

Assessment Of Antimicrobial Efficacy Of Kabasura Kudineer Choornam Against Common Oral Microbes - A Randomised Invitro Study On Biofilm Model

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Abstract

Aim: To assess the antimicrobial efficacy of Kabasura Kudineer Choornam against common oral microbes in an invitro biofilm model. **Materials and methods:** An experimental in-vitro study was done to assess the antimicrobial property of Kabasura Kudineer Choornam (Test product) against 0.2% Chlorhexidine (Positive control), Distilled water (Negative control). The zone of inhibition and minimum inhibitory concentration was assessed using disk diffusion method. 36 single rooted teeth were collected and the selected organisms such as *S.mutans*, *L.acidophilus*, *E. faecalis*, *C. albicans* were procured and cultured in the selective medium. After superficial removal of debris from the tooth samples, the teeth were stored in Brain Heart Infusion agar and later microbial suspension was added to form a biofilm incubating for 7 days. The tooth samples were then irrigated with saline and randomly divided and placed into test tubes containing, the test product, positive and negative control. The colony-forming units were procured with the microdilution obtained from the tooth samples. The antibiofilm efficacy was assessed on 16 teeth samples, which was then randomly divided into a positive and negative control. Under the positive control, the teeth samples were subjected to the microbial suspension along with the nutrient broth while the teeth samples in the positive control were subjected initially to the test product along with the nutrient broth followed by the microbial suspension and both the controls were incubated for 7 days. The antibiofilm efficacy was tabulated through the colony forming units using the microdilution from the tooth samples. **Results:** The test product Kabasura kudineer choornam failed to prove to an antimicrobial efficacy compared to the positive control 0.2% chlorhexidine mouthwash on a matured biofilm formed by the oral microbes. While assessing the ability of the test product against the biofilm formation, the test product had an antibiofilm effect on the teeth samples. **Conclusion:** Kabasura Kudineer Choornam proved to have less effect against the common oral microbes compared to the gold standard 0,2% chlorhexidine.

Keywords: Kabasura kudineer choornam, antimicrobial efficacy, in-vitro study, biofilm

INTRODUCTION

A growing tendency to go natural still exists today. Apart from allopathy, other forms of medicines such as Ayurveda, Siddha, Unani are still being chased due to its cost effectiveness, availability and cultural acceptance. The knowledge in herbal medicine have transferred from generation to generation which set the root of allopathic medicine. According to World health organization, it is estimated that 80% of the world's population still depend mainly on traditional medicines for their health care.³

Oral health is an integral part to general health and relates to the Quality-of-Life (QoL). The link between oral diseases and the activities of several microbial species that form a part of the micro biota is well established. Despite several chemical agents being commercially available today, these can alter oral micro biota and have undesirable side effects such as vomiting, diarrhea and teeth staining.¹

Traditional knowledge of Ayurveda should be integrated to that to modern dentistry and for this, the active principles of plants should be incorporated into modern oral health-care practices and dentists should be encouraged to use natural remedies in various oral health treatments. This will make dentistry much safer, affordable and more accessible for the lower socio-economic groups in society.¹

The search for alternative products continues still today. The natural phytochemicals isolated from plants used in traditional medicine are considered to be good alternatives to synthetic chemicals which may lead to the development of novel preventive or therapeutic strategies for the betterment of oral health.

“**Kabasura Kudineer Choornam**” is a poly herbal formulation of 15 ingredients in equal proportions which was recommended by the Government of India for combating respiratory viral infections in human such as cold, cough, breathing difficulty and flu. “**Kaba**” denotes kapha dosha which means fever due to excess accumulation of kapha (mucus, phlegm), “**Asura**” means herbs that alleviate the symptoms, “**Kudineer**” denotes decoction and “**Choornam**” indicates powder. This preparation was reported to possess an antibacterial, anti-inflammatory and antipyretic properties.⁴

