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EVALUATION OF ANTIMICROBIAL ACTIVITY OF LEAF EXTRACT OF *TINOSPORA CORDIFOLIA* ON URINARY TRACT INFECTION (UTI) CAUSING PATHOGENIC BACTERIA

Madan Mohan Gunda*[1] and Rani Kommidi[2]

[1]Dept of Biotechnology Govt Degree & PG College (Autonomous) Siddipet. T.S.
[2]Dept. of Botany. Govt Degree & PG College (Autonomous) Siddipet. T. S

***Corresponding Author: Madan Mohan Gunda**

Dept of Biotechnology Govt Degree & PG College (Autonomous) Siddipet. T.S.

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ABSTRACT

Urinary tract infection (UTI) has become a more grievous problem today, due to multidrug resistance of infecting Gram-positive (GP) and Gram-negative (GN) bacteria, sometimes even with multiple infections. This study examines effectivity of *Tinospora cordifolia* Tropical flowering plant. *T. cordifolia* or "Guduchi" is a well-known medicinal plant studied extensively since ancient times. This article specifically describes the role of *T. cordifolia* as an antimicrobial agent and as an immunity enhancer plant. Pathogenic bacteria were isolated from urine samples of 70 school going children and were sent to AMPATH for Urine culture Antibiograms of 4 isolated bacteria (*Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia and Enterococcus faecalis).were* isolated from urine samples were ascertained by the disc-diffusion method, and antibacterial effectivity of plant extracts was monitored by the agar-well diffusion method. Isolated bacteria were floridly MDR (Multi Drug Resistant Bacteria) to most antibiotics of the day. Methanol extracts of *T. cordifolia* plant were used, and extracts at least caused 25–29 mm as the maximum size of zone of inhibition on bacterial lawns. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of methanol extracts of *T. cordifolia* plant were recorded. The Ethanol extract of *P. aeruginosa,* had 4.08 mg/ml as the lowest MIC value and 8.37 mg/ml as the lowest MBC value, against signifying effectivity; but it had the highest MIC value of 9.68 mg/ml. and the highest MBC value of 24.07 mg/ml for most other MDR bacteria including *Escherichia coli*. Qualitative phytochemical analysis was done for these *T. cordifolia* plant, which can be promoted as complementary medicine. In the present study, we examined the anti-microbial effects of dried and powered leaves of *T. cordifolia*. were extracted with Ethanol, Ethyl acetate, and hexane Solvents were used, process followed by soxhelt extraction

KEYWORDS: Medicinal plant; *Tinospora cordifolia*; solvent extracts; anti-oxidant activity and, Ethanol extract.

INTRODUCTION

Urinary tract infections (UTIs) are largely caused by Gram-negative (GN) bacteria, mainly *Escherichia coli* and by mixed infections of (Gram-positive, GP) *Staphylococcus aureus, Entrococcus faecalis*, other Gram-negative (GN) bacteria, *Klebsiella pneumoniae, Enterobacter aerogens, Proteus mirabilis, Citrobacter frendi, Proteus vulgaris* and *Klebsiella oxytoca*, in declining order, when monitored, Institute of Medical Sciences, Sume Hospital, Medical Sciences. for example (Mishra *et al.,* 2013).

Infected bacteria usually involve the faecal flora and the UTI episode begins, when there are obstructions to urinary flow in a person, such as complications, calculi, tumours, prostatic hypertrophy, vesicourethral reflux, diabetes, anal disease, pregnancy, catheterization, some surgical procedure at the urinogenital region and cystoscopy (Saint *et al.,* 2002). Infected bacteria attack the urethra and bladder with the body's defence mechanism and reduce urine flow. Under these conditions, bacteria pass into the bladder mucosa through urination, colonizing, multiplying and causing inflammation; It causes unbearable pain, burning, frequency and urgency of urination, nocturia, foul-smelling, cloudy urine and haematuria. True, at the beginning of the problem, the patient reports to the doctor and empirical treatment is initiated prior to obtaining a culture report of the urine sample.

If any infection is not controlled in the patient, the infected microorganisms become resistant to the internally applied antibiotics and the drug-resistant cell survives and becomes infected with the bacterial genetic modification (McMurry & Levy, 2011). In short, there are many aspects of antibiotic resistance in pathogenic

bacteria and this condition has become a clinical illusion. A physician often prescribes some high-grade antibiotics in experiential therapy to prevent failure from treatment failure arising from the presence of MDR bacteria. Frequent attacks mainly on women are a more serious problem than UTI injected, some complementary / supportive / synergistic treatment strategy is guaranteed.

