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EVALUATION OF ANTI MICROBIAL ACTIVITY OF SIDDHA POLYHERBAL FORMULATION- *KARISALAI MATHIRAI*

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ABSTRACT

Our environment is blessed with lot of herbs, many of them having medicinal value. The traditional system of medicine makes use of such medicinal herbs showing their potency in treatment of various diseases. One such traditional system in India is the Siddha system of medicine, which insists treatment with medicinal plants on its first priority *Karisalai mathirai*, a siddha polyherbal formulation was evaluated for its antimicrobial action against several bacterial species name Pseudomonus aeruginosa, E.coli, Staphylococcus aureus, Bacillus subtilis,Proteus vulgaris and Fungal species name Aspergillus flavus and Candida albicans.

KEYWORDS: *Karisalai mathirai*, antimicrobial, zone of inhibition.

INTRODUCTION

Microbes are omnipresent. Some are beneficial to humans while others are harmful. Screening of antimicrobial activity of herbal formulations is essential for scientific validation of the medicines formulated from evidences based on literature. Pseudomonas aeruginosa is a gram-negativebacillus, which can cause diarrhoea, dysentery, UTI, meningitis, urinary tract infection. P.aureus causes various skin lesions such as abscess, carbuncles and other infections such as osteomyelitis, pyoderma, pneumonia, endocarditis, septicaemia, food poisoning, toxic shock syndrome, etc. Agar cell diffusion method was used for the study. Streptomycin was used as a standard for antimicrobial screening and fluconazole for antifungal activity.

MATERIALS AND METHODS

The drug *Karisalai mathirai* is mentioned in the Siddha text book ANUBAVA VAITHIYA DEVA RAGASIYAM ”-Second Edition:1991 Author:J. Seetharamprasad, Page No:53.

DRUG: *KARISALAI MATHTHIRAI* INGREDIENTS

TAMIL NAME	BOTANICAL NAME/FAMILY	PART USED
Karisalai	*Eclipta prostrate (Asteraceae)*	*Leaf*
Milaku	*Piper nigrum (Piperaceae)*	*Dried fruit*

METHOD OF PREPARATION

Purification and Preparation of Karisalai maththirai

All these drugs will be purified as per classical siddha texts. All this drugs are pulverised into fine powder and bottled up.

DRUG COLLECTION AND AUTHENTICATION

All ingredients of the drugs were bought from Nagarkovil and authenticated at Department of Botany, Government Siddha Medical College, Palayamkottai.

PROCEDURE

Antibacterial Activity Procedure

Dilution: 1mg in 1ml

Test Organism

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).

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 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)

ANTIFUNGAL ASSAY BY DISC DIFFUSION METHOD (Bauer *et al*., 1966)

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.

RESULTS AND DISCUSSION

The anti microbial activity of the KARISALAI MATHTHIRAI was evaluated in vitro against Bacteria – Staphylococcus aureus, Bacillus subtilis, Proteus vulgaris, E.coli, Pseudomonas aeruginosa and Fungal-Candida albicans, Aspergillus flavus.

Sample Code	Strains Name						
	Staphylococcus aureus **(G+) MTCC 916**	***Bacillus subtilis*** **(G+) MTCC 1134**	***Proteus vulgaris*** **(G-) MTCC 426**	***E.coli*** **(G-) MTCC 1671**	**Pseudomonas aeruginosa (G-) MTCC 741**	**Aspergillusflavus (F) MTCC 535**	**Candida Albicans (F) MTCC 183**
KM. M.50	16	13	11	12	18	13	16
KM.M. 100	21	16	16	19	21	17	19
KM. M Aq. 50	9	-	8	11	15	-	11
KM. M Aq. 100	11	7	10	14	19	-	13
PC	19	19	18	19	18	28	26
NC	-	-	-	-	-	-	-

PC (Bacterria) - *Positive control (Streptomycin- S 25),*

PC (Fungi) - *Positive control (fluconazol),*

NC - Negative (plain disc),

\- - No Zone,

Mm - Millimetre,

G+ - Gram Positive - G-Gram Negative .

ANTI BACTERIAL ASSAY

IN METHANOL EXTRACT

As per table no 1 & FIg no 1-5

The Methanol extract of 100ul in both Gram+ve & Gram-ve bacteria strains shows more equal zone of inhibition in Staphylococcus aureus & pseudomonas aeruginosa (21mm) followed by E.coli(19mm) and equal zone of Bacillus subtitles & proteusvulgaris(16mm) respectively showed lesser inhibition and possess least activity.