Recently, population rise, inadequate supply of drugs, prohibitive cost of treatments, side effects of several synthetic drugs and development of resistance to currently used drugs for infectious diseases increased the use of plant materials as medicines for a wide variety of human diseases.²

Since the ingredients of Kabasura Kudineer are purely herbal in origin and have been widely advocated for systemic use by the Government of Tamil Nadu to improve the immunity of the subjects during the COVID 19 pandemic, this study was designed to know the impact of kabasura kudineer on common oral microbes. Further, it is a product that is abundantly available and culturally acceptable. Hence the aim framed for the present study is to assess the antimicrobial efficacy of Kabasura Kudineer Choornam against common oral microbes in an invitro biofilm model.

MATERIALS AND METHODS

An in vitro experimental study was conducted to assess the antimicrobial efficacy of Kabasura Kudineer Choornam against *S.mutans*, *L.acidophilus*, *E. faecalis*, *C. albicans*. A biofilm was used to determine the antimicrobial property of Kabasura Kudineer choornam was then compared with 0.2% chlorhexidine mouthwash. The ethical clearance for conducting the study was obtained (MADC/IRB-XXXIV/2020/567).

CONTROL GROUPS:

The positive control for the study was framed to be 0.2% chlorhexidine mouthwash against the *S.mutans*, *L.acidophilus* and *C.albicans* while 2% chlorhexidine was tested for *E.faecalis*. Distilled water was considered to be the negative control and Kabasura kudineer choornam as the test product in the present study.

PREPARATION OF EXTRACT

The Kabasura kudineer powder was purchased from the IMPCOPS store for siddha medicine. The Kabasura Kudineer aqueous extract was prepared with 5gms of the choornam with 300ml of water and heated at 50-60°C till the water reduces to 1/4th of its volume upto 30ml. The concentrated aqueous extract of Kabasura Kudineer Choornam was stored at 2-5°C until the completion of the study.

DISC DIFFUSION METHOD

The disc diffusion method was used to obtain the zone of inhibition and the minimum inhibitory concentration at three concentrations of the test product, negative and positive controls. The 100%, 50% and 25 % dilution of the test product and the positive control were obtained using 2 fold dilution method. Sterile discs of 4mm diameter

was taken and soaked to the prepared dilutions. The brain heart infusion agar was used for enterococcus faecalis, thio agar for lactobacillus, nutrient agar for streptococcus mutans and sabouraud dextrose agar for candida albicans. The agar plates were divided into 4 compartments to test the zone of inhibition and the minimum inhibitory concentration. The discs were placed into the agar plates with which each plate represented a microorganism. An agar plate consisted of discs of 100%, 50% and 25% dilution of the test product and the corresponding positive control and negative control.

BIOFILM MODEL

The biofilm model was formulated using extracted teeth samples. Forty eight single rooted mandibular premolars devoid of caries were included while those extracted teeth with caries, fracture roots and being RCT treated were excluded. The extracted tooth samples were firstly subjected to an ultrasonic bath of 17% Ethylene diamine tetra acetic acid (EDTA) followed by 5.25% sodium hypochlorite and later with distilled water for 10 minutes each and autoclaved at 121°C for 30 minutes

ANTIMICROBIAL EFFICACY OF THE KABASURA KUDINEER CHOORNAM

From the autoclaved tooth samples, thirty two teeth were used to test the antimicrobial efficacy of Kabasura kudineer choornam. The microbial suspension of 2.5ml was then subjected into the test tubes which was adjusted to 0.5 Mc Farland Standard (1.5×10^8 microorganisms/ml) along with the nutrient broth. Later, the test tubes were incubated for 7 days at 37°C. On the third day, the nutrient broth was renewed to prevent nutrient depletion. At the end of the 7th day, the test tubes were subjected for 30 minutes against Kabasura kudineer choornam, 0.2 % chlorhexidine and 2% chlorhexidine and distilled water. The test tubes were then well aggitated manually for 2 minutes. Following the exposure, the tooth samples were diluted using 2ml of saline which was used to subculture on nutrient agar plates to attain the colony forming units pertaining to each organism. (Table 1)

