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RESEARCH ARTICLE

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

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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 KAKcan be prescribed as the medicine for skin diseases due to *Staphylococcus aureus* and *Aspergillus flavus* infection.

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INTRODUCTION

Staphylococcus aureus has emerged as the dominant pathogen causing the blood stream infections in last5 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 (Scheidegger, 2003). The incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) infections in patients admitted to the intensive care unit has dramatically increased in recent years, with an associated increase in morbidity and mortality and the costs of caring for patients with MRSA infections. Although indiscriminate and inappropriate use of antibiotics has contributed to this phenomenon (Sista *et al*., 2004). Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a major public health threat (Xiaoyan Song *et al.,* 2010). Invasive aspergillosis is uncommon in immune competent hosts but is the second most common opportunistic fungal infection in immune compromised patients (Koss *et al*., 2002). Karappan kudineer is poly herbal formulation. KAK contains four herbs namely Capparis sepiaria, Acalypha fruticosa, Piper nigram and Gymnema sylvestre. Karappan kudineer *has been in used for all skin diseases in the OPD of ATSVS Siddha Medical College for last 20 years.* Ethanolic fruit extracts of Capparis sepiaria showed antimicrobial activity against *Bacillus subtilis*, *Escherichia coli*, *Enterococcus faecalis*, *Klebsiella* sp., *Pseudomonas aeruginosa* and *taphylococcus aureus* (Kalpana, 2015). Stem extracts of *Capparis sepiaria* possess anti-inflammatory and anti microbial

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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 cephazolin 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 nigram

Table 1. Ingredients of karappan kudineer

S.No.	Plant name	Botanical name	Part used	Quantity
1	Karunchoorai	*Capparis sepiaria*	Bark	1 part
2	Chinni	*Acalypha fruticosa*	Leaf	1 part
3	Milagu	*Piper nigram*	Fruit	1 part
4	Siru kurinjan	*Gymnema sylvestre*	Root	1 part

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^{o}c continuously for 3 hours until the extract does not leave residue in the shipphon 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 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 Aqueousextract 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

Organisms	ZOI of KAK	ZOI of Streptomycin
Staphylococcus aureus	9 mm	13mm
Streptococcus mutans	NZ	14 mm

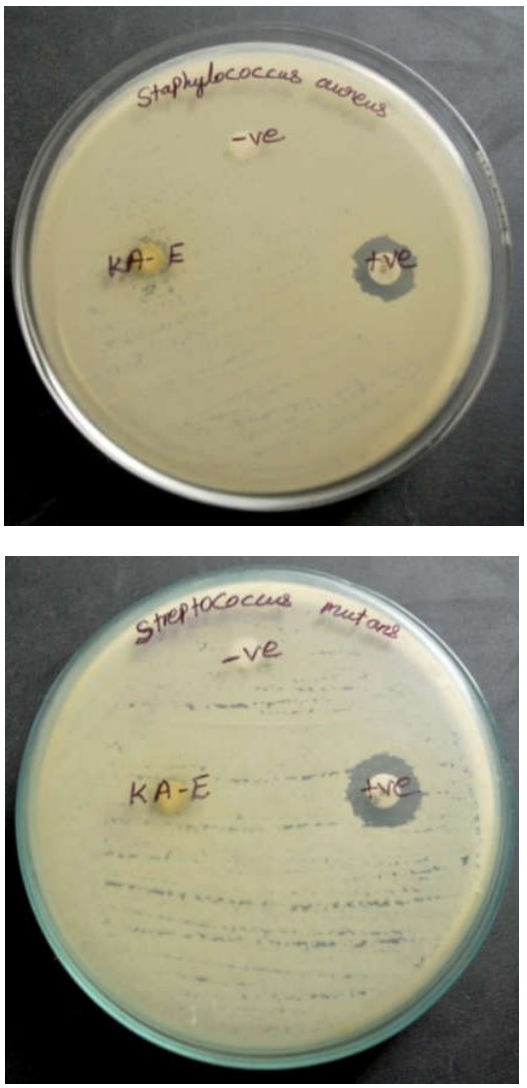

Fig.2. *Staphylococcusaureus* and *Streptococcus mutans*

Table 3. Anti fungal activity of aqueous extract of KAK

Organisms	ZOI of Aqueous Extract of KAK	ZOI of Fluconozole
Aspergillus niger	*NZ*	15 mm
Aspergillus flavus	11mm	12 mm
Candida albicans	NZ	22 mm

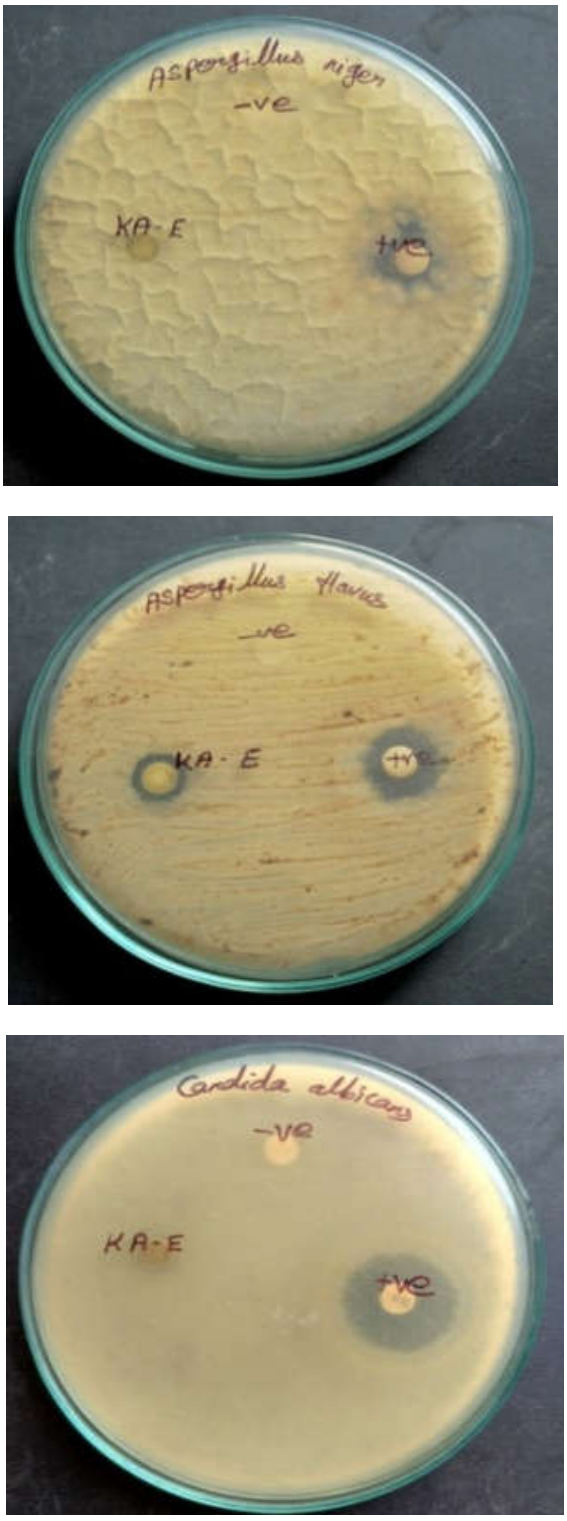

Fig.3. *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans*

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 Duraipandiyan *et al*. (2006) reported that *Acalypha fruticosa* has the potencey 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. *Aspergillous 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 KA*K* 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.

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Conflict of interest

Authors declare that there was no Conflict of interest.

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