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Anti-microbial screening of a Siddha formulation *Kottikkizhangu* Chooranam (Aponogeton Monostachyon)

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ABSTRACT

Siddha Medicine is the traditional medicine originating in ancient Thamilagam in south India. Eighteen siddhars laid the foundation for this system of medicine. The siddha system is largely therapeutic and prophylactic in nature. The aim of the work is to prove the anti-microbial activity of the *Kottikkizhangu* chooranam which is mainly indicated for *Thaemal* (Leucoderma) and *Vellai* (Leucorrhoea) mentioned in Materia Medica- Siddha. *Kottikkizhangu* chooranam (Aponogeton monostachyon) is a Siddha formulation. It has *Kulirchiundakki (Refrigerant),Uramakki (Tonic) actions.* Antibiotic susceptibility tests were determined by Agar disc diffusion (Kirby-Bauer) method. This drug was tested for antimicrobial property against *Proteus vulgaris, Staphylococcus aureus, Streptococcus mutans, Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli* by Agar disc diffusion methods. Streptomycin is used as a positive control for this study. The results obtained from the study showed that *Kottikkizhangu* chooranam has antimicrobial property against Streptococcus mutans are more effective.

KEYWORDS

Kottikkizhangu chooranam, *Aponogeton monostachyon* Antimicrobial, Siddha formulation.

INTRODUCTION

Antimicrobial agent destroys or inhibits the growth of microorganisms. Antimicrobial agents from plants are plentiful such as Penicillin, *Cycloartenol, Stigmasterol, Fusarubin*. Plants produce

secondary metabolites which protect from fungi, bacteria and insects. These compounds include flavonoids, phenols, phenolic glycosides, unsaturated lactones, sulphur compounds, Saponins, glycosides and glucosinalates (Gomez Garibay et al., 1990, Osvourne, 1996). The antimicrobial activity of plants extracts and phytochemical was evaluated with antimicrobial susceptibility test. *Kottikkizhangu chooranam* is a siddha formulation indicated for leucoderma, Leucorrhoea etc. This drug was tested for antimicrobial property against proteus vulgaris, staphylococcus aureus, Streptococcus mutans, Bacillus subtilis, klebsiella pneumonia, E.coli by Agar Disc diffusion method. The results obtained from the study showed that *Kottikkizhangu chooranam* has antimicrobial property against the specific micro organisms.

MATERIALS AND METHOD

Test drug

The drug kottikkizhangu chooranam was selected from Gunapadam mooligai (Siddha Materia Medica- Vegetable section). The drug was purchased from Raw drug store, Nagercoil.

Purification

The rhizome of the plant is washed and dried then outer layer of it is removed.

Preparation

The purified drug is demolished and made into fine powder by sieving.

Micro organisms

The test micro organisms *Proteus vulgaris, Staphylococcus aureus, Streptococcus mutans, Bacillus subtilis, Klebsiella pneumoniae, Escherichia coli* were purchased from Microbial Type Culture Collection (MTCC) and Gene Bank, Chandigarh. The bacterial strains were maintained on Nutrient Agar (NA).The Positive Control was Streptomycin.

Antimicrobial activity procedure

Dilution : 0.1g in 1ml

Test Organism

The test microorganisms used for antimicrobial analysis microorganism are Proteus vulgaris, Staphylococcus aureus, Streptococcus mutans, Bacillus subtilis, Klebsiella pnecumonia, Escherichia 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 0 C 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. Microorganism name 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 strains (Fungi Name) 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. At the end of incubation, inhibition zones were examined around the disc and measured with transparent ruler in millimeters.

Table.1 Organisms used for Anti-Bacterial Activity

Sl.No.	Organisms	Type
1	Proteus vulgaris,	Gram negative
2	Staphylococcus aureus,	Gram positive
3	Streptococcus mutans,	Gram positive
4	Bacillus subtilis,	Gram positive
5	Klebsiella pneumoniae,	Gram negative
6	Escherichia coli	Gram positive

RESULTS

Table.2 Zone of inhibition data of Anti-Bacterial Activity

Sample code	Bacteria Strains					
	Proteus vulgaris	**Staphylococcus aureus**	**Streptococcus mutans**	**Bacillus subtilis**	**Klebsiella pneumonia**	**E.coli**
Test drug	17	16	14	11	11	12
PC	27	30	15	15	19	17
NC	-	-	-	-	-	-

In concentration of test drug 10mg the Antimicrobial zone of inhibitions are Proteus vulgaris -63%, Staphylococcus aureus-53%, Streptococcus mutans-94%, Bacillus subtilis-73%, Klebsiella pneumonia-58% and Escherichia coli-70%

I) Staphylococcus Aureus

Staph. aureus is an important pyogenic organisms and lesions are localised in nature staphylococcal diseases may be classified as cutaneous and deep infections, food poisoning, nosocomial infections, skin exfoliative disease and toxic shock syndrome.

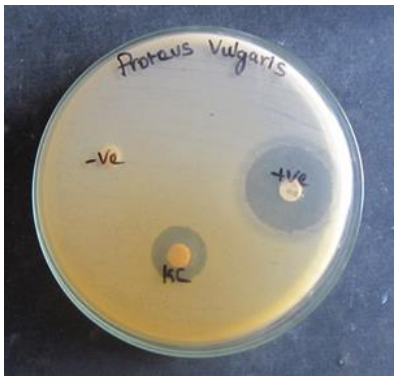
i) Cutaneous infections

Superficial infections includes pustules, boils, carbuncles, abscesses, styes, impetigo, pemphigus neonatorum, wound and burn infections.

ii) Deep infections

It causes urinary tract infections especially in association with local instrumentation or diabetes. It also causes osteomyelitis, tonsillitis, pharyngitis, sinusitis, pheumonitis, empyema, endocarditis, meningitis, bacteriaemia, septicaemia and pyaemia.

