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ANTIMICROBIAL ACTIVITY OF *TINOSPORA CORDIFOLIA* EXTRACTS AGAINST URINARY TRACT INFECTIONS CAUSING BACTERIA

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ABSTRACT

The frequent, increasing drug resistance among urinary tract infections (UTIs) causing bacteria has made therapy difficult and also lead to greater use of expensive broad-spectrum drugs. The aim of the study was to assess the antimicrobial activity of *Tinospora cordifolia* stem extracts, a broad-spectrum therapeutic plant investigated against bacteria causing UTIs, most common diseases infecting from the neonate to the geriatric age groups. The two isolated uropathogens:1.Gram-negative, *Escherichia coli;* and 2.Gram-positive, *Staphylococcus aureus* were tested against two standard antibiotics, used as a positive reference to determine the sensitivity of the test strains. The extracts of *T. cordifolia* were prepared from alcoholic extracts, and water reflux for aqueous extract. The antibacterial activities of extracts were evaluated using the disc diffusion method. The study showed that all three solvent extracts of *T. cordifolia* reveal different antibacterial activity against both uropathogenic isolates. The antibacterial effect of *T. cordifolia*, which were observed for zone of inhibition (ZOI) could be arranged in decreasing order as follows: ethanolic (maximum) >methanolic (moderate) >aqueous (poor). The evaluated results reveals that plant extracts have significant potential of antibacterial properties and seems promising for the development of the safe herb derived medicinal preparation for treating UTIs.

KEYWORDS: Antimicrobial activity; *Tinospora cordifolia;* Urinary tract infections (UTIs); Disc diffusion assay; Uropathogen; Zone of inhibition (ZOI).

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1. INTRODUCTION

One of the most prevalent problems faced by healthcare services is the increasing prevalence of antimicrobial resistance. UTIs are the most common bacterial infections affecting humans throughout their lifetime. They are the frequent cause of morbidity in outpatients as well as most frequently involved in the cause of nosocomial infection in many hospitals [1]. Urinary tract infections are a serious health problem affecting millions of people each year. A urinary tract infection (UTI) is very common infection that occurs when bacteria enter and multiply anywhere along the normally sterile urinary tract. UTI has a number of causes. Most are caused by bacteria normally present on the skin or in the intestinal tract that invade the urinary tract. Leading etiological agents of UTI's include *Escherichia coli, Candida albicans, Enterococcus faecalis, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Proteus mirabilis* [2]. Women are especially prone to UTIs for reasons that are not yet well understood. One woman in five develops a UTI during her lifetime. The incidence of acute uncomplicated UTI is estimated to exceed 0.5 episodes per annum among women between 18 - 20 years [3,4]. Urinary microorganisms anywhere in the urinary tract and is perhaps the single commonest bacterial infection of mankind [5,6]. The commonest urinary pathogen accounting for over 80% of community-acquired infection is due to *Escherichia coli* [7]. Multiple drug resistance in human pathogenic microorganisms has been developed due to indiscriminate use of commercial antimicrobial drugs commonly used in the treatment of infectious diseases. The development of antibiotic resistance is multi factorial, including the specific nature of the relationship of bacteria to antibiotics, the usage of antibacterial agent, host characteristics and environmental factors. This situation has forced scientists to search for new antimicrobial substances from various sources as novel antimicrobial chemotherapeutic agents, but the cost production of synthetic drugs is high and they produce adverse effects compared to plant derived drugs [8]. Some plants are known as medicinal because they contain active substances that cause certain reactions from relenting to the cure of human diseases [9]. Therefore, different countries used plants medicinally as a source of many potent and powerful drugs [10] from different plant parts including root, stem, flower, fruit, twigs and modified plant organs [11]. Medicinal plants play a key role in health care with about 80% of the world's populations relying on the use of traditional medicine, which is pre-dominantly based on plants [12]. Bacterial genetic ability to transmit and acquire resistance to drug and therapeutic agents created a global problem of antimicrobial resistance [13]. This obstacle can be resolved through the new and innovative antimicrobials from plants [14]. *Tinospora cordifolia* (Guduchi) 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 [15]. The extract of the plant contains several bitter principles, glucosides, alkaloids, a glycoside-giloin, a non-glucoside-gilenin, gilosterol, alkakoid tinosporin, tinosporic acid, tinosporol, berberine, tinosporidine, sitosterol isolated, cordifol, heptacosanol, octacosonal and a new furanoid diterpene-tinosporide [16]. A variety of phytochemicals constituents have also been isolated from *T. cordifolia* plant such as vitamins (A, C, E and K), carotenoids, terpenoids, flavonoids, polyphenols, tannins, saponins, pigments, enzymes and minerals that have antimicrobial and antioxidant activity [17]. The stem of *T. cordifolia* is used as an ingredient in Ayurvedic preparations used in general debility, dyspepsia, fevers and urinary diseases. The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency. Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant [13]. Thus, the present study was undertaken to evaluate *In vitro* antimicrobial effects of medicinal plant, *T.*

cordifolia extracts on urinary tract infections causing bacteria.