Tinospora cordifolia (Willd.) Miers ex Hook.F. & Thoms. (Family: Menispermaceae) is an important drug of Indian Systems of Medicine (ISM) and used in medicines since times immemorial. *T. cordifolia* (Guduchi) is one of the most versatile rejuvenate herbs belonging to the family Menispermaceae. It is also called as 'Amrita or nectar of life', as it strengthens the immune system of the body and maintains the functions of its various organs in harmony (Nostro *et.al.,* 2000). In the ancient literature *T. cordifolia* preparations were used in fever, diabetes, dyspepsia, jaundice, urinary problems, skin diseases and chronic diarrhoea and dysentery. It has also been indicated to be useful in the treatment of heart diseases, leprosy, helmenthiasis and rheumatoid arthritis (Misra and Bhava Prakash 1969. Kirtikar and Basu 1933 Sharma and Dravya 1969).

Plant extracts of *T. cordifolia* have been reported to have potential against microbial infections. The anti-bacterial activity of *T. cordifolia* extracts have been assayed against various Gram positive and Gram-negative organisms. The antimicrobial activity of TC stem extracts was investigated against bacteria causing UTIs viz. uropathogens, *Escherichia coli* and *Staphylococcus aureus*. The study conducted using disc diffusion method showed that all three solvent extracts of TC reveal different antibacterial activity against both uropathogenic isolates with decreasing order as ethanolic (maximum) > methanolic (moderate) > aqueous (poor).

T. cordifolia and its phytochemicals are known and studied for its antimicrobial properties. In the last few years, a number of studies have been conducted in different countries to prove such efficiency (Priyanka Singh 2015). *T. cordifolia* is well known for its immunomodulatory response. This property has been well documented by the scientists. A variety of compounds which are responsible for immunomodulatory activity are isolated and studied from the plant (Tripathi and Sharm 1997 and Bishayi *et.al.,* 2002).

Urinary tract infections (UTl) are one of the most common infectious diseases caused by bacterial entry and multiplication along the normally sterile urinary tract. UTI can progress to become a complicated infection in an individual with functional, metabolic or structural abnormalities of the genitourinary tract. Recurrent UTI is also common with relapse or re-infection. *E. coli* is the commonest cause and other organisms involved are Klebsiella, Proteus, Enterobacter, Citrobacter, Serratia and Pseudomonas. UTI significantly impacts the quality of life of patients with a psychological burden because they live with the anxiety of sudden acute episodes. Synthetic antimicrobial agents are equally efficacious against UTI however, these drugs have limited therapeutic utility and the organisms rapidly develop resistance. Complicated UTIs normally require a longer course of antibacterial therapy that is associated with the various side effects (LE Nicolle 2015 and Priyanka Singh 2015). This warrants the need of an alternative therapy with less side-effects or which can be used as an adjuvant therapy along with the standard line of treatment with this view we here reporting the specific antimicrobial and immunomodulatory activity of *T. cordifolia* and its relevance in urinary tract infections.

MATERIAL METHOD

Fresh and healthy leaves of *T. cordifolia* were collected from local growers. The leaves were washed thoroughly in distilled water and the surface water was removed by air drying under shade. The leaves were subsequently dried in a hot air oven at 40^0 C for 48h, powdered and used for extraction. *T.cordifolia* leaf extract preparation Ethanol, Ethyl acetate, and hexane Solvents were used, process followed by soxhelt extraction.

Test bacterial pathogens Four UTI causing pathogenic bacteria, *E. coli, P. aeruginosa, K. pneumonia* and *E. faecalis* were isolated from urine samples Isolation of UTI causing pathogenic bacteria Culture media and inoculums preparation Bacteria grown in Nutrient Broth in pH 7.4 at 37°C. Culturing bacterial strain in Nutrient agar/Broth and inoculated in it. Further incubated at 37°C for 72 hrs. Testing for antibacterial activity Well diffusion was method employed and antibacterial activity of prepared extracts were done *in-vitro* by well diffusion method. Statistical analysis (Mean) of obtained data was done to determine the significance of extract for antimicrobial activity of *T. cordifolia*. The values were expressed in mean. Urine samples were collected from 70 school going children and were sent to AMPATH for Urine culture.

Statistical analysis

Kruskal–Wallis H test for data of zone of inhibition as antibacterial activities in agar-cup method of *T. cordifolia* plant against four bacteria was done using the Statistical Package for Medical Science version 17.0.

RESULTS

Ethnomedicinal information on selected medicinal plant *T. cordifolia* are documented along with the details of modalities on crude extracts as medicine for many ailments used by local ethnic aborigine groups (Fig-I). Most of these plants are used for infectious diseases and were found edible as medicines by the aborigine society. The immuomodulatory property of *T. cordifolia* is well documented. (Tripathi *et. al.,* 1997, Subramanian *et.al.,* 2002) Active compo unds 11- hydroxymustakone, N-methyl-2-pyrrolidone, N-formylannonain, cordifolioside *m magno florine*, tinocordiside and syringin (Sharma

et.al., 2012) has been reported to have potential immunomodulatory and cytotoxic effects.(Kapil and Sharma 1997) They have been reported to function by boosting the phagocytic activity of macrophages, production of reactive oxygen species (ROS) in human neutrophil cells.

