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RESEARCH ARTICLE

ANTI BACTERIAL ACTIVITY OF *TINOSPORA CORDIFOLIA* EXTRACTS ON CLINICAL ISOLATES FROM HIV INFECTED PATIENTS

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ABSTRACT

The present study was carried out to evaluate the *in vitro* antibacterial activity of aqueous leaf and stem extracts of *Tinospora cordifolia* on HIV related opportunistic bacterial pathogens. The leaves and stems of *T. cordifolia* were powdered and subjected to aqueous extraction. Preliminary phyotochemical screening was done by using standard procedures. The antibacterial activity of the aqueous extract of leaves and stems of *T. cordifolia* was studied using disc diffusion method against *Escherichia coli, Enterococcus faecalis, Streptococcus pneumoniae, Salmonella typhi and Staphylococcus aureus* these were isolated from blood and urine samples of HIV infected patients. The aqueous extracts of *T. cordifolia* exhibited effective antimicrobial activity against all tested microorganisms, while the leaf aqueous extract of *T. cordifolia* exhibited maximum zone of inhibition against *Enterococcus faecalis* (28 mm) and *Salmonella typhi* (26 mm) at 50 mg/ml concentration. The stem aqueous extract of *T. cordifolia* exhibited maximum zone of inhibition against *Enterococcus faecalis* (23 mm) and *Streptococcus pneumonia* (24 mm) at 50 mg/ml concentration. The present results suggest that the aqueous extracts of *T. cordifolia* have significant antibacterial activity against HIV related opportunistic bacterial pathogens.

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INTRODUCTION

In the present time multi-drug resistant microbial strains are continuously increasing due to indiscriminate and repetitive use of antimicrobial drugs. In addition, synthetic drugs are more expensive for the treatment of diseases. Therefore there is a need to develop new infection fighting strategies to control microbial infections (Rajesh *et al*., 2007; Sieradzki *et al*., 1999). Medicinal plants represent a rich source of antimicrobial agents. Among the estimated 250,000-500,000 plant species, only a small percentage have been investigated phytochemically (Mahesh and Satish, 2008). Recently, the plant *T.cordifolia* is of great interest to researchers across the globe because of its reported medicinal properties like anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory and anti-neoplastic activities (Soham and Shyamasree, 2012). *T. cordifolia* is climbing shrub, which belongs to family Menispermaceae, widely distributed in India, China, Burma, Ceylon, Myanmar and Sri Lanka, which is known by the common names Heart-leaved Moonseed, Guduchi and Giloy. Anti-HIV effects of *T.cordifolia* extract (TCE) was revealed by reduction in eosinophil count, stimulation of B lymphocytes, macrophages and polymorphonuclear leucocytes and hemoglobin percentage revealing its promising role in management of the disease (Soham and Shyamasree, 2012; Kalikar *et al*., 2008; Akhtar, 2010). The antibacterial activity of TCE has been assayed against *Escherichia coli, Staphylococcus aureus, Klebsiella pneumoniae, Proteus vulgaris, Salmonella typhi, Shigellaflexneri, Salmonella paratyphi, Salmonella typhi murium, Pseudomonas aeruginosa, Enterobacter aerogene,* and *Serratia marcesenses* (Jeyachandran *et al*., 2003; Tambekar *et al*., 2006; Narayanan *et al*., 2011). In mice models, TCE has been reported to function in bacterial clearance and improved phagocytic and intracellular bactericidal capacities of neutrophils (Thatte *et al*., 1992).

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MATERIALS AND MEHODS

Collection of plant material

T. cordifolia samples were collected from Pamidi Kothapalli Village, Anantapur District, Andhra pradesh, India. The plant was authenticated by a botanist, S.K.University.

Extraction of selected plant material by Maceration method

The leaves and stems of *T.cordifolia* were shade dried for 5 days and were ground into powder separately. Exposure to sunlight was avoided to prevent loss of active components. 100 gms of each leaf and stem powders were soaked in 1000ml of double distilled water separately and kept at room temperature for 3 days with intermittent shaking. The extracts were filtered through muslin cloth. The extracted liquids were subjected to water bath evaporation to remove the water. For water bath evaporation, liquid extracts were placed in a beaker and subjected to water bath evaporation at 60°C temperature for 7-10 hr daily for 3-4 days until as emisolid state of extracted liquid is obtained. The semisolid extracts produced were kept in the deep freezer at -20°C overnight and then subjected to freeze drying. Extracts obtained by this method were then weighed and percentage yield was found to be stem 16.73 %, leaf 10.73%. The above aqueous extracts were stored at 4 °C until further use.

