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In vitro Anti-microbial activities of various extracts of Parangipattai Kudineer - Siddha formulation

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ABSTRACT

Aim: *The aim of this study is to screen the In vitro Anti-microbial activities of various extracts of Parangipattai Kudineer (PPK)-A Poly herbal Siddha formulation.*

Methodology: *PPK was collected from Siddha OPD, The TN Dr. M. G. R. Medical University. Ethanol, acetone and aqueous extract of PPK were prepared by soxhlet method. In vitro antimicrobial activity of above said three extracts of PPK were screened against Staphylococcus aerus, Streptococcus mutans, Aspergillus niger, Aspergillus flavus and Candida albicans using disc diffusion method. The microorganisms were collected from the Microbial Type Culture Collection (MTCC). Sterilized discs were soaked in ethanol, acetone and aqueous extract of PPK individually at the concentration of 25 mg/ disc. Anti-bacterial and anti-fungal suspension was inoculated in Muller-Hinton Agar Media and Potato Dextrose Agar Media respectively. Streptomycin and fluconozole was used as standard drug for the Antimicrobial study. Zone of Inhibition was measured and recorded.*

Result: *Ethanol and Acetone extract of PPK showed more anti-bacterial activity against Streptococcus mutans (8mm) and ethanol extract of PPK showed anti-fungal activity against Aspergillus flavus (12 mm).*

Key Words*: Parangi pattai kudineer, Anti-microbial activity, smilax china, skin diseases.*

1.Introduction

Siddha system of medicine is the oldest form of healthcare which is nourishing the mankind since thousands of years for all types of health issues. WHO estimates that about 4 million people, 80% of population are using traditional medicine in present senerio.[1] The prevalence of skin infection are increasing now-a-days in people of all age groups. Skin diseases are hard to get accustomed, to especially when it occurs in the region that is difficult to conceal like face, neck and hands. Common man find difficult to get a correct solutions from such infection. Siddha system has numerous solutions for microbial infection one among them is Parangipattai kudineer. It has been in practice for years together in Siddha Practice to manage skin diseases like Granthi (abscess),Vandukadi (insect bite), Padaigal (fungal infections), Viranangal (wounds).

Ingredients of Parangi pattai kudineer is Acorus calamus , Azadirachta indica, . Coscinium fenestratum, Curcuma longa, Embilica officinalis, Foeniculum vulgure, Tinospora cardifolia, Rubia cordifolia, Smilax china, Terminelia chebulla and Terminelia bellarica.

Acorus calamus and azadirachtra indica have been studied for anti-microbial properties against staphylococcus auerus and pseudomonas.[3,4] Ethanolic extract of Coscinium fenestratum has anti microbial activity against acne inducing bacteria.[5] Curcuma longa is tested for anti-microbial activity against bacillus sps and azotobacter.[6] Embilca officinalis has activity against gram positive bacteria and Candidia albicans.[7]

Tinospora cordifolia and smilax china showed anti-microbial properties against staphylococcus aureus, E-coli and proteus vulgaris.[8,9]The fruit extracts of triphala are rich in anti-microbial properties against Aspergillus *flavus* [10] .Methonolic extract of *Rubia cardifolia* showed anti-microbial activity against *Bacilus subtilis* and *staphylococcus auerus* [11]. Each single drug added in *parangipattai kudineer* has anti-microbial properties individually. Therefore, in this study, it was planned to screen the in vitro anti-microbial activity of Ethanol, Acetone and Aqueous extracts of PPK against *Staphylococcus aerus, Streptococcus mutans, Aspergillus niger, Aspergillus flavus and Candida albicans* to prove its efficacy scientifically.

2.Materials and Methods

Parangipattai Kudineer was selected from the Siddha literature for this anti-microbial study.

Source of *Parangipattai Kudineer*: PPK was collected from Siddha OPD, The TN Dr. M. G. R. Medical University for screening of anti-microbial properties. Ingredients of PPK are 1.*Acorus calamus* , 2. *Azadirachta indica,* 3. *Coscinium fenestratum*, 4.*Curcuma longa,* 5.*Embilica officinalis,* 6.*Foeniculum vulgure,* 7. *Tinospora cardifolia,* 8.*Rubia cordifolia,* 9.*Smilax china,* 10. *Terminelia chebulla,* 11.*Terminelia bellarica.*

Preparation of extract of PPK

The extract was prepared by taking 30gms coarse powder of PPK and 180 ml of water in soxhlet apparatus and the extract is obtained by hot continuous extraction at 90 degree Celsius. The same procedure was followed to get ethanol and acetone extract of PPK but the temperature was optional. The extract obtained with each solvent was filtered with whatmann filter paper no: 40nm, then the solvents are allowed to evaporate by placing it in water bath at 60 degree Celsius. The end product is collected and preserved in airtight container. The different solvent extracts of the PPK were tested for antimicrobial activity using disc diffusion method.

Culture and Media preparation for bacteria

The microbial strains used for this study are *Staphylococcus aureus and Streptococcus mutans.* The microorganisms were collected from the Microbial Type Culture Collection (MTCC), Chandigarh, India and maintained in the laboratory by periodic subculture.

Disc preparation: Antibacterial Assay Sterilized discs were soaked in ethanol extract of *Parangipattai Kudineer* (EEP) at the concentration of 25 mg/ disc and kept overnight in room temperature. The same procedure was followed to prepare the disc with acetone extract of *Parangipattai Kudineer* (ACEP) and aqueous extract of *Parangipattai Kudineer* (AQEP). Then the soaked discs were dried aseptically to ensure evaporation of solvents.

