RESEARCH ARTICLE

IN VITRO ANTI-MICROBIAL ACTIVITIES OF AQUEOUS EXTRACT OF KARAPPAN KUDINEER – SIDDHA FORMULATION

1Bahulayan Janani, *1Kullappan Shanmugam Uma, 2Sekkizhar Geethalakshmi, 1Natarajan Kabilan, 2Thiyagarajan Balasubramanian and 3Thankaiah Mohan Raj

1Department of Siddha, The TN Dr. M. G. R. Medical University, Chennai-32, Tamilnadu, India  
2The TN Dr. M. G. R. Medical University, Chennai-32, Tamilnadu, India  
3ATSVS Siddha Medical College, Munchirai, Kanyakumari Dist. Tamilnadu, India

ABSTRACT

Aim: The aim of this study is to screen In-vitro Anti-microbial activities of aqueous extracts of Karappan Kudineer (KAK) - A Siddha formulation.

Methodology: KAK was collected from the pharmacy of ATSVS Siddha Medical College, Munchirai, Kanyakumari Dist. Aqueous extract of KAK was prepared by soxhlet method. In-vitro antimicrobial activity of extract of KAK was screened against Staphylococcus aureus, Streptococcus mutans, Aspergillus niger, Aspergillus flavus and Candida albicans using disc diffusion method. The micro-organisms were collected from the Microbial Type Culture Collection (MTCC). Sterilized discs were soaked in aqueous extract of KAK individually at the concentration of 25mg/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: Aqueous extract of KAK showed more anti-fungal activity against Aspergillus flavus (11mm) and showed anti-bacterial activity against Staphylococcus aureus (9mm)

Conclusion: It is concluded that KAK can be prescribed as the medicine for skin diseases due to Staphylococcus aureus and Aspergillus flavus infection.

Copyright©2017, Bahulayan Janani et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Staphylococcus aureus has emerged as the dominant pathogen causing the blood stream infections in last 5 years. (Atul K Patel et al., 2010). Staphylococcus aureus is a major human pathogen that causes a wide range of clinical infections. It is a leading cause of bacteremia and infective endocarditis as well as osteoarticular, skin and soft tissue, pleuropulmonary, and device-related infections. Clinical infections with S. aureus will likely remain both common and serious and also increasing antimicrobial resistance (Tong et al., 2015). S. aureus frequently causes infections of eyelids and conjunctiva, (Ramesh et al., 2010). Aspergillus flavus is a fungus. Growth of the fungus on a food source often leads to contamination with aflatoxin, a toxic and carcinogenic compound. Aspergillus flavus is also the second leading cause of aspergillosis in humans (Hedayati et al., 2007). Patients infected with A. flavus often have reduced or compromised immune systems.

*Sidha Medicine, Karappan kudineer, Gymnemasyvestre, Piper nigrum, Capparis sepiaria, Acalypha fruticosa, Anti-microbial, Staphylococcus aureus, Aspergillus flavus, Skin diseases.

*Corresponding author: Kullappan Shanmugam Uma, Department of Siddha, The TN Dr. M. G. R. Medical University, Chennai-32, Tamilnadu, India.
activities. (Satyanarayana et al., 2010) The aqueous leaves extract of Gymnema sylvestre recorded an intermediate antimicrobial activity against S. aureus. (Beverly, C. et al., 2013). Piper nigrum L has Analgesic and anti-inflammatory activities. (Farhana Tasleem et al., 2014). Both aqueous and ethanol extracts of black pepper were screened for antibacterial activity against a penicillin G resistant strain of Staphylococcus aureus and showed antibacterial activity, which was determined by the agar-well diffusion method, using cephalixin as a standard antibiotic. (Amit Kapoor et al., 2015). In this scenario. It was planned to identify the efficacy of Siddha formulation- Karappan kudineer against Pathogenic organism like Staphylococcus aureus, Streptococcus mutans, Aspergillus niger, Aspergillus flavus and Candida albicans to develop scientific evidence for the karappan kudineer.

MATERIALS AND METHODS

Karappan Kudineer is collected from the pharmacy of ATSVS Siddha Medical College, Munchirai, Kanyakumari Dist. The ingredients of Karapan Kudineer were presented in Table 1 & Figure 1.