MIC and MBC of plant extracts

The Ethanol leaf extract of *T. cordifolia* had the lowest MIC value, 4.08 mg/ml and the lowest MBC value 8.37 mg/ml against, *P. aeruginosa*; MIC value of 4.27 mg/ml and MBC value of 9.63 mg/ml against *E. coli*, while MIC value of 8.27 mg/ml and MBC value of 20.76 mg/ml were recorded by *K. pneumoniae*. On the other hand, the highest MIC value of 9.68 mg/ml, and the highest MBC value of 24.07 mg/ml due to *E. faecalis* extract were noted (Table 1). A lower MIC/MBC value signifies that a minimum amount of *T. cordifolia* plant extract is used, whereas, a higher value signifies the use of comparatively more amount of plant extract for the control of any bacterium (Table-1 and Fig-II).

Table 1: MIC and MBC values of selected *T. cordifolia* medicinal plant and of gentamicin as the positive control against bacteria due to Ethanol Extract.

Sl. No	Name of the Bacterium	Values of MIC	Values of MBC
1	*Escherichia coli,*	4.27	9.63
2	*Pseudomonas aeruginosa,*	4.08	8.37
3	*Klebsiella pneumonia*	8.27	20.76
4	*Enterococcus faecalis*	9.68	24.07

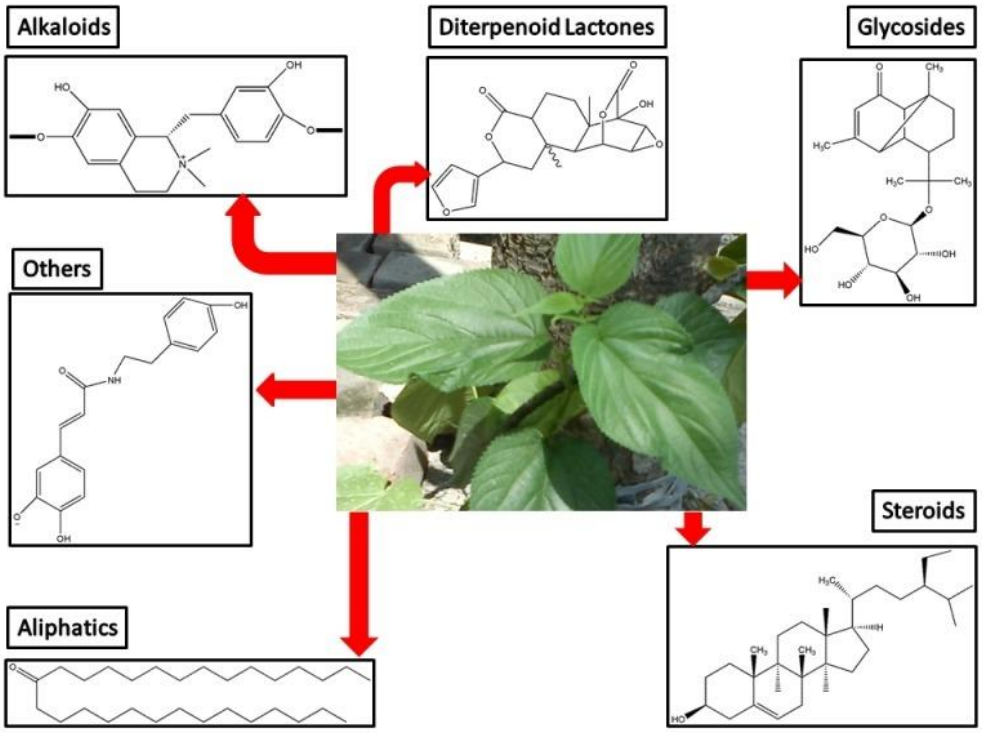


Fig. I: Major Active compounds from *T.cordifolia*

DISCUSSION

T. cordifolia plant was used by an ethnic tribe, the of Karimnagar district, since time immemorial for primary healthcare needs specifically for infectious diseases. It was seen that, four bacteria isolated from urine samples were resistant to the following: aminoglycosides, b-lactams (amoxyclav and ampicillin), two cephalosporins (ceftriaxone and cefpodoxime) as well as, chloramphenicol, signifying most bacterial strains as resistant to most antibiotics. *T. cordifolia* had control capacity on all the four strains of MDR bacteria.