ANTIMICROBIAL EFFICACY TESTED ON 32 TEETH SAMPLES

Randomly divided into 4 groups with 8 teeth samples each

Group 1

Group 2

Group 3

Group 4

The teeth samples are then subjected BHI broth and the microbial suspension respective to their groups

Incubation for 7 days at 37°C. Broth media renewal done on

2 teeth samples exposed to four products, The test product Kabasura kudineer choornam, 0.2% chlorhexidine, 2% chlorhexidine and distilled water

Microdilution taken and subcultured

Colony forming units attained

Table 1: Flowchart on the methodology used for assessing antimicrobial efficacy.

Test product : Kabasura kudineer choornam

Positive control: 0.2% chlorhexidine mouthwash and 2% chlorhexidine (E.faecalis)

Negative control : Distilled water

ANTIBIOFILM EFFICACY OF THE KABASURA KUDINEER CHOORNAM

Sixteen tooth samples after autoclaving randomly divided into two groups with 8 teeth samples each. Under the negative control, four test tubes were taken with 2 teeth samples each. Each test tube was then subjected to nutrient broth with each microbial suspension onto the test tubes. While in the positive control, 4 test tubes with 2 teeth samples were initially subjected to a nutrient broth being prepared with the Kabasura kudineer powder to prevent its dilution into the test tubes. Following which, the test tubes were exposed to the microbial suspension into respective test tubes. The negative and the positive control were then incubated for 7 days at 37°C. On the third day, the nutrient broth was renewed in the negative and the positive control. At the end of the seventh day, the teeth samples were microdiluted with saline. Subculture on nutrient agar plates were done to obtain the colony forming units. (Table 2)

