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Studies on Antimicrobial Activity of Leaf Extract of *Tinospora Cordifolia* on Urinary Tract Infection (UTI) Causing Four Pathogenic Bacteria

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Abstract
The present study was carried out to evaluate the extracts of *T. cordifolia* was done by using standard procedures. The antibacterial activity of the aqueous extract of leaves of *T. cordifolia* on four pathogenic bacteria namely *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia and Enterococcus faecalis* for Urinary tract infection (UTI). The leaves of *T. cordifolia* were powdered and subjected to aqueous extraction. Preliminary phytochemical screening was done by using standard procedures. The antibacterial activity of the aqueous extract of leaves of *T. cordifolia* was studied using disc diffusion method against *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia and Enterococcus* faecalis these were isolated from 70 school going healthy children. The aqueous extracts of *T. cordifolia* exhibited effective antimicrobial activity against all four pathogenic bacteria. More specifically, higher percent growth inhibition was obtained in presence of aqueous extracts in comparison to solvent extracts, which was much higher than synthetic antibiotics while the leaf aqueous extract of *T. cordifolia* exhibited maximum zone of inhibition against *Escherichia coli,* (28mm) *Pseudomonas aeruginosa* (26mm), *Klebsiella pneumonia*(23mm) *and Enterococcus* faecalis (22mm). The present results suggest that the aqueous extracts of *T. cordifolia* have significant antibacterial activity against Urinary tract infection (UTI) related opportunistic bacterial pathogens.

Keywords
Antibacterial activity, Phytochemicals, inhibition zone, *Tinospora cordifolia*.

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INTRODUCTION:
Many of the plant materials used in traditional medicine are readily available in rural areas at relatively cheaper rates than modern medicines (Mann *et al.,* 2008). Medicinal plants represent a rich source of secondary metabolites, many of which are antimicrobial agents (Mahesh and Satish, 2008). Medicinal plants have been used since a long time as a source of medicine to combat various ailments including infectious diseases. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs (WHO, 1999). In developing countries, medicinal plants are used in traditional medicine. Such plants have been investigated for better understanding of their medicinal properties. Number of medicinal plants has been used for their antifungal and antibacterial properties (Adamu *et al.,* 2005) and in the treatment of a wide range of infections (Mongalo *et al.,* 2013). *T. cordifolia* Miers. is one of the most versatile rejuvenative herbs belonging to the family Menispermaceae. It is also called as amrita or nectar

of life, as it is extremely useful in strengthening the immune system of the body and keeping the functions of its various organs in harmony (Desai *et. al.,* 2002). The extract of the plant contains several bitter principles, glucosides, alkaloids, a glycoside-giloin, a non-glucosidegilenin, gilosterol, alkakoid tinosporin, tinosporic acid, tinosporol, berberine, tinosporidine, sitosterol isolated, cordifol, heptacosanol, octacosonal and a new furanoid diterpene-tinosporide (Singh *et. al.,* 2003). The stem of *T. cordifolia* is used as an ingredient in Ayurvedic preparations used in general debility, dyspepsia, fevers and urinary diseases. The root is a powerful emetic and is used for treating visceral obstruction; its watery extract is used in leprosy. Administration of the polysaccharide fraction from *T. cordifolia* was found to be very effective in reducing the metastatic potential of B16F-10 melanoma cells (Leyon and Kuttan, 2004). *T. cordifolia* is reported to have immunostimulatory property (Mathew and Kuttan, 1999). It is considered as a general tonic in Ayurveda. The positive effect of *T. cordifolia* on leucocytes suggests its use as an adjuvant in cancer therapy. Activation of macrophage by *T. cordifolia* leads to increased colony forming units, leading to leucocytosis and improvement in neutrophil function. It was found that the herbal mixture containing this plant was effective in treatment of advanced malignancies. In addition, it helped in diseases like raktapitta, anaemia, cardiac debility, diabetes, sexual debility and spleen disorders (Mathew and Kuttan, 1999).

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. 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 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).

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).

MATERIAL AND METHODS:

Collection of plant material

T. cordifolia samples were collected from Kothapally Village, Karimnagar District, Telangana state, India. The plant was authenticated by a botanist, SRR. Arts & Science College Karimnagar.

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 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 semisolid 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 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 Urine samples were collected from 70 school going children for Urine culture. Preliminary phytochemical analysis the aqueous extracts of *T. cordifolia* subjected to preliminary photochemical screening by using standard procedures to identify the phytoconstituents as described by (Treasa and Evans, 1978).

