In Vitro Antibacterial Effect Of Decoction Of *Thrikatu Kalinga Katuka* Against *Streptococcus Pyogenes*

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ABSTRACT

In clinical practice many herbal preparations have been using in the management of Tonsillitis (*Thundikeri*). *Thrikatu kalinga katuka* is a famous decoction prescribed only by some of the renowned physicians according to their experience. Its efficacy against *Streptococcus pyogenes* is not proved by laboratory investigations up to now. This study was carried out to determine the antibacterial effect of the above decoction against the laboratory specimen of *S. pyogenes* and to determine the most effective concentration against the organism. Methodology was based on anti-bacterial susceptibility test by using laboratory specimen of *S. pyogenes* in Agar well diffusion method. The drug was prepared according to the *Kwatha Paribhasha* of *Sharangadhara Samhita*. First all the ingredients were identified correctly and then measured the required amounts of each drugs. After cleaning the ingredients 3 samples for different decoctions (4-1, 8-1, and 16-2) were prepared. Amoxicillin (01 mg/ml) was used as standard positive control, while distill water was used as negative control. Anti-bacterial activity was obtained by determining the zone of inhibition (ZI) around the well and it was compared with standard drug. Mean ZI values of those three decoction levels were significantly different from each other. According to T-test the p value < 0.05 and null hypothesis was rejected at 0.05 level of significance. It revealed that the most effective decoction (16-2) was not as much as effective than the amoxicillin. It can be concluded that the decoction *Trikatu Kalinga Katuka* is significantly effective against *Streptococcus pyogenes* at the concentration of 16-2 according to the study carried out.

Keywords: Tonsillitis, *Streptococcus pyogenes*, Herbal decoction, Antibacterial activity

INTRODUCTION

Ayurveda medical system is a one of great medical system which can cure and prevent many diseases of humans in world wide. *Ayurvedic* medicines are becoming popular day-by-day and demand for its usage is increasing not only in the country but also worldwide the inherent quality of Ayurveda treatment of having negligible side/after effects, has made great potential for its production. *Ayurvedic* medicines are based on plants, animals extract and minerals both in single ingredient drugs and compound formulations [Devgan et al., 2014].

Many hundreds of herbals worldwide are used in traditional medicine as treatments for bacterial infections. Some of these have also been subjected to in vitro screening but the efficacy of such herbal medicines has seldom been rigorously tested in controlled clinical trials. Conventional drugs usually provide effective antibiotic therapy for bacterial infections but there is an increasing

problem of antibiotic resistance and a continuing need for new solutions. Although natural products are not necessarily safer than synthetic antibiotics, some patients prefer to use herbal medicines. Thus healthcare professionals should be aware of the available evidence for herbal antibiotics.

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades, these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses not, only because many of them produce toxic reactions, but also due to emergence of drug-resistant bacteria. It is essential to investigate newer drugs with lesser resistance. Drugs derived from natural sources play a significant role in the prevention and treatment of human diseases. In many developing countries, traditional medicine is one of the primary healthcare systems. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented. About 61% of new drugs developed between 1981 and 2002 were based on natural products and they have been very successful, especially in the areas of infectious disease and cancer. Recent trends, however, show that the discovery rate of active novel chemical entities is declining. Natural products of higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action. The effects of plant extracts on bacteria have been studied by a very large number of researchers in different parts of the world.

Streptococcus pyogenes is a species of bacteria. Like other streptococci, it is clinically important in human illness. It is an infrequent, but usually pathogenic, part of the skin flora. It is the sole species of Lancefield group A and is often called group A streptococcus (GAS), because it displays streptococcal group A antigen on its cell wall.

Group A streptococcal infection can cause illness, which typically produces small zones of beta-hemolysis, a complete destruction of red blood cells. (A zone size of 2-3 mm is typical). It is thus also called group A (beta-hemolytic) streptococcus.

Streptococcus pyogenes is a round bacterium. The name derives from the Greek word 'streptos,' meaning 'twisted chain,' because streptococcal cells tend to link together in chains, which resemble a string of pearls when viewed under the microscope. Streptococci are catalase-negative and gram-positive. *S. pyogenes* can be cultured on blood agar plates. Under ideal conditions, it has an incubation period of about 1 to 3 days.