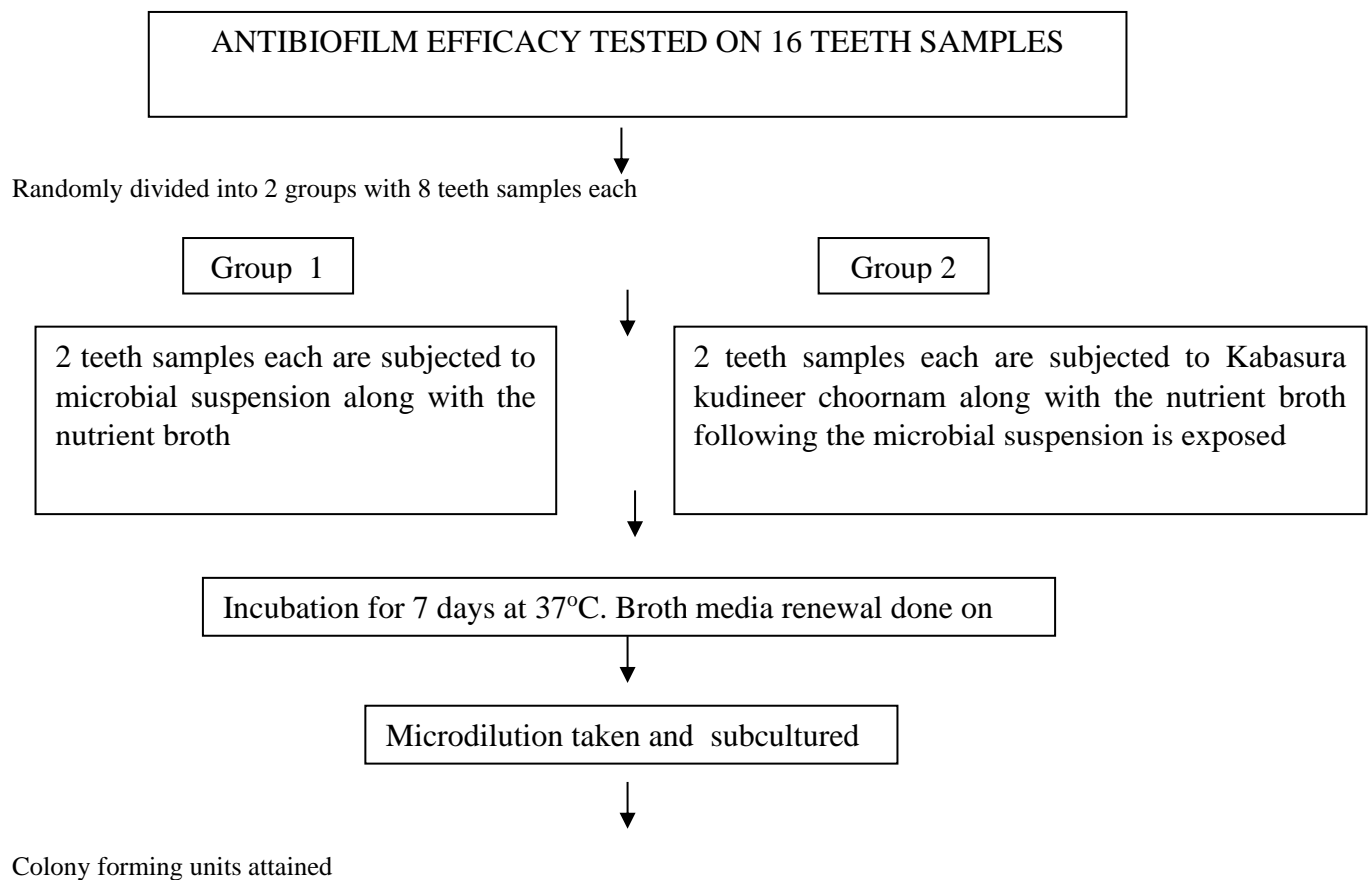


Table 2: Flowchart on the methodology used for assessing antibiofilm efficacy. Test product : Kabasura kudineer choornam (Positive control)

STATISTICAL ANALYSIS:

The collected data were tabulated in Microsoft excel and analysed using Statistical Package for Social Sciences (SPSS) version 19. Kruskal wallis test followed by post hoc analysis was carried out to compare between the three groups. p value less than 0.05 was considered to be statistically significant.

RESULTS

The study revealed that, Kabasura kudineer choornam didnt show any zone of inhibition against the oral microbes S.mutans, E.faecalis, Lactobacilli and C.albicans. Later the results were compared to that of the positive and the negative control in the study and it proved that 0.2% chlorhexidine proved to show antimicrobial efficacy with S.mutans, Lactobacilli and C.albicans. (Table : 3)

The antimicrobial efficacy of the test product showed that the colony forming units was found to be confluent with regard to S.mutans, E.faecalis, C.albicans and Lactobacilli when treated with Kabasura kudineer choornam but it was much less with regard to 0.2% chlorhexidine. (Table : 4)

The antibiofilm efficacy of the test product study showed that Kabasura kudineer proved to show an antibiofilm efficacy in inhibiting the formation of a biofilm. The antibiofilm efficacy of Kabasura kudineer showed a statistical significance with regard to E. faecalis (p = 0.02) while other comparisons didnt prove any statistical significance. (Table : 5)

Table 3 : Zone of inhibition and Minimum Inhibitory Concentration (mm)

	KSK(100%)	KSK (50%)	KSK (25%)	0.2% CHX	2% CHX	DW
S.mutans	Nil	Nil	Nil	18mm	-	Nil
E.faecalis	Nil	Nil	Nil	-	16mm	Nil
Lactobacilli	Nil	Nil	Nil	21mm	-	Nil
C.albicans	Nil	Nil	Nil	17mm	-	Nil

KSK – Kabasura kudineer, DW – Distilled water ,CHX – Chlorhexidine

Table 4 : Antimicrobial efficacy of Kabasura kudineer compared to the positive control and negative control on the teeth samples (CFU/ml)

