

**EFFECTIVENESS OF *TINOSPORA CORDIFOLIA* ON  
STAPHYLOCOCCUS, STREPTOCOCCUS, KLEBSIELLA AND  
CANDIDA SPECIES AMONG HIV INFECTED CHILDREN - A  
RANDOMISED CONTROL TRIAL.**

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**ABSTRACT**

Acquired Immuno Deficiency Syndrome (AIDS) is one of the major global health concerns. Lack of immunity and opportunistic infections are the major threat to these patients. Ayurveda has been of immense help in managing these type of patients. The present study was conducted to assess the anti bacterial and anti fungal effectiveness of *Tinospora cordifolia* and to compare its effect with chlorhexidene. Saliva and swab specimens were collected from 12 people living with HIV infection. Study subjects were divided into two groups and one group was given chlorhexidene and other group *Tinospora cordifolia* leaves. Saliva and swab samples were taken before and after, and sent

for microbial analysis. Statistical analysis of the results revealed that there is no statistical significance between the two groups. ( $p > 0.05$ ). From the present study, we can conclude that both *Tinospora cordifolia* and chlorhexidene have antibacterial and antifungal effectiveness and since *Tinospora cordifolia* is locally available, it can be used as an adjuvant to oral hygiene practice, especially in case of AIDS patients who are more prone to opportunistic infections.

**KEY WORDS :** *Tinospora cordifolia*, Chlorhexidene, microbial counts, AIDS.

## INTRODUCTION

Human Immuno deficiency Virus (HIV) infection induces a wide array of immunologic alterations resulting in the progressive development of opportunistic infections and malignancy, which results in AIDS.<sup>[1]</sup> HIV infected people and AIDS patients often seek complementary therapies including herbal medicines due to reasons such as unsatisfactory effects, high cost, non-availability, or adverse effects of conventional medicines.<sup>[2]</sup>

AIDS is one of the major global health concerns, highly infectious and stands first as a sexually transmitted and incurable disease. Anti Retro viral Therapy has been successful to an extent in managing AIDS patients. It reduces the mortality and the morbidity of HIV infection, but it is expensive and inaccessible in many countries.<sup>[2]</sup> For the process of rejuvenation and improving the immunity, Ayurveda has a unique therapy – Rasayana Therapy. One of the main constituent of this is *Tinospora cordifolia*.<sup>[3]</sup>

*Tinospora cordifolia* commonly known as “Amrita” or “Guduchi” is an important drug in Indian Systems of Medicine (ISM) and has been used in medicine since time immemorial. The drug has been prescribed in fevers, diabetes, dyspepsia, jaundice, urinary problems, skin diseases and chronic diarrhoea and dysentery. It is also indicated in the treatment of heart diseases, leprosy, helmenthiasis and rheumatoid arthritis. The starch obtained from the stem known as “Guduchi-satva” is highly nutritive and digestive and is used in many diseases.<sup>[4]</sup>

### History and Traditional Uses

The Ayurvedic drug Guduchi or Amrita is mentioned in various classical texts of Ayurvedic System of Medicine, viz. Charak, Sushrut and Ashtang Hridaya and other treatises like Bhava Prakash and Dhanvantari Nighantu under other various names, viz. Amara, Amritvalli, Chinnarruha, Chinnodebha and Vatsadani etc. In Sushurta Samhita, it is mentioned under “Tikta-Saka Varga” and claimed to be useful in treating Kustha (leprosy), Maha-jvara (a kind of fever), Svasa (asthma) and Aruchi (anorexia).<sup>[5]</sup>

### Chemical Constituents

The plant *Tinospora cordifolia* has been subjected to chemical investigations extensively and a number of chemical constituents belonging to the different groups, viz. terpenoids, alkaloids, lignans, steroids have been reported.<sup>[6]</sup> It is distributed throughout the tropical