It causes numerous infections in humans including Pharyngitis, Tonsillitis, Scarlet fever, Cellulitis, Rheumatic fever etc. But people have to face more complications due to careless of those infections. Tonsillitis is an inflammation (swelling) of the tonsils presenting with features of difficulty in swallowing, ear pain, fever, sore throat, tenderness of the jaw and throat.

It is called as *Thundikeri* in Ayurveda medical system. Some Ayurveda physicians use the decoction of *Trikatu kalinga katuka* by their experience to cure Tonsillitis. So there should be antibacterial effect, but it is not proved scientifically.

MATERIALS AND METHODS

Ingredients of decoction of Trikatu kalinga katuka

त्रिकटु कलिन्ग कटुका हरीतकी विभीतकामलकैः
ध्वंशयति कन्टकुब्जं व्रश रजनीद्वय संयुतः कषायः

(Sri Dewamiththa, 1994, page 36)

Viyali iguru - *Zingiber officinalae*
Gammiris - *Piper nigrum*

Thippili - *Piper longum*
Kelida sahal - *Holarrhena antidysentrica*
Katukarosana - *Picrorhiza kurroa*
Aralu - *Terminalia chebula*
Bulu - *Terminalia bellerica*
Nelli - *Phyllanthus emblica*
Adathoda - *Adhatoda vasica*
Viyali kaha - *Curcuma longa*
Venival geta - *Coscinium fenestratum*

Material	**Part used**
Zingiber officinalae	Rhyzome
Piper nigrum	Seeds
Piper longum	Seeds
Holarrhena antidysentrica	Seeds
Picrorhiza kurroa	Roots
Terminalia chebula	Pericarp
Terminalia bellerica	Pericarp
Phyllanthus emblica	Pericarp
Adhatoda vasica	Leaves, Bark
Curcuma longa	Rhyzome
Coscinium fenestratum	Bark

Table 1 – Part used of each ingredient for the decoction

Drug preparation

The drug was prepared at the the pharmacy of GWAI, University of Kelaniya, under the supervision of *Bhaisajya Kalpna* Unit, Dept. of *Dravyaguna Vignana* by using original ingredients. The research drug was prepared under the instructions in authenticated text, *Kashaya Sangrahaya.*

Authentication of raw materials

All the ingredients were collected from the market at Gampaha district, Sri Lanka on 02nd June 2016. The materials were authenticated by Dept: of *Dravyaguna Vignana*, GWAI, University of Kelaniya, Sri Lanka.

Preparation of the Decoction

The drug was prepared according to the *Kwatha Paribhasha of Sharangdhara Samhita.*

Preparation method

- First all the ingredients were identified correctly and then measured the required amounts of each drugs.
- Sample C - 60g of drug, 960ml of water, boiled under moderate heat to obtain 120ml of the decoction.
- After cleaned the ingredients prepared 3 samples for different decoctions.
- Sample A - 60g of drug, 240ml of water, boiled under moderate heat to obtain 60ml of the decoction.
- Sample B - 60g of drug, 480ml of water, boiled under moderate heat to obtain 60ml of the decoction
- Prepared the positive controller (+VE) by dilute 10mg of Amoxicillin into 1ml of distilled water.

Sample	Raw material amount	Water amount	Obtain amount
A (4 -1)	60g	240ml	60 ml
B (8-1)	60g	480ml	60 ml
C (16- 2)	60g	960ml	120ml

Table 2 - Preparation method of the decoction

Disc Preparation for ABST

All of glass wears were sterilized with aluminum foil wrapping by using hot air oven at least 2hr of 160℃. All of aqueous solution were prepared with sterile distilled water. Medias and distill water were sterilized by autoclave with steam at a pressure about 15 psi; temperature 121℃ in 15 minutes before experimental procedures.

Preparation of MHA plates

17.5g of MHA (CM0337, Oxoid ltd, England) mixed in a conical flask with 250ml of sterile distilled water and boiled the mixture up to dissolve the medium completely. Then the media was sterilized non absorbable cotton wad as mentioned in above. The sterile media allowed to cool up to 45℃ in water bath for 15 minutes and poured in to sterile petri dishes up to 4mm in height in a sterile environment without bubbling and transferred to the refrigerator for ambient temperature at 4℃ for 18hrs.