Collection of microorganisms

All the test cultures were procured from ART Center, RDT Hospital, Bathalapalli, Anantapuramu district. These are clinical isolates from blood and urine samples of HIV infected patients.

Preliminary phytochemical analysis

The aqueous extracts of *T.cordifolia* subjected to preliminary phyotochemical screening byusing standard procedures to identify the phytoconstituents as described by Trease and Evans, 1978.

Antibacterial activity evaluation by Agar well diffusion assay

In vitro antibacterial activity was evaluated by Agar well diffusion method using Mueller Hinton Agar (MHA) (Bauer *et al*., 1996).

Working stock was prepared as 1ml of each bacterial strain was initially inoculated in100 ml of sterile Mueller Hinton broth and incubated for 37°±1°C for 24 hr respectively. Then 0.2ml of the each test organisms from the working stock were seeded into 100ml sterile MHA medium and cooled to 48°C to 50°C in a sterile Petri dish respectively. When the MHA solidifies, six holes of uniform diameter (7 mm) were made using sterile alluminium borer. Then, 70µl of each leaf, stem aqueous extracts standard solution (10, 20, 30, 40, 50 mg/ml) respectively and control (Ciprofloxacin 25mg/ml) were placed in each hole separately under aseptic condition. The plates were then maintained at room temperature for 2 hr to allow the diffusion of the solution into the medium. All the bacterial plates were then incubated at 37°±1°C for 18 hr and the zone of inhibition was measured (including the diameter of the bore (7 mm)) and the results were recorded.

RESULTS

The result of preliminary phytochemical analysis presented in Table 1, clearly indicated the presence of plant secondary metabolites like alkaloids, flavonoids, proteins, phenolics, tannins, carbohydrates and glycosides. Antimicrobial activity against the pathogens isolated from HIV infected patients, such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, *Enterococcus faecalis* and *Streptococcus pneumonia* were presented in Table 2 and in the Fig.1 (1-5) are the images for inhibitory zones of leaf aqueous extract and (6-10) are the images for inhibitory zones of the stem aqueous extract respectively. The aqueous extracts of *T. cordifolia* exhibit effective antimicrobial activity against all tested microorganisms, like leaf aqueous extract of *T. cordifolia* exhibited maximum zone of inhibition against *E. faecalis* (28 mm) and *S. typhi* (26 mm) at 50 mg/ml concentration (Fig.2), while the stem aqueous extract of *T. cordifolia* exhibited maximum zone of inhibition against *E. faecalis* (23 mm) and *S. pneumonia* (24 mm) at 50 mg/ml concentration (Fig.3).

Table 1. Phytochemical analysis

S. No	Phytoconstituents	Stem Aqueous Extract	Leaf Aqueous Extract
1	**Alkaloids**		
	Mayer's test	+	+
	Hager's test	+	+
	Dragondorff's test	-	+
	Wagner's test	-	+
2	**Flavanoids**		
	Shinoda test	+	+
3	**Proteins & amino acids**		
	Biuret test	+	+
	Millons test	-	+
	Precipitation test	+	+
	Ninhydrin test	+	+
4	**Carbohydrates**		
	Molish test	+	+
5	**Phenolics and tannins**		
	Ferric chloride test	-	+
6	**Glycosides**		
	Keller- Killiant test for cardiac glycosides	+	-
	Borntrager's test for anthraquinone glycosides	-	-
	Foam test for saponin glycosides	+	+
	Alcohol extract test for coumarin glycosides	+	+
	Cyanic glycosides	-	+