Indian subcontinent and China, ascending to an altitude of 300 m. In Hindi, the plant is commonly known as Giloe, which is a Hindu mythological term that refers to the heavenly elixir that has saved celestial beings from old age and kept them eternally young. The term amrita is attributed to its ability to impart youthfulness, vitality and longevity.<sup>[7]</sup> *Tinospora cordifolia* has been used in Ayurvedic preparations for the treatment of various ailments throughout the centuries. It is used as a rasayana to improve the immune system and body resistance against infections. The whole plant is used medicinally; however, the stem is approved for use in medicine as listed by the Ayurvedic Pharmacopoeia of India. This is due to higher alkaloid content in the stems than in the leaves.<sup>[8]</sup>

It is a common misconception that Ayurvedic medicines are always safe. In fact, they also pose serious health risks either in the form of adverse reactions or in the form of drug interactions. In a clinical study, *Tinospora cordifolia* has been shown to be effective as antibacterial and antifungal at a dose of 500 mg/day for a period of 21 days in healthy individuals.<sup>[9]</sup> It has also been shown not to exert any remarkable adverse effects on the cardiovascular system, renal system, central nervous system and gastrointestinal system.<sup>[9]</sup>

*Tinospora cordifolia* has been used in Ayurvedic preparations for the treatment of various ailments throughout the centuries. It is used as a rasayana to improve the immune system and body resistance against infections. The methanolic stem extract of *Tinospora cordifolia* is used as an anti-bacterial and anti-fungal agent.

HIV infected patients are more prone to various opportunistic infections due to the decreased immunity. It is important to maintain a good oral hygiene to minimize the risk of these infections. The accessibility to good oral health care services is not present everywhere. Hence the role of the Ayurvedic plant *Tinospora cordifolia* is important especially to these patients. The present study is conducted to assess the anti-bacterial and anti-fungal activity of *Tinospora cordifolia*.<sup>[10]</sup>

### **Aim Of The Study**

The aim of the study is to

1. To assess the effectiveness of *Tinospora cordifolia* on Staphylococcus, Streptococcus, Klebsiella and Candida species among HIV students.
2. To compare its effectiveness with chlorhexidene mouth wash.

### **Methodology**

Reference population were selected from Citizen Alliance for Rural Development and Training Society (CARDTS), Samvedana, Mangalore. CARDTS authorities were informed about the procedure and prior permission was taken from them and after obtaining an informed Consent, the study subjects were examined.

### **Ethical Clearance**

Ethical clearance for the study was obtained from the Ethical Committee of A.J. Institute of Medical Sciences, Mangalore. From a group of 20 inmates, 12 study subjects were selected based on the following inclusion and exclusion criterias.

### **Inclusion criteria.**

1. Subjects with more than 60% of teeth remaining and above 18 years of age.
2. Subjects consenting to participate in the study.

### **Exclusion Criteria.**

1. Subjects with CD4 count less than  $400/\text{mm}^3$
2. Subjects under any antibacterial, antifungal therapy within last 6 months.
3. Subjects using any other Ayurvedic medications.

### **Sterilization**

On the day of examination, adequate numbers of autoclaved instruments and materials was carried to CARDTS, Samvedana, Mangalore. Sterile containers was used to collect saliva and swab samples from every subjects. Appropriate infection control measures was practiced for the clinical examination and sample collection from each study subjects.

### **Methods**

From a group of 20 inmates, 12 study subjects were enrolled into the study meeting the inclusion criteria. Patients with any type of acute opportunistic infections were excluded from the study.

### **Randomisation**

The 12 study subjects were divided randomly into two groups of 6 study subjects each by flipping a coin method.

### Saliva and swab collection

Unstimulated saliva samples were collected from both control and test group prior to the study by asking the patient to expectorate into the sterile container for 2 minutes under aseptic precautions. Swab samples were taken from the buccal mucosa and dorsum of the tongue with the help of a sterile cotton swab<sup>[11]</sup> and was mixed with the saliva in the sterile container. It was then numbered and was stored in an ice box at 4 degree celsius. Saliva and swab samples together is needed to isolate the maximum number of microorganisms in Streptococcus, Staphylococcus, Klebsiella and Candida species.