Preparation of nutrient media

The standard *S. pyogenes* bacterial culture from laboratory specimen slants at MRI was used in the present study. With the help of a sterile inoculating loop, loopful of bacterial culture was inoculated in a laminar unit to rejuanate, in a sterile 100ml flask containing sterile nutrient agar (NA) broth (CM0003, Oxoid ltd, England) at 37c for 18hrs to ensure the proliferation of test organism. This culture was used for susceptibility test and then stocked at 4c prior to sub culture. The inoculum size of the bacterial culture was standardized according to the National Committee for clinical laboratory standard guide lines of USA. The turbidity of the inoculums was attained with 0.5 McFarland units of turbidity to comparable with suspension of bacterial solates 1.5×10^8 CFU/ml as approximate cell density. (Tambekar & Dahikar, 2010)
Sensitivity of S. pyogenes in each decoction samples was measured in terms of IZ using modified Well Diffiution Assay (MWDA). (Almazeb et al., 2013)

Preparation of the inoculum

Prepared the dilution series using the broth which are the maximum growth of *Staphylococcus pyogenes.*

- -1 dilution series
  put 1ml broth into 9ml of peptone water
- -2 dilution series
  put 1ml -1 dilution series into 9ml of peptone water
- -3 dilution series
  put 1ml -2 dilution series into 9ml of peptone water

According to the (0.5 McFarland standards) selected the -1 dilution series for the ABST test.

Seeding the plates

All of MH plates were allowed to warm up to room temperature before seeding from the refrigerator. Hence any excess moisture will be absorbed into the medium.

From nutritional broth, 18hr old bacterial culture was taken and the inoculum were seeded over the solidified MHA plates using a sterile cotton swab in order to get a uniform microbial lawn. Then the plates left on a sterile area for excess fluid to be absorbed. (Murray et al., 2007)

The wells were bored using sterile copper cup borer of 5mm in diameter, 4mm in deep and about 3cm apart from each well. There were four wells in some plates and 3 wells in others.

A bacterial positive controller and antibiotic control were kept for comparative study. Amoxicillin (01 mg/ml) was used as a standard drug and served as positive control, while distill water was used as negative control. (NCCLS 2002). The plates were left at ambient temperature for 15 minutes to allow excess pre-diffusion of extract prior to incubation at 37℃ according to the optimum temperature required for *S. pyogenes* culture. Approximately 100μl of the decoction samples were transferred into each well which filled them respectively to fullness by using homogenous micropipette. The setups were allowing to stabilize for 3hr before being incubated at 37 for 18hr as described previous.

Anti-bacterial activity was obtained by determining the zone of inhibition around the well and it was compared with standard drug.

OBSERVATION

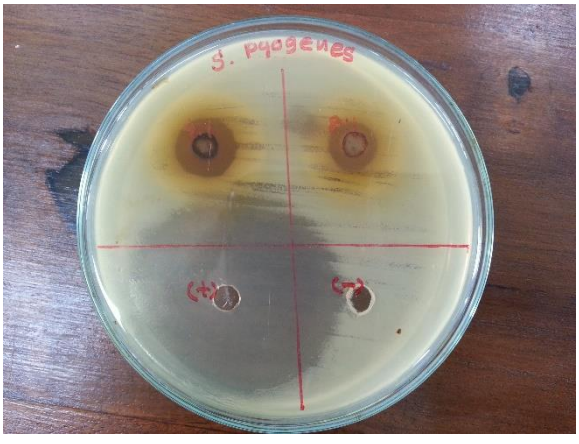

Figure 1 - ZI of sample A (60g/240ml) & sample B (60g/480ml)

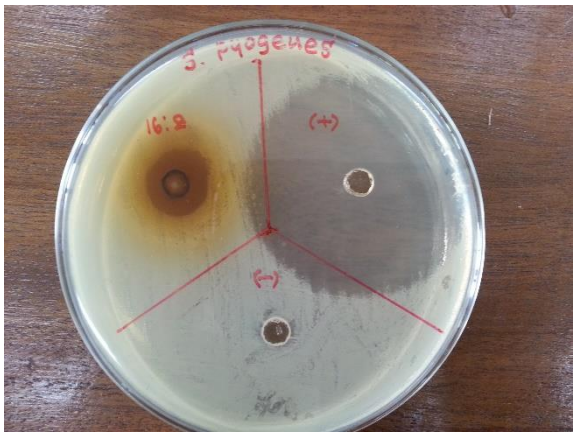

Figure 2 - ZI of sample C (60g/960ml)