(-): Absence, (+): Presence

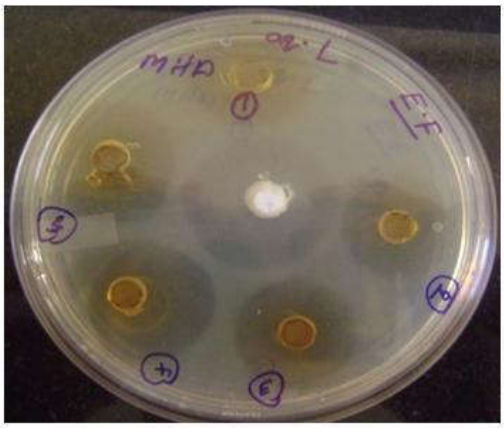
Table 2. Antimicrobial activity of extracts of *T.cordifolia*on pathogens isolated from HIV infected patients

S. No.	Microorganism (Strain)	Zone of inhibition measured in mm											
		Leaf aqueous extract (mg/ml)						Stem aqueous extracts(mg/ml)					
		Control (25)	10	20	30	40	50	Control (25)	10	20	30	40	50
1	*E. coli*	24	10	12	14	17	18	24	12	14	17	18	19
2	*E.faecalis*	27	19	21	24	25	28	27	16	18	20	21	23
3	*S.pneumoniae*	26	11	14	18	20	24	26	16	18	21	23	24
4	*S. typhi*	30	14	18	20	24	26	30	07	08	13	15	17
5	*S. aureus*	28	12	13	15	18	20	28	11	14	15	16	16