The extraction was continued until complete extraction was affected (24 to 30 h) and the solvent was removed at the reduced pressure with the help of rotary vacuum evaporator to yield a viscous dark green or brown residue. Biochemical and phytochemical analysis Both biochemical (carbohydrates, tannins, protein) and phytochemical

(alkaloids, saponins) content in the three medicinal plants were carried out using standard methods (Bharathi *et al.,* 2012; Patel *et al.,* 2011). 100 mg of the crude extracts were taken and mixed with 1 ml methanol. From this stock, different concentrations were prepared, namely, 6, 10, 30, 50 and 100 mg/ml. These concentrations were used for antimicrobial test. Various solvents such as ethanol and petroleum ether have been used for extraction of secondary metabolites.

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 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 aluminium borer. Then, 70µl of each leaf 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 AND DISCUSSION:

Many of the infectious diseases are still a major challenge to health issues all over the world. The emergence of resistance to antibiotics has further compounded the problem (Alli *et al.,* 2011). The need for new antimicrobial compounds has become imperative. The ethnobotanical importance of the tested medicinal plants has been highlighted and it is used for various diseases. The result of preliminary phytochemical analysis presented in (Table-1), clearly indicated the presence of plant secondary metabolites like alkaloids, Carbohydrates, Proteins, tannins, Saponins and Amino acids.

Table 1. Phytochemical analysis of the plant extract derived from *T. coridifolia*,

Sl. No	Plant constituents	Test/Reagent	Observation
1	Alkaloid	Mayer's test	+ve
2	Carbohydrates	Molish test	+ve
3	Proteins	Burette and Millions test	+ve
4	Tannins	Ferric chloride and Lead acetate	+ve
5	Saponins	Foam and NaOH	+ve
6	Amino acids	Ninhydrine	+ve

Table 2. Antimicrobial activity of Leaf aqueous extracts (mg/ml) of *T. cordifolia* on four pathogens isolated from 70 school going healthy children.

Sl. No	Microorganism (Strain)	Zone of inhibition measured in mm					
		Control (25)	10	20	30	40	50
1	*Escherichia coli*	24	10	12	14	18	28
2	*Pseudomonas eruginosa*	26	12	14	20	23	26
3	*Klebsiella pneumonia*	29	17	20	18	20	24
4	*Enterococcus faecalis*	31	18	18	20	26	20

A

B

C D

Figure 1: Antibacterial activity of Leaf aqueous extracts against *E. coli, P. aeruginosa, K. pneumonia* and *E. faecalis* of conical flask with leaf extracts; (B.C and D) NAM plates showing ZOI by well diffusion method

Antimicrobial activity against the pathogens isolated from 70 school going children, such as *E. coli, P. aeruginosa, K. pneumonia and E. faecalis* for Urinary tract infection (UTI). were presented in (Table 2) and in the (Fig.1) are the images for inhibitory zones of leaf aqueous extract and (6-10) are the images for inhibitory zones of the leaf 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. coli* (28 mm) *P. aeruginosa* (26 mm) at 50 mg/ml concentration, Leaf aqueous extract of *T. cordifolia* exhibited maximum zone of inhibition against *K. pneumonia* (24 mm) and *E. faecalis* (24 mm) at 50 mg/ml concentration (Fig.1).

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 day 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 different concentrations of crude extracts were tested against the pathogenic bacteria, namely, *E. coli, P. aeruginosa, K. pneumonia* and *E. faecalis* (Figures -1). It was observed that *T. cordifolia* leaf extract showed maximum zone of inhibition of 28.0 mm against the bacterium *E. coli* at the concentration of 50mg/ml (Figure- 1).

The preliminary phytochemical analysis of leaf aqueous extracts of *T. cordifolia* revealed the presence of alkaloids, carbohydrates, tannins, amino acids, proteins, and Saponins may also account for its very high antimicrobial activity against test microorganisms.

Traditional medicine has demonstrated its contribution to health through reduction of disabilities caused by diseases such as Urinary tract/HIV/AIDS, malaria, tuberculosis and other microbial infections (Elujoba *et al.,* 2005). From the experiment the results suggested that *T. cordifolia* aqueous leaf extract can be used for treatment of opportunistic infections in Urinary tract 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 Urinary tract disease patients.

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