

II. TAXONAMY

Kingdom	:	Plantae
Division	:	Magnoliopsida
Class	:	Magnoliopsida
Subclass	:	Eurosid
Order	:	Brassicales
Family	:	Salvadoraceae
Genus	:	Azima
Species	:	Azimatetracantha

Description

Azima tetracantha is a perennial shrub, much branched, spiny, evergreen shrub growing upto 3m tall. It particularlygrows on alkaline or saline soil near lakes and seasonal rivers. The plant is dioecious, erect shrub with 1-2 spines and ½- 1 inch long in each leaf axil[1]; branchlets are terete or quadrangular, glabrous to densely hairy. The leaves of the plant are stiff, shining, sharplymucronat or spinescent, simple, elliptical in shape and are rigid, pale green colored. The flowers aresmall, greenish, white or yellow coloured, unisexual in axillary fascicles.[4] Fruit are globular, white shiny. Seeds are compressed, circular.[7] The plant is

harvested from the wild mainly for local use as a medicine. It is sometimes cultivated for medicinal use and is grown as a hedge and oranament.[9]

III. MATERIALS

3.1 Collection of raw drug

The Sangan leaves (*Azima tetracantha*) were collected in and around the areas of Palayamkottai and Tirunelveli. The plant was identified and authenticated by the Medical botanist experts at Government Siddha Medical College and Hospital, Palayamkottai.

3.2 Preparation of formulation

Stalks and unnecessary parts of leaves were removed. And the leaves were dried in shade and processed to fine powder. Finally the powder was sieved using cloth and it is stored in air tight container.

3.3 Culture of pathogens

The microbial strains used in the sensitivity assay were *Staphylococcus aureus*(MTCC 916), *Bacillus subtilis*(MTCC1134), *Klebseilla pneumonia*(MTCC 530), *E.coli*(MTCC1671), *Pseudomonas aeruginosa*(MTCC1671), *Aspergillus flavus*(MTCC 335), *Aspergillus niger* (MTCC 281). The test microorganisms used for antimicrobial analysis Microbial strains were purchased from Microbial Type Culture Collection and Gene Bank (***MTCC***)Chandigarh. The bacterial strains were maintained on Nutrient Agar (NA).

3.4 Standard controls

The standard drugs used as control were Streptomycin *(S25*) for anti-bacterial study and Fluconazole for anti-fungal study.

IV. METHODS

4.1 Test procedure

The anti-microbial and anti-fungal study was conducted at Inbiotics, William hospital campus, MS road, Nagercoil, KK district, Tamil nadu.

4.2 Cleansing and Sterilization

The glass-wares used cleaned with cleaning solution and sterilized in hot air oven to 180ºC for 3 hours. All nutrient media were sterilized by autoclave (121ºC, 15psi for15-20 mins).

4.3 Preparation of test drug samples

1gram of test drug was diluted in1ml 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 4ºC to avoid the fermentation of the sample. Then the extracts were subjected to anti-microbial assay.

4.4 Anti-bacterial assay

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.

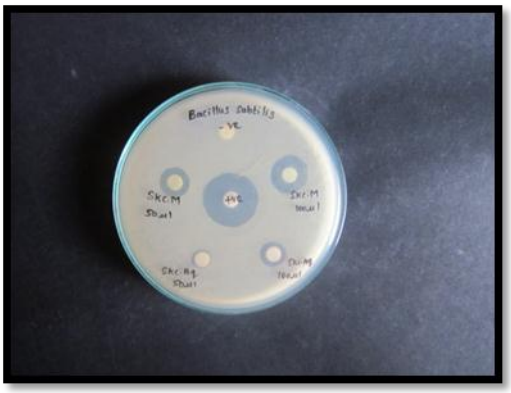

Fig no 1: Bacillus subtilis.

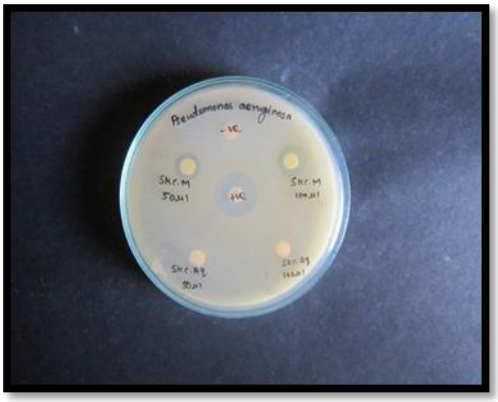

Fig no 2: Pseudomonas aeruginosa.

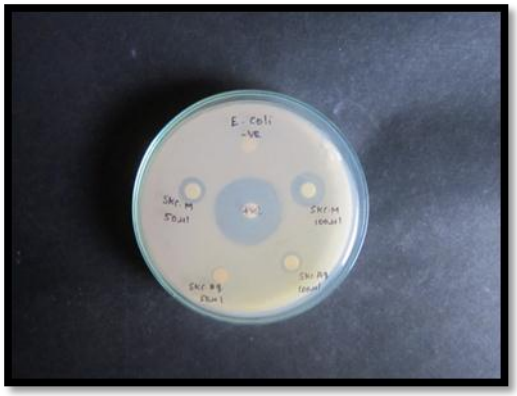

Fig no 3: E.coli.