1

Escherichia coli

2

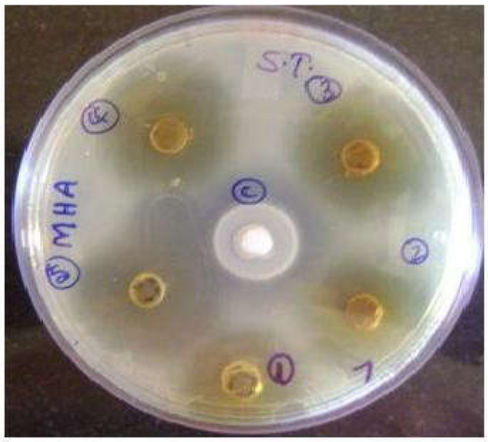


Enterococcus faecalis

3

Streptococcus pneumoniae

4



Salmonella typhi

5

Staphylococcus aureus

6

Escherichia coli

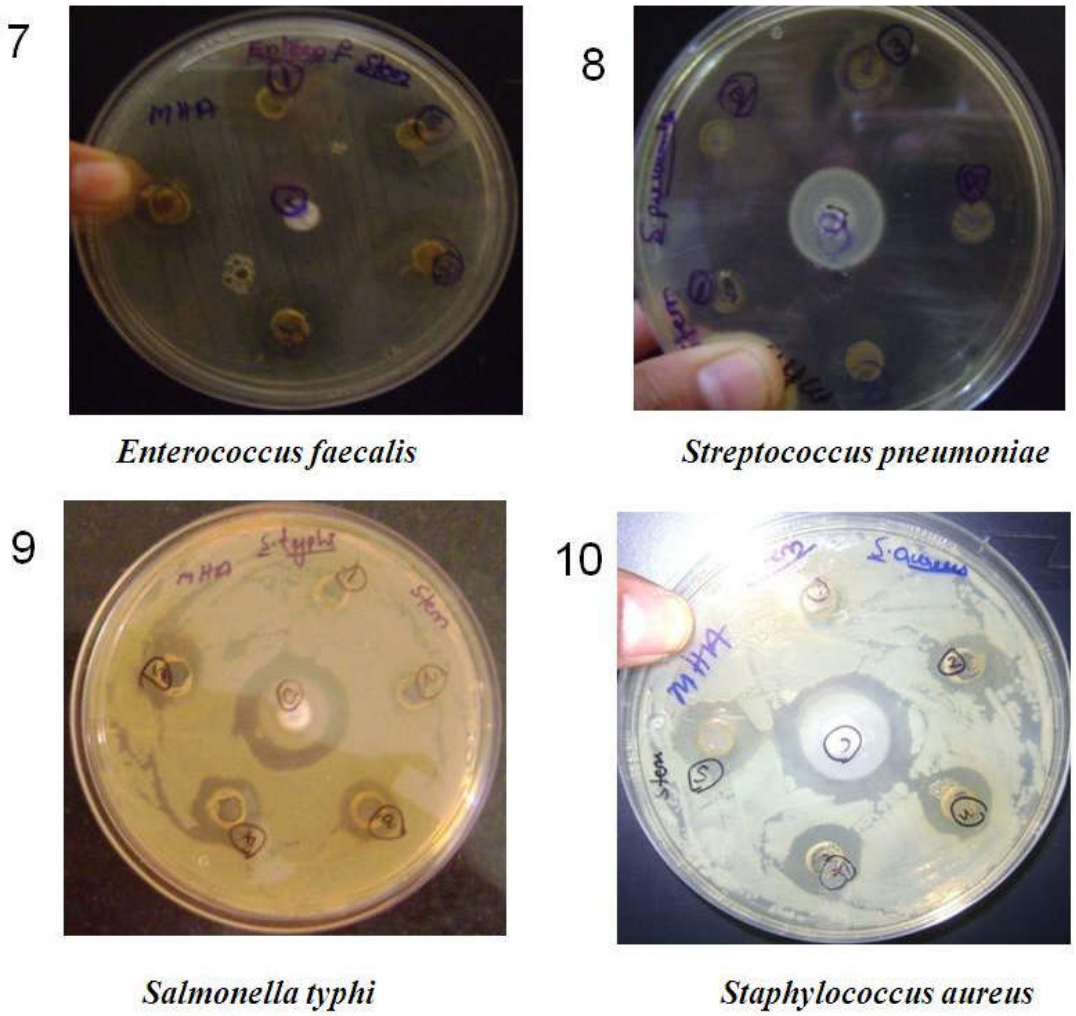


Fig. 1. Antimicrobial activity of extracts of *T. cordifolia* on pathogens isolated from HIV infected patients

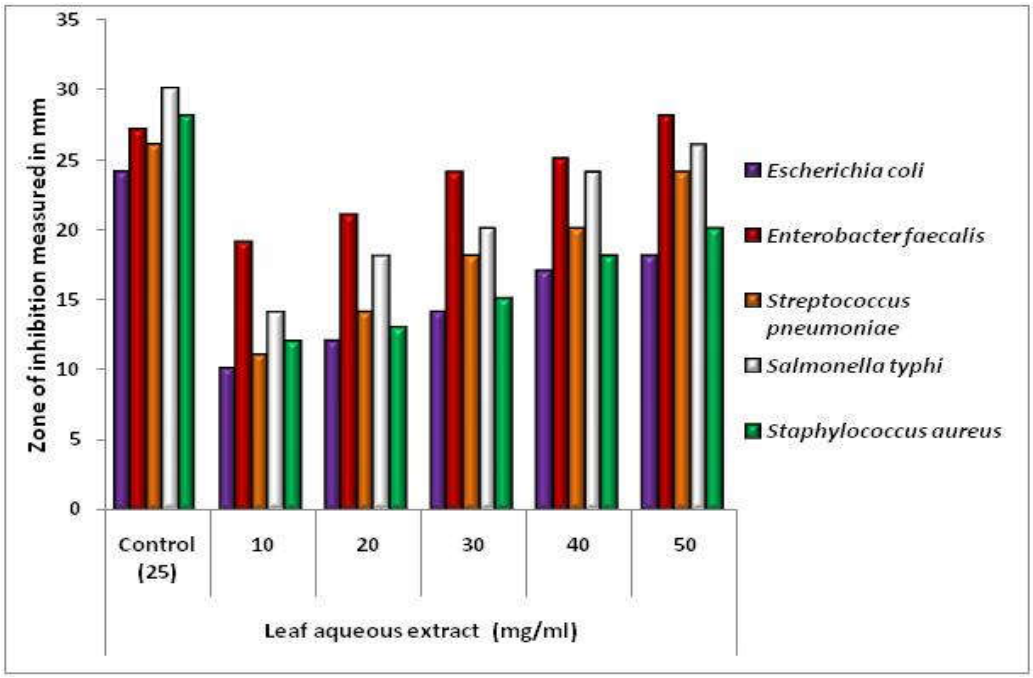


Fig. 2. Graphical representation of antimicrobial activity of leaf aqueous extract of *T. cordifolia* on pathogens isolated from HIV infected patients