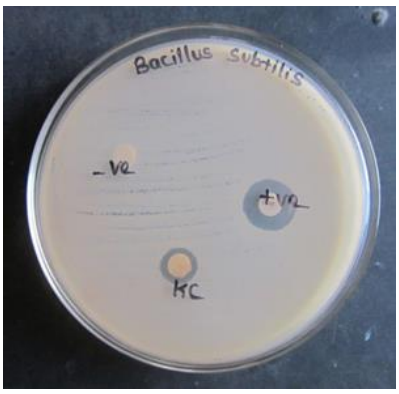
Proteus Valgaris



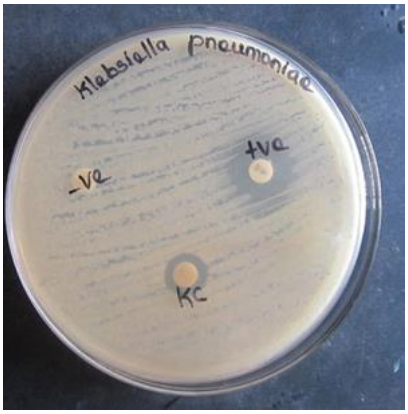
staphylococcus Aureus

Streptococcus Mutans

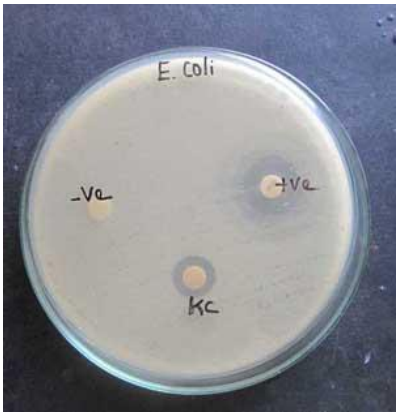
Bacillus Subtilis



Klebsiella Pneumoniae



Escherichia coli



iii) Food poisoning

Staphylococcal food poisoning may follow 2-6 hrs after ingestion of contaminated food which contain perfomed entertoxins of staph.aureus.

iv) Nosocomial infections

v) Skin exfoliative Diseases

vi) Toxic shock syndrome (TSS)

vii) Respiratory Disease

II) Klebsiella pneumoniae

Klebsiella pneumoniae is the flora of intestine of humans. It is responsible for severe bronchopneumonia, UTI, nosocomial infections, wound infections, septicaemia, meningitis and

rarely diarrhoea. Klebsiella Pneumoniae cause serious disease with high care fatality. It has been associated with atrophic rhinitis (Ozaena).

III) Escherichia coli

E.coli forms the normal intestinal flora of man and animal. There are four major types of clinical syndromes:

i) UTI

E.coli is the commonest organism responsible for urinary tract infection. Most frequent O Serotype of E.coli causing UTI include O1,O2,O4,O6,O7,O18 and O75. They are also named as nephritogenic strains.

ii) Diarrhoea

The Enterotoxin producted by E.coli are important in the pathogenesis of diarrhoea. E.coli cause diarrhoeal disease of 5 types

- Enteropathogenic Esch. coli (EPEC)
- Enterotoxigenic Esch.coli (ETEC)
- Enteroinvasive Esch.coli (EIEC)
- Enterohaemorrhagic Esch.coli (EHEC)
- Enteroaggregative Esch.coli (EAEC)

iii) Pyogenic infections: E.coli may cause wound infection, peritonitis, cholecystitis and neonatal meningitis.

iv) Septicaemia

E.coli is a very common cause of septicaemia in many hospitals and leads to fever, hypotension and disseminated intravasular coagulation (endotoxic shock).

IV) Bacillus subtilis

B.Subtilis is anthracoid or pseudoanthrax bacilli due to it resembling B.anthracis. It is aerobic spore bearing bacilli. Bacillus subtilis may act as opportunistic pathogen causing eye infection and septicaemia.

V) Proteus vulgaris

Proteus species are swarming growth on agar. It indentified as PPA test. It is commensal in intestine. It is saprophytic. It cause UTI and nasocomial infection. Pyelonephritis due to proteus is

particularly toxic as the ammonia produced by the organism interfers with complement and other defence mechanisms.

VI) Streptococcus mutans

It is normal commensal in month. It cause mainly dental caries. It break down dietary sucrose producing acid and a tough adhesive dextran. As the plaque formation progresses, it leads to the formation of a sponge like structure that gets soaked in acid. Acid than slowly diffuses into the tooth from overlying cariogenic plaque penetrates the enamal and finally attacks the dentin that leads to the destruction of root canal lined with nerve and blood cells.

CONCLUSION

Streptomycin was used as the standard Antibacterial agent. From this results of the present study it was concluded that the sample *Kottikkizhangu* chooranam shown antimicriobial properties against staphylococcus aureus, Streptoccus mutans, E.coli, proteus vulgaris, Bacillus subtillis, klebsiella pneumoniae. The test drug is more effective against Streptococcus mutans when compared to other bacteria . The diseases caused by streptococcus mutans such as Dental plaque, Subacute infective endocarditis,tooth decay can be cured by *Kottikkizhangu* chooranam. Further works are to be done in this drug to evaluate its potency.

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