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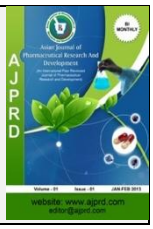

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Research Article

Evaluation of Anti Microbial Activity of Classical Siddha Medicine *Seenthil Choornam*

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A B S T R A C T

Seenthil Choornam is a Classical Siddha medicine having its ingredients with herbal andanimal origin. The raw drugs- *Seenthil* (*Tinospora cordifolia* .sp), Karisalai *(Ecilpta Albas*p), *and Poonagam(Eudriluseugeniae.*sp) were purified and combined as per theliterature. Aim: - The aim of the study is to evaluate the anti-microbial activity of *Seenthil Choornam.*

Materials and methods: - Raw drugs were collectedfrom Keralaand Tamilnadu .The identification and authentication of plant

drugs were obtained fromGovt.Siddha Medical College, Palayamkottai. The authentication of *Poonagam (Eudrilus eugeniae*sp.*)* was obtained from Advanced Centre of Environmental Studies and Sustainable Development-Inter University Centre – Mahatma Gandhi University, Kottayam...Anti-bacterial and Anti-fungal activity of aqueous and ethanolic extract of drug is studied for its activity against four Gram positive, three Gram negative bacteria and five fungi in concentrations of 25ul, 50ul, 75ul and 100ul , using Disc diffusion method. The standard drug for comparison of anti-bacterial activity is Streptomycin and for Anti-fungal activityis Fluconazole. Result:- The aqueous extract of drug in concentrations of 75ul & 100ul has zone of inhibition against *Bacillus subtilis* (G+ve)- (*19mm, 21mm*) ,*Enterococcusfeacalis*-**(**G+ve)in conc.75ul&100ul - (11mm, 13mm), *Klebsiella pneumonia* (G-ve) in conc.75ul &100ul- (11mm ,13mm) , *Staphylococcus aureus* (G+ve)in conc. 75ul &100ul-(10mm,11mm**)** and towards fungus -*Aspergillu**s** flavus*in conc. 75ul &100ul (12mm, 13mm) .From the above study it is evident that the classical medicine*Seenthil Choornam*is effective against human pathogens.

Key words: - Anti bacterial, Anti-fungal, Seenthil Choornam.

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INTRODUCTION:-

Indian system of Medicines is one of the mainstays of world's traditional medical system.SIDDHA system of medicine was introduced and propagated by the great Saints "*Siddhars*". This system of medicine uses Herbal, Animal, Mineral, Herbo- mineral and Herbo- Animal drug formulations in treating, acuteand chronic illness. *Seenthil Choornam* is a Herbo –animal formulation with its reference *Agathiyarparipooranam-400.*It contains *Seenthil*-(*Tinospora cordifolia* .sp*Menispermaceae*, family -Bentham &Hooker cl), *Karisalai* (*EcilptaAlbas*p, *Asteraceae*, family - Bentham &Hooker cl) and *Poonagam* (*Eudrilus eugeniae*.sp,*Eudrilidae*, family, Phylum-*Annelida).* This formulation contains a number of bioactive molecules

likeFlavonoids, Alkaloids& Glycosides. This study deals with the Anti-Microbial activity of *SeenthilChoornam* in its ethanolic and aqueous extracts at various concentrations against Gram Positive, Gram Negative bacteria and Fungi. The standard drug for comparison of Anti –Bacterial activity is Streptomycin and for Anti-Fungal activity is Fluconazole. Chronic diseases like,autoimmunediseases, Skin diseases, Respiratory system diseases, due to constant medications and other consecutive diseases, there will be decreased immune system functioning resulting in other opportunistic Infections. It is better to treat chronic diseases with drugs which can compromise both the illness and other following diseases. Siddha medicines always containdrugs, in which the adverse effect of one drug is alleviated by the other drug. So, Siddhars had formulated each medicine by foreseeing this aspect. So, Antimicrobialstudy of the drugs, used for treating chronic ailments can definitely discover its efficacy in managing the diseases.

MORPHOLOGY:-

Tinospora cordifolia, which is commonly known as *Seenthil* (Tamil), *Guduchi*(Sanskrit), *Amrita,Giloy* is a deciduous climbing shrub growing up to height of 3-4 feet height. It is indigenous to South Asia. It has fleshy aerial branch roots. Stem: - Greyish brown color, cylindrical with a circumference of 5mm to 25mm. Leaf: - Simple, 5-10cm long and rounded. Bark: - it is grey in color and easy to peel.Flower: - Unisexual, yellow or yellowish green coloured. Fruit: - Drupe, Reddish and fleshy. Seed: - Curved seeds, so the plant is called heart leaved moon seed plant. It has a bitter taste. Mostly matured stem, sometimes whole plant is used for medicinal preparations.