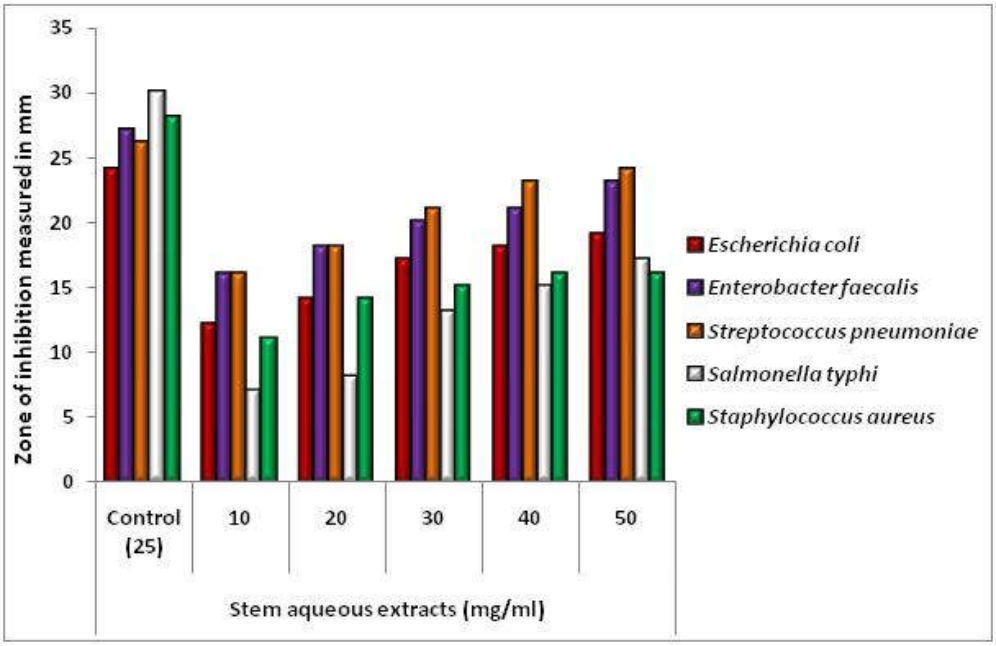


Fig. 3. Graphical representation of antimicrobial activity of stem aqueous extract of *T. cordifolia* on pathogens isolated from HIV infected patients

DISCUSSION

The Emergence and widespread of Multidrug resistance bacterial and fungal diseases has been the major concern now plants have been a cornerstone in traditional folk medicine to treat microbial infections. A days and has been scientifically validated for their safety, efficacy and irrespective of the presence of conventional medicine (Dieudonné *et al*., 2015; Ravi Kant *et al*., 2011). The leaf and stem aqueous extracts is potential source for purification of phytochemicals, and they have good antimicrobial activity against several bacterial strains (Ravi Kant *et al*., 2011). The preliminary phytochemical analysis of leaf and stem aqueous extracts of *T. cordifolia* revealed the presence of alkaloids, flavonoids, carbohydrates, tannins, phenolic compounds, amino acids, proteins and glycosides may also account for its very high antimicrobial activity against test microorganisms. Acquired immune deficiency syndrome (AIDS) is a fatal illness caused by human immune deficiency virus (HIV), which breaks down the host immune system, leaving the subject vulnerable to life-threatening opportunistic infections. Opportunistic infections and associated complications account for a considerable proportion of mortality (Anonymous, 2007). Traditional medicine has demonstrated its contribution to health through reduction of disabilities caused by diseases such as HIV/AIDS, malaria, tuberculosis and other microbial infections (Elujoba *et al*., 2005). From the experiment the results suggested that *T. cordifolia* aqueous leaf and stem extract can be used for treatment of opportunistic infections in HIV/ AIDS patients.

Conclusion

It is concluded that *T. cordifolia* plant is a richest source of phytochemical constituents and has antimicrobial activity on pathogenic organisms. The results of the present study are encouraging as all the tested pathogens exhibited sensitivity to aqueous extracts of *T.cordifolia*, and also support the traditional uses of the plants for the treatment of opportunistic infections in HIV/ AIDS patients.

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Conflicts of interest

There are no conflicts of interest.

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