However, the plant, *T. cordifolia* has the famous antimicrobial agent, quercetin, which has an effective drug likeness score of 0.93; but this plant did not have the best antibacterial activity, in this study or with these bacteria Antibiotic sensitive pathogens have a limited capacity of virulence as the employed antibiotic controls them *in vivo.*

At a particular density, the host defence system too helps control pathogens, when the later are in a limiting number. As known, for the internal protection, antibiotic producing organisms harbour antibiotic resistant genes in plasmids and chromosomes, as well as the transfer mechanisms remain active (Mamelli *et al.,* 2009). Therefore, such genes and/or transposon are taken up, horizontally by the susceptible group of bacteria, through

bacterial transformation and/or conjugation (Pages *et al.*, 2008; Warnes *et al.*, 2012).

It is because a suitable emulating agent for the control is absent, and if plant-based antimicrobial would be present in parallel along with the employed antibiotic, there would be the coveted blithesome result, since no bacterium how much genetically well-equipped be it may as in a cohort of MDR bacteria, can never over-ride complexities of phytochemicals for survival. This fact is repeatedly seen in vitro with several plant extracts (Mishra and Padhy, 2013; Rath and Padhy 2014). Thus, those in a coalesced manner, as in a crude extract, have a combined controlling effect. It has been demonstrated with *Salmonella enterica* serotype typhimurium (Alekshun and Levy, 1999). Moreover, MDR *Neisseria gonorrheae* had been known to acquire 'MTR and SAP A MDR' systems of genes, from *S. enterica* serotype typhimurium (Hagman and Shaferm, 1995). Discovery and development of antibiotics in the last century have not only saved countless human lives, but have provided assurances in clinical management all over. But, concomitant development of antibiotic-resistance mainly in bacteria has dismayed both preventive and therapeutic potencies of antibiotics today. In the odyssey of drug development, antibiotics are introduced continually and a few of them are modified suiting to the need to overcome bacterial resistance. Eventually, today there are a large number of antibiotics in use. The demand for newer antibiotics for MDR bacteria in colossal scale has arisen, which has become difficult to meet, as these small molecules are extremely complex in functionality linked to chemical structure. Secondly, an antibiotic ensconced for a typical set of infections cannot ordinarily be abandoned as an obsolete drug, due to reports of dogmatic/realistic resistance in a geographical zone; rather, along with the same antibiotic the introduction of complementary or adjuvant drug could be aimed, when considered with contemplation the problem of morbidity/mortality from infections due to MDR bacteria (Davies and Davies, 2010).

Figure II (A): Antibacterial activity exhibited by ethyl acetate leaf extract against *E. coli, P. aeruginosa, K. pneumonia* and *E. faecalis of conical flask with leaf extracts*; (B.C) NAM plates showing ZOI by well diffusion method.

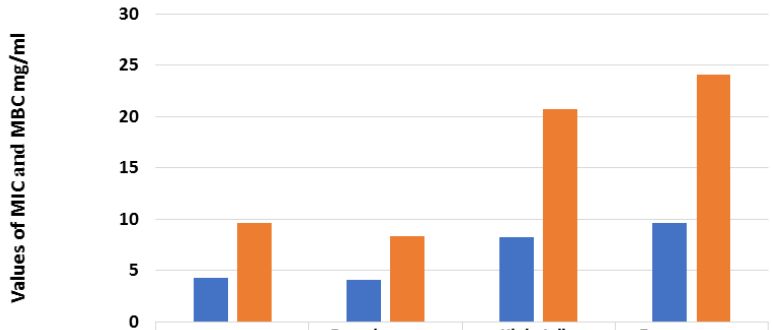
DISCUSSION

- Screening of 70 *Children* for UTI showed that presence of UTI causing pathogenic bacteria in 30 children.
- Ethanol and ethylacetate extracts have better activity against all the four pathogenic microorganisms.
- Ethanol extract was more effective against *E.coli.*
- Ethyl acetate extract was more effective against *E.coli and K.pneumoniae.*

CONCLUSION

Antibiotics of four isolated pathogenic bacteria with six antibiotics of the day all confirmed that MDR was adequate. Individual *T. cardifolia* plant in controlling all MDR strains of bacteria. The effort made on the is clear, mostly with low MIC and MBC values. These used antibiotics have ethnomedicinal uses and can be promoted as a single best plant supplement. Phytocompounds, stigmasterol and luteolin-7-o-glucoside have already been isolated from the second and third-best antibacterial plant and have drug drug-comparison scores, respectively. Therefore, the currently used *T. cardifolia* can be considered the best plant as the most effective plants studied for further consideration for complementary medicine as a non-microbial anti-microbial source against the bacteria that cause MDR UTI.It suggests that *Tinospora cordifolia* plant could be a potential source for production of drug with broad spectrum of activity.

Graph : I MIC and MBC values of selected *T. cordifolia* medicinal plant



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