Anti bacterial Activity

Culture Media used: Muller-Hinton Agar Media

Standard drug Used: Streptomycin

The prepared Muller-Hinton Media was poured in each petri dish and allowed to cool. Cotton swabs charged with each test bacterial suspension were inoculated on Muller-Hinton agar plates and were spread over agar surface to make a lawn. Then the plates were allowed to dry for 20 minutes.

The sterile dried antimicrobial discs impregnated individually with each extract EEP, ACEP and AQEP at the concentration of 25 mg /disc were carefully dispensed with uniform distances placed on Muller-Hinton agar plates and incubated for 18-24 hours at 37°C. Streptomycin was used as standard drug for anti bacterial screening. The zone of inhibition was measured with the scale from the centre of disc to the clear zone in millimetre and the results were recorded [12].

Culture and Media Preparation for Fungus

The different solvent extracts like EEP, ACEP and AQEP were tested for antifungal activity using disc diffusion method. The microbial strains used for current study are *Aspergillus niger, Aspergillus flavus and Candida albicans*. The microorganisms were collected from the Microbial Type Culture Collection (MTCC), Chandigarh, India and maintained in the laboratory by periodic subculture.

Disc preparation: Antibacterial Assay Sterilized discs were soaked in ethanol extract of *Parangipattai Kudineer* (EEP) at the concentration of 25 mg / disc and kept overnight in room temperature. The same procedure was followed to prepare the disc with acetone extract of *Parangipattai Kudineer* (ACEP) and aqueous extract of *Parangipattai Kudineer* (AQEP). Then the soaked discs were dried aseptically to ensure evaporation of solvents.

Anti fungal Activity:

Culture Media used: Potato Dextrose Agar Media

Standard drug Used: Fluconozole

The prepared Potato Dextrose Agar Media was poured in each petri dish and allowed to cool. Cotton swabs charged with each test fungal suspension were inoculated on potato dextrose agar plates and were spread over agar surface to make a lawn. Then the plates were allowed to dry for 20 minutes. The sterile dried antimicrobial discs impregnated with EEP, ACEP and AQEP of 25 mg/disc were carefully dispensed with uniform distances placed on potato dextrose agar plates and incubated for 24-48 hours at 27° C. Fluconozole was used as standard drug for screening anti fungal activity. The zone of inhibition was measured from the centre of disc to the clear zone in millimetre and the results were recorded.[12]

Results

The results of *In vitro* anti microbial assay indicates that Ethanol and Acetone extract of PPK showed more anti-bacterial activity against *Streptococcus mutans* and ethanol extract of PPK showed anti-fungal activity against *Aspergillus flavus* as par

with the positive control. Results were expressed in Figure 1 & 2 and Table 1 & 2

Figure.1: Zone of inhibition (mm) of PPK against Bacteria

Table 1: Zone of Inhibition of PPK and Standard drug against bacteria

Bacteria	ZOI-EEP	ZOI-ACEP	ZOI-AQEP	Positive Control
Staphylococcus aureus	**NZ**	**NZ**	**NZ**	**12mm**
Streptococcus mutans	**8mm**	**8mm**	**NZ**	**15mm**

Figure 2: Zone of inhibition (mm) of PPK against fungus

Table 2: Zone of Inhibition of PPK and Standard drug against fungi

Fungus	ZOI - EEP	ZOI-ACEP	ZOI - AQEP	Positive Control
Aspergillus Niger	**NZ**	**NZ**	**NZ**	**16mm**
Aspergillus Flavus	**12mm**	**NZ**	**NZ**	**16mm**
Candidias Albicans	**NZ**	**NZ**	**NZ**	**22mm**

Note: PPK - *Parangipattai kudineer*, EEP- Ethanol extract of *Parangipattai Kudineer*, ACEP- Acetone extract of *Parangipattai kudineer*, AQEP- aqueous extract of *Parangipattai kudineer.*

DISCUSSION

PPK is extracted with water, acetone, and ethanol and subjected to anti-microbial studies. There was no scientific data available on *Parangipatti kudineer*. Therefore, antimicrobial activity of ingredients of PPK was discussed in this study.

Shah S et al investigated that Neem extract have antibacterial effect against Streptococcus *mutans.*[13] Neem is one of the ingredients of PPK. This study report showed antibacterial activity against *Streptococcus mutans* which is similar with results of study done by Shah S et al.

This study result supports the statement that *Aspergillous flavus* is the main causative agent for keratitis. [13] and PPK is indicated for *padai* (fungal infection) in the siddha text.[2]

Shah S et al investigated that Neem extract have antibacterial effect against Streptococcus *mutans.*[14] Neem is one of the ingredients of PPK. This study report showed antibacterial activity against *Streptococcus mutans* which is similar with results of study done by Shah S et al. *S. mutans* plays a major role in tooth decay.[15] Therefore, PPK may be given for tooth decay . This study result similar with the reports of study done by Ayuchi Kojima et al[16] and the indication for PPK in literature. [2] Most of the skin diseases are treated with PPK in Out Patient Department, The Tamil Nadu Dr.MGR Medical University. But this study result showed no ZOI in AQEP. Shelf life of the PPK is 3 hrs.[17] The extract was prepared in October 2016 and the antimicrobial test was performed on May 2017. This may be the region for negative result with AQEP.

Conclusion: The antimicrobial study should be done immediately after taking aqueous extract of PPK. PPK should be screened for antimicrobial activity with some other micro organism to prove the efficacy scientifically.

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Conflict of interest: Authors declare that there was no Conflict of interest.

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