Figure 1: Whole plant –*Seenthil* (*Tinospora cordifolia*)

Ecilpta alba, commonly known as *Karisalai (*Tamil), *Bhringaraj* (Sanskrit), *TrailingEcilptaplant* (English), is an erect or prostate annual herb growing up to height of 30 - 40cm. Stem :- Cylindrical with small white hairs . Leaves: - opposite, single, 2.0cm to 6.2 cm long with hairs on both leaf surfaces. Flower: - White solitary unisexual flowers. Fruit: - Cypsela, one seed with a narrow wing. Plant is growing throughout India mostly in moist places. Whole plant is used for medicinal preparations.

Figure:2 *Karisalai*(*Eclipta Alba*)

Eudrilus eugeniae (Kinberg, 1867) collected from Madurai District, Tamilnadu belongs tofamilyEudrilidae. External: - Color, reddish to purple or dark mauve. Setae Lumbricine. Prostomium epilobus tongue open. Copulatory chamber apertures, transverse slits centered at or lateral to setae *b*, slightly in front of 17/18. Vaginal apertures, transvers slits centered at or median to setae c, presetal in segment 14. Clitellum on segments 14-18, intersegmentfurrows faintly indicated setae visible. Nephridopores lateral to c setal lines. Genital markings absent.

Internal: - Septa, all present from 4/5, 6/7 and several sub-sequent septa slightly strengthened .Pigment, red, in circular muscle layer. Gizzard, in segment 5. Intestinal origin, close to segment 14/15. Typhlosole and caeca, absent. Dorsal blood vessel, aborted in front of hearts of 7.Testis sacs, unpaired ventral. Prostate, long, duct short slender and muscular.

Figure: 3 *Poonagam* (*Eudrilus eugeniae)*

MATERIALS AND METHODS:-

Raw drug Collection and medicine preparation:-

1. ***Seenthil (Tinospora cordifolia***) was collected from Kottarakkara, Kollam District, Kerala. The matured stem of the plants were taken and are cleaned by washing them with

water for 21 times and soaking in butter milk as per "*Agathiyar Paripooranam 400*". The purified raw drugs were dried in sunlight.

2. ***Karisalai* (*Ecilpta Alba*)** were collected from Tirunelveli District, Tamilnadu .The whole plants were cleaned by washing with water and dried in sunlight.

The identification and authentication of the plant raw drugs were obtained from Department of Botany, Government Siddha Medical College, Palayamkottai, Tirunelveli.

3. ***Poonagam* (Eudrilus eugeniae)** is collected from Vadipatti, Madurai District, and Tamilnadu. The worms were washed thoroughly with water and were soaked in milk for 3 hours to remove engulfed soil particles from body of earthworm. After that lime water was sprinkled over the earth worm. Then it was placed in sunlight until it was dried well. The identification and authentication of the earth worm were obtained from Advanced Centre of Environmental Studies and Sustainable Development-Inter University Centre – Mahatma Gandhi University. All the above medicines were finely powered and filtered. All the powered medicines were kept in air tight containers. Above three medicines were mixed in a ratio of 3:3:1 as per the literature.

TEST SAMPLE PREPARATION:

The given sample was dissolved in concentration of 0.1g/1ml of distilled water and ethanol for makingextracts. Extracts is prepared using Soxhlet extraction method.

ORGANISMS FOR SCREENING OF ANTIMICROBIAL ACTIVITY:-

The microorganisms –Bacteria and Fungi were isolated from clinical samples.

BACTERIA: -

Gram Positive bacteria: - 1. *Staphylococcus aureus*(Gram +ve),2.*Bacillus subtilis* (Gram+ve), 3.*Enterococcus feacalis* (Gram +ve), 4. *Streptococcus mutant* (Gram +ve).

GRAM POSITIVE BACTERIAE:-

Figure: 4 - ***Streptococcus mutant***

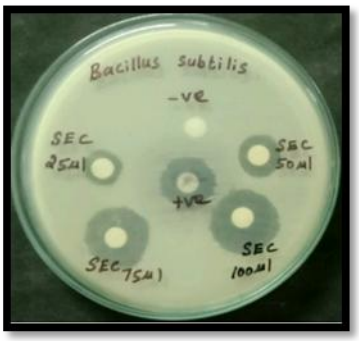

Figure 5: ***Bacillus subtilis***

Gram Negative bacteria: -1.*Escherichia coli* (Gram -ve), 2. *Klebsiella pneumonia* (Gram -ve), 3. *Pseudomonas aeruginosa* (Gram –ve).