### Intervention

1. Control group (Chlorhexidene).
2. Test group (*Tinospora cordifolia*)

Chlorhexidene mouth wash was obtained with the concentration of 0.12% for the study. It was used for rinsing for a period of 2 minutes.<sup>[12]</sup> *Tinospora cordifolia* leaves were freshly plucked on the day of the study. It was washed and cleaned and was carried in separate sterile plastic containers. Control group were given chlorhexidene mouth wash (0.12%) for rinsing for 2 minutes. Test group were given two small *Tinospora cordifolia* leaves to each study subject for chewing for 3 minutes. After half an hour, saliva and swab samples were collected again from the study subjects in sterile containers was numbered. It was stored in the ice box at 4 degree celsius and was transported to Department of Microbiology, A.J. Institute of Medical Sciences for microbial analysis

### Microbial Analysis

Saliva and swab samples from the sterile containers were placed in brain heart infusion broth for 30 minutes. A 4mm inoculation loop was dipped in the broth and spread over Blood agar and Mac Conkeys agar plates to isolate Streptococcus, Staphylococcus and Klebsiella species. Sabouraud's Dextrose agar plates was used for isolation of Candida species. The plates were incubated at 37<sup>0</sup>C for two days. Bacterial and fungal identification was done using colony morphology, gram staining and biochemical tests. Colony counts were determined using QUEBEC colony counter. 4 ml loop holds about 0.01 ml of broth. So after counting the number of colonies manually, it was multiplied by a dilution factor of 100 units to give the results as CFU /ml of broth.

### Statistical Analysis

The results were subjected to statistical analysis using Mann Whitney U test for comparison

between *Tinospora cordifolia* and Chlorhexidene groups. Data were analysed using SPSS version 17.0. A difference was considered to be of statistical significance if the p value was <0.05.

## RESULTS

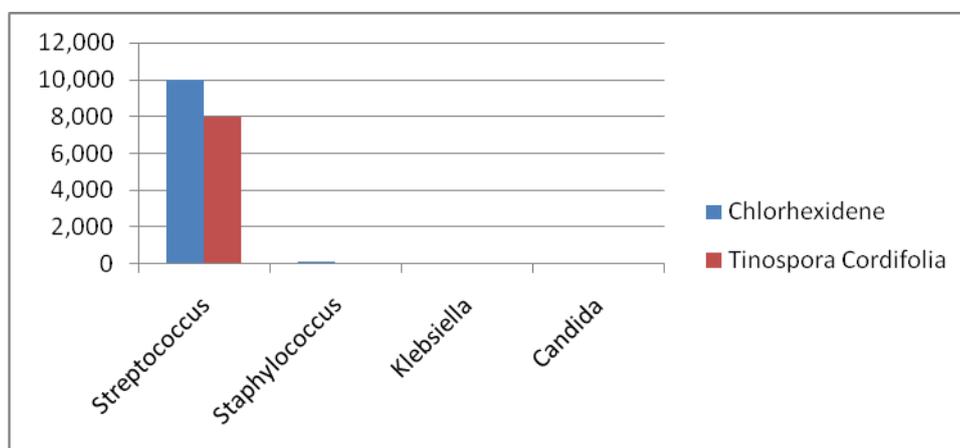
A total of 12 study subjects participated in the study out of which all were females and the mean age of the study subjects was 18.7 years. Results of the present study shows that both *Tinospora cordifolia* and Chlorhexidene has antibacterial action against Staphylococcus, Streptococcus, Klebsiella and anti fungal action against Candida species. Table 1 ( Graph 1) shows the mean reduction of microbial counts in CFU / ml among Streptococcus, Staphylococcus, Klebsiella and Candida species in control and test groups. Though the microbial count reduction was higher with chlorhexidene, there was no statistical significant difference. (  $p = 0.468$  ).