As per table no 1 & Fig 1-5

The Methanol extract of 50ul in both Gram+ve& Gram-ve bacterial strains shows more inhibition in pseudomonus aeruginosa (18mm)& Staphylococcus aureus (16mm) & Bacillus subtilis(13mm) & E.coli(12mm) and proteus vulgaris (11mm) showed lesser inhibition and possess least activity.

IN AQUEOUS EXTRACT

As per table no1&Fig 1-5

The aqueous extract of 100ul in both Gram+ve & Gram-ve bacterial strains shows more inhibition in pseudomonus aeruginosa (19mm) & E.coli (14mm) & staphylococcus aureus (11mm) & proteus vulgaris(10mm) and Bacillus subtilis(7mm)showed lesser inhibition.

As per table no1&Fig 1-5

The aqueous extract of 50ul in both Gram+ve &-ve bacteria strains shows more inhibition in pseudomonus aeruginosa (15mm)&E.coli(11mm) & staphylococcus aureus (9mm) & proteus vulgaris(8mm) and no zone of inhibition observed in bacillus subtilis.

ANTI FUNGAL ASSAY

IN METHANOL EXTRACT

As per table no 1&Fig no 6&7:

In Methanol extract of 100ul shows more inhibition in Candida albicans (19mm) & Aspergillus flavus(17mm).

As per table no1&Fig no6&7:

In Methanol extract of 50ul shows more inhibition in Candida albicans(16mm), Aspergillus flavus(13mm).

IN AQUEOUS EXTRACT

As per table no1&Fig no 6&7

In aqueous extract of 100ul shows more inhibition in Candida albicans(13mm)and no zone of inhibition observed in Aspergillus flavus.

As per table no1&Fig no 6&7:

In aqueous extract of 50ul shows more inhibition in Candida albicans (13mm) and no zone of inhibition observed in Aspergillus flavus.

Fig no 1:E.coli

Fig no 2: Staphylococcus aureus

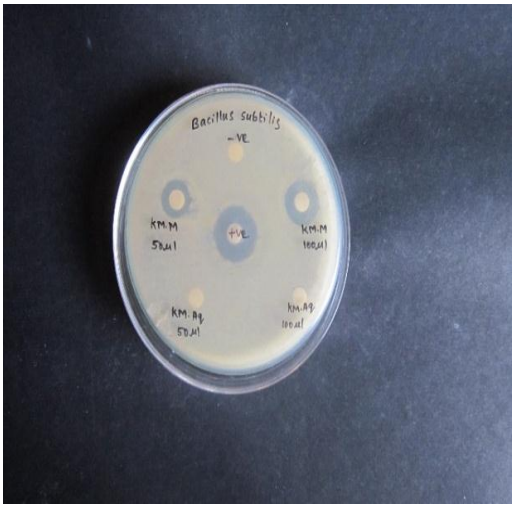

Fig no 3:Bacillus subtilis

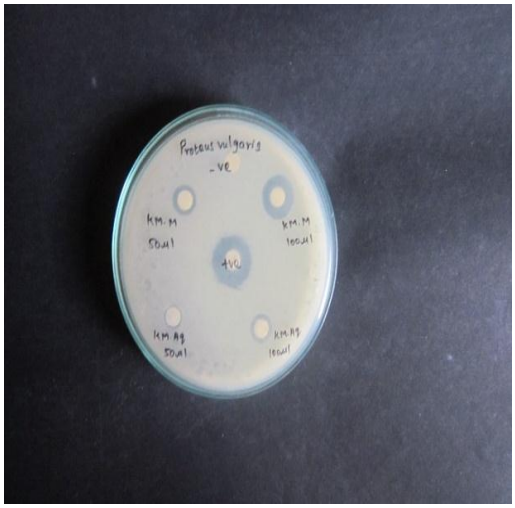

Fig no 4:Proteus vulgaris

Fig no 5:Pseudomonas aeruginosa

Fig 6:Candida albicans

Fig no 7:Aspergillus flavus

The zone of inhibition for P.aeruginosa is higher, followed by E.coli and S.aureus.

CONCLUSION

From the study, it is clear that the drug has some antimicrobialactivity. A further study regarding its constituents, phytochemicals and pharmacological studies is to be done for the evaluation of its therapeutic efficacy. The high zone of inhibition present in staphylococcus aureus and Pseudmonas aeuroginosa(21mm) respectively, moderate zone of inhibition seen in E.Coli and Candidaalbicans(10mm)respectively. Mild zone of inhibition seen in Bacillus subtillis(7mm).

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