FUNGI: - 1. *AspergillusNiger*, 2. *Aspergillus flavus*, 3. *Aspergillus terreus*, 4. *Pencilliumnotatum* 5. *Rhizhopus sp.*

Nutrient Brothpreparation: - Pureculture from the plate was inoculated into Nutrient Agar plate and sub cultured at 37^0c for 24hours. Inoculum was prepared by aseptically adding the fresh culture into 2ml of sterile 0.145mol/l saline tube and the cell density was adjusted to 0.5Mcfarland turbidity standard to yield a bacterial suspension of 1.5x108cfu/ml. Standardized inoculum is used for Antimicrobial test.

ANTI BACTERIAL TEST:-

The medium was prepared by dissolving 38gms of Muller Hinton Agar Medium (Hi Media) in 1000ml of distilled water. The dissolved medium was autoclaved at 15Lbs pressure at 121^0c for 15 minutes (p^H 7.3). The autoclaved medium was cooled, mixed well and poured petriplates (25ml/plate) the plates were swabbed with pathogenic bacterial culture viz. Staphylococcus aureus, Streptococcus mutant, Bacillus subtilis, Enterococcus feacalis, Pseudomonas aeruginosa, Klebsiella pneumonia, E.coli. Finally, about 10ul of sample (N.S and N.B) was loaded into the disc then placed on the surface of Muller Hinton medium and the plates were kept for incubation at 37^oc for 24hours.At the end of the incubation ,inhibition zones were examined around the disc and measuredwith transparent ruler in millimeters. The size of the zone of inhibition (including disc) was measured in millimeters .The absence of zone of inhibition was interpreted as the absence of activity. The activities are expressed as resistant ,if the zone of inhibition was less than 7mm, intermediate (8-10mm), and sensitive if more than 11mm**.**

Figure:-6 ***Staphylococcus aureus***

Figure :-7***Enterococcus feacalis***

GRAM NEGATIVE BACTERIAE*:-*

Figure: 8 ***Klebsiella pneumonia***

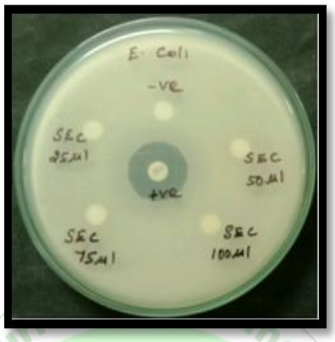

Figure:-9 ***Escherichia coli***

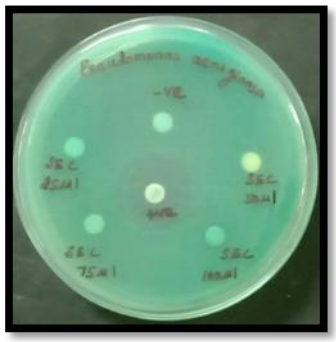

Figure: 10 ***Pseudomonas aeruginosa***

ANTI FUNGAL ASSAY:-

Antibiotic susceptibility tests were determined by agar disc diffusion method (Kirby-Bauer) .Fungalstrains were swabbed using sterile cotton swabs in SDA agar plate.10ul of each sample were introduced in the sterile discs using sterile pipettes respectively. 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 22^0c for 48 hours. At the end of incubation period, the inhibition zones were examined around the disc and measured with transparent ruler in millimeters.

Figure 11:- *Pencillium notatum*

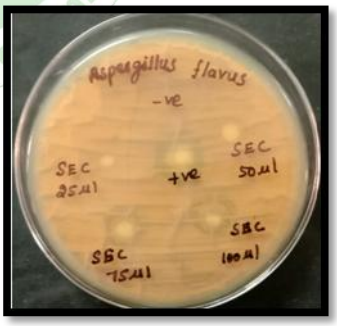

Figure 12: *Aspergillus flavus*

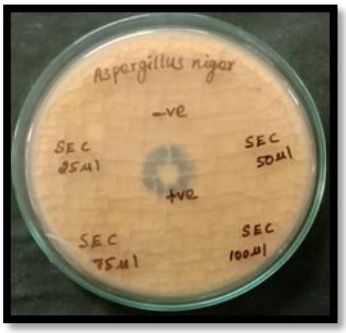

Figure 13:- *Aspergillus terreus*

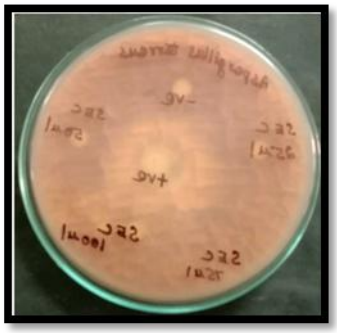

Figure 14: *Aspergillus Niger*

Figure 15: *Rhizhopus sp*

RESULT:-

After the prescribed incubation period, the petridishes were carefully taken and examined the inhibition zone around the disc and measured with a transparent scale in millimeters.The antimicrobial activity is assessed as follows: - Absence of zone of inhibition –absence of activity, if the zone of inhibition is less than 7mm –resistant**,** zone of inhibition is between 8-10mm – intermediate**,** Zone of inhibition is more than 11mm –sensitive**.**