	KSK	DW	0.2% CHX	2% CHX
S.mutans	Confluent	Confluent	Nil	Nil
E.faecalis	53300	Confluent	500	Nil
Lactobacilli	700	7400	Nil	Nil
C.albicans	8900	11700	Nil	Nil
p = 0.124				

p < 0.05 is statistically significant

KSK – Kabasura kudineer, DW – Distilled water, CHX – Chlorhexidine

Table 5: Antibiofilm efficacy of Kabasura kudineer (positive control) compared to the negative control on the teeth samples (CFU/ml)

	KSK	CONTROL	p value
S.mutans	1,012.5	Confluent	0.195
E.faecalis	50	Confluent	0.02*
Lactobacilli	25	7400	1.00
C.albicans	337.5	11700	0.195

p < 0.05 is statistically significant

KSK – Kabasura kudineer, DW – Distilled water, CHX – Chlorhexidine

DISCUSSION

A siddha formulation which took the spotlight specially during this covid pandemic was Kabasura kudineer choornam. As a boon to overcome less complications, this test product Kabasura kudineer choornam was highly recommended to use by the Government as an immunity boster. With alarming side effects using synthetic medicine like chlorhexidine mouthrinse which causes loss of taste, dryness and burning sensation and staining of teeth propentiated to explore on the dental benefits of other forms of medicine such as siddha where no side effects are proved. Hence an attempt was made to explore its antimicrobial efficacy against common oral microbes thereby introducing it into dentistry towards preventing the dental diseases which are chronic in nature which eventually leads to a costlier treatment needs.

Dental diseases such as dental caries, periodontal diseases are common among all age groups. Denture related problems, periapical infections due to untreated dental decay are also being common today. Literature has been proved that dental caries is initiated by streptococcus mutans and progressed by lactobacillus. Enterococcus faecalis is a very common pathogen in infected root canals and candida albicans causes fungal infections in the oral cavity and is prominent among elders. Thereby these microorganisms were considered as the common oral microbes for the evaluation of the antimicrobial efficacy of Kabasura kudineer.

Anitha et al in 2015⁵, had concluded in a study, which revealed that Kabasura kudineer has the presence of various secondary metabolites which was eventually the reason for its therapeutic activity. Hence an invitro study was designed to prove the antimicrobial efficacy of Kabasura kudineer choornam against common oral microbes such as streptococcus mutans, enterococcus faecalis, lactobacilli acidophilus and candida albicans at the recommended concentration suggested by the government.

The present study showed no zone of inhibition and minimum inhibitory concentration against the oral microbes while there existed a promising result in regard to chlorhexidine mouthwash. This result could be due to the fact that, under the recommended dose to use this siddha formulation, no antimicrobial action was evident. Similarly a study done by Prabhu Manju et al in 2015¹¹, had done a study evaluating the invitro efficacy of a poly herbal mouthrinse formulation and proved its efficacy against streptococcus but not on candida albicans.

While considering on the biofilm model, the herbal medicine Kabasura kudineer proved to be less efficient to the 7 day old biofilm formed in regard to the four microorganisms compared to 0.2% chlorhexidine mouthrinse. A similar study done by J.Prabhakar et al in 2014⁹, had proved an antimicrobial efficacy using triphala as the herbal product, on a 7 day old biofilm formed by streptococcus mutans which showed similar result to 0.2% chlorhexidine mouthrinse. A contrasting result initiated to assess the antibiofilm activity of the herbal product which means the ability to inhibit a biofilm formation on the tooth substrate.

The present study used the aqueous extract Kabasura kudineer choornam as the herbal medicine in interest. There exists literature pertaining to other forms of siddha formulations such as nilavembu kudineer, adathodia kudineer, notchi kudineer proving its antiphenolic component which is a secondary metabolite being the reason for its action. Triphala is an ayurvedic herbal formulation which was also experimented for its antimicrobial efficiency. The result from the study being formulated proposes that, the antimicrobial efficiency of Kabasura kudineer could have not been evident on a matured biofilm of 7 days old due to its low concentration being used in the study. This was noted to be a reason to highlight when Saravana J et al in 2015³ proved the antibacterial effect present in the same herbal formulation Kabasura kudineer against Bacillus subtilis, E coli, Pseudomonas aeruginosa and Staphylococcus aureus in a higher concentration than used in the present study. The strength of the study was that, it was first of its kind to assess the antimicrobial efficacy on an immature and matured biofilm at the recommended dose of Kabasura kudineer. The study was resolved over a herbal drink which took its path during this recent times where a majority of them were aware of this drink and its pharmacological actions.