Fig. 1. Ingredients of Karappan Kudineer. 1. Capparis sepiaria 2. Gymnema sylvestre, 3. Acalypha fruticose 4. Piper nigrum

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Plant name</th>
<th>Botanical name</th>
<th>Part used</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Karunchooriai</td>
<td>Capparis sepiaria</td>
<td>Bark</td>
<td>1 part</td>
</tr>
<tr>
<td>2</td>
<td>Chinni</td>
<td>Acalypha fruticosa</td>
<td>Leaf</td>
<td>1 part</td>
</tr>
<tr>
<td>3</td>
<td>Milagu</td>
<td>Piper nigrum</td>
<td>Fruit</td>
<td>1 part</td>
</tr>
<tr>
<td>4</td>
<td>Siru kurinjan</td>
<td>Gymnema sylvestre</td>
<td>Root</td>
<td>1 part</td>
</tr>
</tbody>
</table>

Method of preparation of Karappan Kudineer

The above said drugs to be first purified and then it should be ground into coarse powder asper mentioned in the Siddha text. (Thiyagarajan, 2006)

Preparation of extract of Karappan kudineer

120 ml of water was taken in a round bottomed flask. Karapan kudineer coarse powder was filled in the Thimple of Soxhlet Apparatus. The condenser of Soxhlet Apparatus was fixed to inlet and outlet tube for flow of water and then the apparatus was allowed to run at a temperature of 100°C continuously for 3 hours until the extract does not leave residue in the shippin tube. The extract was collected and filtered, then it was dried by keeping in water bath and it was preserved in a airtight container and it was stored in refrigerator for further use. The extract was used for testing Anti-Microbial 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 aqueous extract of KAK at the concentration of 25 mg/ disc and kept overnight in room temperature. 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 extract of KAK at the concentration of 25 mg /disc and kept overnight in room temperature. Then the plates were allowed to dry for 20 minutes. The sterile dried antimicrobial discs were screened individually with extract of KAK at the concentration of 25 mg /disc and kept overnight in room temperature. The sterile dried antimicrobial discs impregnated individually with extract of KAK at the concentration of 25 mg /disc and kept overnight in room temperature. The sterile dried antimicrobial discs impregnated individually with extract of KAK 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.

Culture and Media Preparation for Fungus

Aqueous extract of KAK was 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 aqueous extract of KAK at the concentration of 25 mg / disc and kept overnight in room temperature. 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 aqueous extract of KAK 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 (Drew Lawrence, W., et al., 1972).

RESULTS

The results of In vitro anti-microbial assay indicates that aqueous extract of KAK showed more anti-fungal activity against Aspergillus flavus as par with the positive control and anti-bacterial activity against Staphylococcus aureus. Results were expressed in Figure 2 & 3 and Table 2 & 3.

Table 2. Anti bacterial activity of karappan kudineer

<table>
<thead>
<tr>
<th>Organisms</th>
<th>ZOI of KAK</th>
<th>ZOI of Streptomycin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus</td>
<td>9 mm</td>
<td>13 mm</td>
</tr>
<tr>
<td>Streptococcus mutans</td>
<td>NZ</td>
<td>14 mm</td>
</tr>
</tbody>
</table>

DISCUSSION

Aqueous extract of Karappan kudineer (KAK) was subjected to anti-microbial studies. There was no scientific data available on Karappan kudineer. Therefore, antimicrobial activity of ingredients of KAK was discussed in this study. Satdive, R.K.
et al. (2003) reported that Leaf extract of Gymnema sylvestre possess Antimicrobial activity. KAK showed more anti-fungal activity against Aspergillus flavus as par with the positive control, hence it approves with the above study result. Veeramuthu Duraiapandiyan et al. (2006) reported that Acalypha fruticosa has the potency of Antimicrobial activity. KAK showed anti-bacterial activity against Streptococcus aureus. Both the study results were same. Acalypha fruticosa is one of the ingredient of KAK. Aspergillus flavus is the main causative agent for keratitis (Hedayati et al., 2007). And KAK is indicated for padai (fungal infection) in the siddha text. Piperine showed maximum zone of Inhibition against Staphylococcus aureus (18 mm) (Shiva Rani et al., 2013). Aqueous extract of KAK result supports the above results. Piper nigrum also one of the ingredient of KAK. Karappan kudineer has been dispensed by the Siddha physician for last 20 years for the skin diseases in the OPD of ATSVS Siddha Medical College. KAK is widely used by the Siddha practitioners for more 50 years for the all types of skin diseases. But this study revealed that KAK can be prescribed to the diseases due to Staphylococcus aureus and Aspergillus flavus infection.

Conclusion

From this study result, It is concluded that KAK can be prescribed as the medicine for skin and other diseases due to Staphylococcus aureus and Aspergillus flavus infection. KAK should be screened for antimicrobial activity with some other microorganism to prove the efficacy scientifically. In vivo antimicrobial activity of KAK may be conducted in future.

Acknowledgement

Authors thank the Vice chancellor, Registrar, Professor, Department of Siddha, The Tamil Nadu Dr. M. G. R. Medical University for permitting to carry out this study.

Conflict of interest

Authors declare that there was no Conflict of interest.

REFERENCES