Table 1: Anti-Bacterial activity against Gram Positive Bacteria

Sample Code	Gram Positive Bacterial Strains			
	Staphylococcus aureus (G+ve)	*Bacillus subtili* (G+ve)	*Enterococcus feacalis*(G+ve)	*Streptococcus mutant* (G+ve)
SEC. Aq.25ul	-	11mm	-	-
SEC .Aq.50ul	-	13mm	-	-
SEC .Aq.75ul	10mm	19mm	11mm	-
SEC.Aq.100ul	11mm	21mm	13mm	-
SEC.E.25ul	-	-	-	-
SEC.E.50ul	-	-	-	-
SEC.E.75ul	-	-	-	-
SEC.E.100ul	-	-	-	-
Positive Control	18mm	16mm	19mm	15mm
Negative Control	-	-	-	-

Key words: - **PC**-Positive control (Streptomycin), **NC**- Negative control, "-"No Zone,**Aq**-Aqueous extract**, E**- Ethanol extract, **Mm**- Millimeter, **G+ve** –Gram positive

Antibacterial activity of Aqueous Extract of Sample (SEC) against Gram Positive Bacteria:

(Table -1, Fig: 4-7)

The Aqueous extract of Sample (SEC) in concentrationsof 25ul, 50ul, 75ul and 100ulshows zone of inhibition(11mm ,13 mm , 19mm, 21mm.) respectively against *Bacillus subtilis* (G+ve) bacteria.

In concentration of 75ul & 100ul Aq.extract of sample –(SEC) shows a zone of inhibition of **(**11mm,13mm) towards *Enterococcus feacalis.*

In concentration of 75ul& 100ulof SEC –Aq.extract shows a zone of inhibition of (10mm, 11mm) against *Staphylococcus aureus* (G+ve).

Bacillus subtilis is highly sensitive, *Enterococcusfeacalis* is sensitiveand*Staphylococcus aureus* is also sensitive towards Aq.extract of sample (SEC).

The zone of inhibition is absent against *Streptococcus mutant*. So it is resistant towards aqueous extract of sample (SEC).

Antibacterial activity of Ethanolic Extract of Sample (SEC) against Gram positive Bacteria

:- (Table-1)

The Ethanolic extract of Sample (SEC) in concentrations of 25ul, 50ul, 75ul and 100ul does not exhibit zone of inhibition against all the four Gram positive Bacteria-

Bacillus subtilis, Enterococcus feacalis, Staphylococcus aureus and Streptococcus mutant. The four, Gram Positive bacteria are resistant towards the ethanolic Extract of sample (SEC).

Table.2: **Antibacterial activity against Gram Negative Bacteria**

Sample Code	Gram Negative Bacterial Strains		
	Escherichia coli (G –	*Klebsiella pneumonia (G* –	*Pseudomonas aeruginosa*(G –
SEC .Aq.25ul	-	-	-
SEC .Aq.50ul	-	-	-
SEC.Aq.75ul	-	11mm	9mm
	-	13mm	10mm
SEC .E.25ul	-	-	-
SEC .E.50ul	-	-	-
SEC.E.75ul	-	-	-
SEC.E.100ul	-	-	-
Positive	20	17	25
Negative	-	-	-

PC= positive control (Streptomycin), NC= Negative control, "-"= no zone, G –ve= gram negative, E= ethanol, Aq=aqueous.

Antifungal activity of Sample (SEC) (Table :3, Fig: (11-15)

The Aqueous extract of the sample in concentrations of 75ul and 100ul exhibit zone of Inhibition (-12mm& 13mm) against *Aspergillus flavus*. *Aspergillus flavus* is sensitive towards Sample (SEC) and all other fungal strains were not resistant towards the aqueous and ethanolic extract of Sample (SEC).

CONCLUSION:-

By the above study, it is revealed that aqueous extracts of Seenthil Choornam have significant anti-microbial activity against gram positive bacteria –*Bacillus subtilis*, *Staphylococcus aureus* and *Enterococcus feacalis* and gram negative bacteria *Klebsiella pneumonia* and *Pseudomonas aeruginosa*. Also the medicine has very effective activity against fungi- *Aspergillus flavus.* So this medicine can be used to treat infectious diseases, especially associated with chronic inflammatory diseases.

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