The limitation of the present study was that, it was done on a very small sample of forty eight tooth samples and the study didn't consider to assess the antimicrobial efficacy on a multispecies model. Being a study of first of its kind, the study restrained to compare on other concentrations of chlorhexidine mouthwash such as 0.12%, 0.1%. Considering the microorganism enterococcus faecalis, the minimum time required for a matured biofilm to form is 21 days, while the present study has followed a methodology pertaining for 7 days for the biofilm formation. Data relaying on the microbial load at multiple time interval could have been evaluated for this herbal medicine against the microbes being considered.

The present study could conclude that Kabasura Kudineer Choornam has the potential to be used as an oral agent. Its antibacterial activity on a mature biofilm was significant when compared with 0.2 % chlorhexidine, but it can be used as a regular mouth rinse, which can probably prevent the biofilm formation.

Kabasura Kudineer Choornam can be recommended as a prophylactic agent for caries prone individuals, denture wearers and patients with orthodontic appliances. This result can extend research to conduct in vivo so that this siddha formulation could be introduced in the field of dentistry and can be beneficial to the common man. At the recommended dose to ingest this herbal drink, it is proved through this study that compared to the gold standard, this herbal drink is less efficient in terms of antimicrobial efficacy but has the property to inhibit a biofilm being formed in an immature stage. Hence, Kabasura kudineer has its potential antimicrobial efficacy so that the last sip of this drink can be used as a mouth rinse. Eventually, people belonging to different social strata can prepare this herbal decoction at home on a regular basis and can use it as a mouth rinse to facilitate the reduction of microbial load in the oral cavity. Therefore the government can also promote and distribute this

herbal product at various primary health centres to enhance the oral health of the community. Moreover, it is a product that is abundantly available, cost effective and culturally acceptable.

CONCLUSION

The present invitro study, which was initiated to assess the antimicrobial efficacy of Kabasura kudineer choornam against the common oral microorganisms resulted to show that, the herbal drink wasn't as effective as chlorhexidine mouthwash at the recommended dose suggested by the government promoting as an immunity booster during the covid pandemic.

When evaluating its action on the antimicrobial activity on a matured biofilm formed by the common oral microbes, it was evident that this herbal drink could not substitute the gold standard 0.2% chlorhexidine. The test product had a less efficacy to inhibit the matured biofilm being formed by streptococcus mutans, enterococcus faecalis, lactobacillus acidophilus and candida albicans.

When the biofilm prevention ability was evaluated for the test product, it showed that the test product proved to have an antibiofilm activity, in preventing the biofilm formation on the tooth samples. The assessment was done by comparing the colony forming units being formed and a reduction was evident preventing the biofilm formation with regard to streptococcus mutans, enterococcus faecalis and lactobacillus. The present invitro study, which was initiated to assess the antimicrobial efficacy of Kabasura kudineer choornam against the common oral microorganisms resulted to show that, the herbal drink wasn't as effective as chlorhexidine mouthwash at the recommended dose suggested by government promoting as an immunity booster during the covid pandemic.

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The study results suggested that even though literature has proven on the antibacterial, anti inflammatory, antipyretic activity of Kabasura kudineer, the recommended dose of the product stays less efficient than the gold standard 0.2% chlorhexidine mouthwash towards oral microbes. The present study recommends more research on invivo investigating the benefits of this herbal drink kabasura kudineer choornam and if proved to have any effect, this product can be widely used in the cure of various dental diseases.

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