Fig no 4: Klebseilla pneumonia.

Fig no 5: Staphylococcus aureus.

4.5 Antifungi assay

Antibiotic susceptibility tests were determined by agar disc diffusion (Kirby–Bauer) method. Fungi strains were 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.

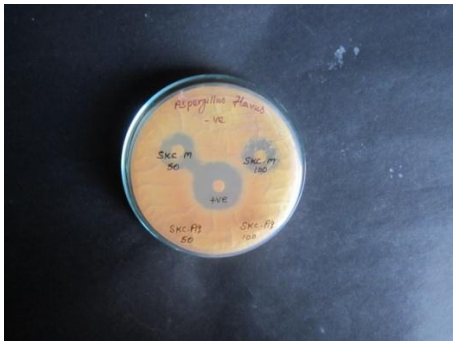

Fig no 6: Aspergillus flavus.

Fig no 7: Aspergillus niger.

V. RESULTS AND DISCUSSIONS

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).

Table no 1: AntiBacterial activity against Gram +ve&ve Bacteria.

Sample Code	Strains Name				
	***Staphylococcus aureus* (G+) MTCC 916**	**Bacillus subtilis (G+) MTCC 1134**	***Klebsilla pneumonia* (G-) MTCC 530**	***E.coli*(G-) MTCC 1671**	***Pseudomonas aeruginosa* (G-) MTCC 741**
SKC. M. 50	13	13	10	11	11
SKC. M. 100	17	16	13	15	13
SKC. Aq.50	10	10	7	7	-
SKC. Aq. 100	12	12	9	10	8
PC	20	24	18	31	18
NC	-	-	-	-	-

PC (Bacteria)-Positive control (Streptomycin- S 25), PC (Fungi) - Positive control (fluconazole), NC - Negative (plain disc) , - - No Zone, Mm - Millimeter, G+ - Gram Positive, G- - Gram Negative.

IN METHANOL EXTRACT

As per **table no 1 & Fig. no1-5:** The methanol extract of 100µl in both Gram+ve & ve bacteria strains shows more inhibition in *Staphylococcus aureus(17mm*) followed by *Bacillus substilis(16mm) & E.coli(15mm*). And equal zone of inhibition in *Klebseilla pneumonia& Pseudomonas aeruginosa*(13 mm) respectively showed lesser inhibition and possess least activity.

As per **table no 1 & Fig. no 1-5:** The methanol extract of 50µl in both Gram+ve &-ve bacterial strains shows more inhibition in *Staphylococcus aureus & Bacillus substilis(13mm)* respectively followed by *E.coli(11m) & Pseudomonas aeruginosa(11mm). Klebseilla pneumonia(10mm)* showed lesser inhibition.

IN AQUEOUSEXTRACT

As per **table no 1 & Fig. no 1-5:** The aqueous extract of 100µl in both Gram+ve & -ve bacterial strains shows more inhibition in *Staphylococcus aureus(12mm) & Bacillus substilis*(12mm) followed by *E.coli*(10mm) & *Klebseilla pneumonia(9mm) and Pseudomonas aeruginosa (8mm*) showed lesser inhibition.

As per **table no 1 & Fig.no 1-5:** The aqueous extract of 50µl in both Gram +ve & -ve bacterial strains shows more inhibition *Staphylococcus aureus & Bacillus substilis(10mm) respectively* and lesser zone of inhibition in *E.coli & Klebseilla pneumonia(7mm)*respectively and no zone of inhibition observed in *Pseudomonas aeruginosa*.

Table no 2: Anti Fungal Assay.

DRUG	Strains Name	
	***Aspergillus flavus*(F) MTCC 335**	***Aspergillus niger* (F) MTCC 281**
SKC. M. 50	20	14
SKC. M. 100	23	18
SKC. Aq.50	-	-
SKC. Aq. 100	-	-
PC	28	14
NC	-	-

As per **table no 2 & Fig.no 6&7**: In Methanol extract of 100µl shows more inhibition in *Aspergillus flavus* (23mm) than *Aspergillus niger*(18mm). In methanol extract of 50µl shows more inhibition in *Aspergillus flavus* (20mm) than *Aspergillus niger(*14mm). There is no zone of inhibition against the fungus *Aspergillus niger & Aspergillus flavus* in aqueous extract.

VI. CONCLUSION

The Siddha formulation *Sangan ilai kudineer* (SKC) has promising action in the management of microbial infection against Staphylococcus aureus(17mm), Bacillus substilis(16mm), E.coli(15), Pseudomonas aeuroginosa(13mm), Klebseilla pneumonia(13mm) and same extracts are very effective in fungus like Aspergillus flavus(23mm) than Aspergillus niger(18mm). The results Anti-microbial tests in methanol and aqueous extracts indicated Azima tetracantha leaves is very effective and maximum inhibitory zone against both Gram +ve bacteria & -ve bacteria and fungus.

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