RESULTS

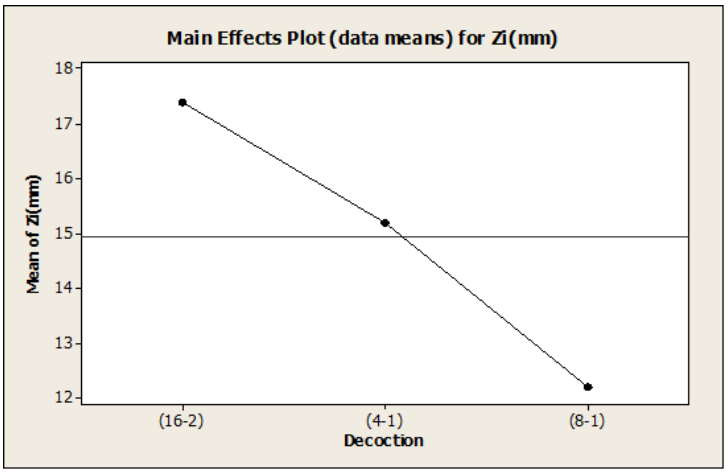

Chart 1 - Mean values of the decoctions

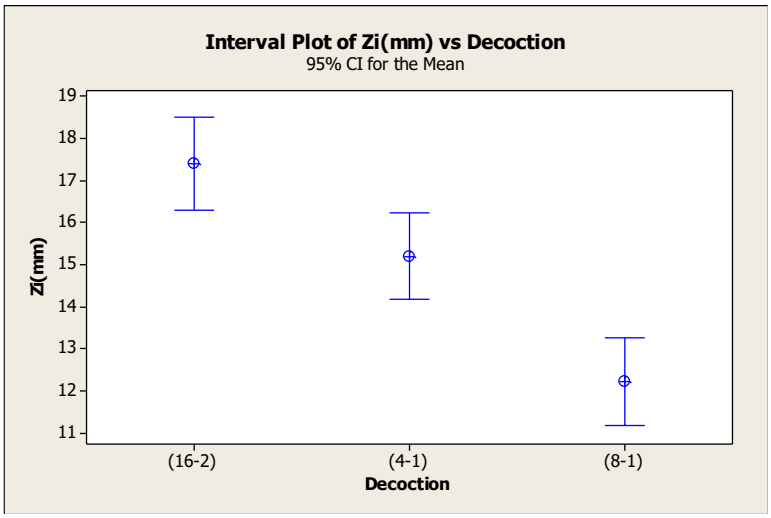

Chart 2 - Mean intervals of each decoctions

According to Tukey multiple comparisons, mean effects plot and interval plot can be concluded that decoction (16-1) gives the higher ZI value, decoction (16-1) is the most effective decoction.

The most effective decoction levels out of these three concentrations

Analytical results for comparison of three concentration of the decoction from each other given below chart.

Compared concentrations	P value
16 – 2 Vs 4 – 1	0.0042
16 – 2 Vs 8 – 1	0.0000
4 – 1 Vs 8 – 1	0.0004

Table 3 – Comparison of P value of each decoction

T test to compare the effect of Amoxicillin and the most effective decoction

Drug	Mean	Standard Deviation
Amoxicillin	36.400	0.548
Decoction (16 – 2)	17.400	0.894

Table 4 - Comparison of most effective decoction versus Amoxicillin

DISCUSSION

Anti-bacterial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extract or their active constituents are used as folk medicine in traditional therapies of 80% of the world population.

In the present study, the decoction of *Trikatu Kalinga Katuka* shows significant activity against tested bacteria. The results was compared with standard antibiotic drug. In this work, the decoction of *Thrikatu Kalinga Katuka* was found to be effective against the organism. And also 16-2 concentration of the decoction was found as the most effective concentration than others. 16-2 sample was not effective as much as the positive control (Amoxicillin). It may be due to the low diffusion of the herbal water extract throughout the Agar medium or the heavy thickness of the 16-2 decoction.

CONCLUSION

It can be concluded that the decoction *Trikatu Kalinga Katuka* is significantly effective against *Streptococcus pyogenes* at the concentration of 16-2 according to the study carried out.

SUGGESTIONS

According to the study carried out it is obvious that the selected decoction is effective against the tested micro-organism. Considering the *Ayurvedic* medicinal properties revealed by the literature review there is a high potential of introducing a novel

antibiotic out of this decoction after some further developments. And also another concentration such methanol or ethanol extractions of the decoction can use for the test.

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