**Table 1. Mean reduction of microbial counts in CFU /ml among different species after using Chlorhexidene and *Tinospora cordifolia*.**

Microorganism	Chlorhexidene	<i>Tinospora Cordifolia</i>	P value
Streptococcus	10,000	8000	<b>0.468</b>
Staphylococcus	150	100	
Klebsiella	100	60	
Candida	20	18	

**P > 0.05**

**NS\* Not significant**



**Graph 1. Comparison of mean Reduction of microbial counts in CFU /ml between Chlorhexidene and *Tinospora cordifolia***

## DISCUSSION

In the present study, 12 HIV infected patients were selected from an HIV home in Mangalore. They were divided into two groups, Chlorhexidene group and *Tinospora cordifolia* group. The present study was conducted to assess the antibacterial and antifungal effectiveness of *Tinospora cordifolia*, which can be of immense help to HIV patients to control the various oral infections. The study also compared its effectiveness with Chlorhexidene group. One group were asked to chew the leaves. Other group were asked to rinse the mouth with Chlorhexidene solution. Saliva and swab samples were collected from both the groups. Microbial analysis revealed a microbial count reduction in Streptococcus, Staphylococcus, Klebsiella and Candida species in both Chlorhexidene and *Tinospora cordifolia*.

The present study shows that on comparing *Tinospora cordifolia* with chlorhexidene, since there is no statistical significant reduction, it can be presumed that both *Tinospora cordifolia* and Chlorhexidene could be effective in reducing the bacterial and fungal growth in the oral cavity. Since it is readily available in and around our living environment, chewing of *Tinospora cordifolia* can be used as a supplementary oral hygiene aid, especially in case of HIV patients who are more prone to these type of opportunistic infections.

The result of the present study is in comparison with Ram S M,<sup>[13]</sup> who reported that *Tinospora cordifolia* have anti bacterial and antifungal properties. It also share common properties of amalaki, which is fortified with Vitamin C which is a natural, abundantly available powerful antioxidant, anti inflammatory and free radical scavenger.<sup>[13]</sup> Stanley P and Venugopal P<sup>[13]</sup> reported the anti inflammatory and hepatoprotective properties for *Tinospora cordifolia*. It acts on liver, the chief site of metabolism of food and drugs, normalizing the elevated transaminases and repletes the glutathione sodium dismutases responsible for scavenging of free radical. The leaf extract has memory enhancing and tranquilising properties which treat the depression often associated with HIV patients.<sup>[14]</sup> These all could be the added benefits of *Tinospora cordifolia* when used among AIDS patients. However long term clinical trials should be conducted in this field to prove its effect.

The result of the present study is in concurrence with the study conducted by Suresh D R<sup>[15]</sup>. Herbal preparations are cheap and simple; hence they remain a hope for the HIV/AIDS infected people with opportunistic infections. Saliva can constitute a first line of defense

against free radical mediated oxidative stress. The use of saliva as a diagnostic fluid has become somewhat of a translational research success story.

The *Tinospora cordifolia* leaves used in the present study is non toxic, inexpensive, easy to use and readily available. *Tinospora cordifolia* could be our nature's gift to the AIDS patient as it may have so many unexplored uses especially in case of patients with less immunity. It could be of immense help in dealing with the opportunistic infections and other systemic conditions of AIDS patients. However more studies are required to evaluate its long term effects.

## CONCLUSION

From this present study, we can conclude that both *Tinospora cordifolia* and chlorhexidene have antibacterial and antifungal effectiveness against Staphylococcus, Streptococcus, Klebsiella and Candida species. Since *Tinospora cordifolia* is locally available, it can be used as an adjuvant to oral hygiene practice, especially in case of HIV patients who are more prone to various opportunistic infections. However further clinical studies need to be conducted on this field to assess its long term effects.

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