2. MATERIALS AND METHODS

2.1. Collection of plant materials

Sample of *T. cordifolia* was collected from "Botanical garden, Banaras Hindu University". The collection of plant sample was thoroughly washed with flowing water and then air-dried sample was subjected to drying at 37°C in a hot air oven. The plant material then heated in hot air oven for 3-4 hours with intermittent turning the material to avoid burning. Then dried stem were crushed by hand; the dried material crushed in mixer grinder to coarse powder. The dried powder then stored in airtight bottles at 28^0C for further extraction.

2.2 Aqueous extraction

One grams of dried powder was extracted in 7.0 ml distilled water for 6 h at slow heat. Every 2 h, it was filtered and centrifuged at 5000g for 15 min. The supernatant was collected and concentrated to make the final volume one-fourth of the original volume. It was then autoclaved at 121 ºC and 15 lbs pressure and then stored at 4 ºC [18]

2.3 Solvent extraction

Two g of dried powder was extracted in 20 ml of ethanol/methanol kept on a rotary shaker at 190-220 rpm for 24h. Thereafter, it was filtered and centrifuged at 5000 g for 15 min. The supernatant was collected and the solvent was evaporating volume one-fourth of the original volume.

2.4 Phytochemical analysis

Protein content presence, were analyzed quantitatively by the Lowry's method. Carbohydrate contents were present in the test sample were determined quantitatively by the Phenol-H_2SO_4 method.

2.5 Culture media and incubation conditions

The three pathogenic bacterial strains used in this study were selected from our strain collection, which included 1 Gram-negative bacteria *Escherichia coli* and 1 Gram-positive bacteria *Staphylococcus aureus*. All strains were grown on agar media supplemented with 0.5 % NaCl, 1% peptone, tryptone, yeast extract. All Cultures were incubated at 37^oC for 24 h. Gentamycin Ampicillin and penicillin were used as standard antibiotics against all pathogenic bacterial strain.

2.5.1. Gram staining

The growth of the investigated bacteria was observed during 24 hrs experiments the growth of the bacteria colony was monitored by 100 X emmersion oil microscopes after Grams staining.

2.6 Antimicrobial Susceptibility Testing

For antimicrobial susceptibility methods described below, the bacterial suspensions were prepared by suspending 3-5 well-isolated colonies from appropriate agar plates into 3 ml broth (adjusted to pH 5.9) and the turbidity was adjusted equivalent to a 0.5 McFarland standard.

2.7 Bioassay studies

The disc Agar diffusion method [19, 20] was used for the test.

2.7.1 Disk Diffusion Assay

For the disk diffusion method, the bacterial suspension prepared above was inoculated onto the entire surface of agar plate (pH 5.9) with a sterile cotton-tipped swab to form an even lawn. Eight sterile paper disks impregnated with 20 µl diluted plant extracts were placed on the surface of each plate using a sterile pair of forceps. The plates were incubated aerobically at 37^0C for 24 h. The diameter of inhibition zone was measured after 24 h incubation using a ruler.

2.7.2 Agar Well Diffusion Method

Stock solution of test material: The herbal residues so obtained and stored at 4ºC were dissolved in Dimethyl sulfoxide (DMSO) to give a concentration of 0.2g/ml. These were kept at 28ºC till further use. During diffusion assay, we have taken DMSO as a control. The range of volume of test solution was 10µl-15µl. The wells were then sealed with molten Muller-Hinton Agar (MHA) and kept for 10 minutes. These plates were then swabbed with 0.5 Mc Farland adjusted 16-18 hour old culture of the test organisms and incubated at 37^oC overnight. The inhibition zones

were recorded in the test well as well as the control well. The assay was repeated twice [21].

2.8 Minimum Inhibitory Concentration (MIC)

Minimal inhibitory concentrations (MIC) are regarded as the lowest concentration of extract that inhibits growth of test organisms. The method of Eloff (1999) was used [22]. Gentamycin, ampicillin was used as a positive control in the antibacterial tests. The experiments were performed in triplicate.

2.9 Statistical Analysis & Preparation of Data

All the treatment data were statistically evaluated with SPSS/16.00 software. Hypothesis testing methods included one way Analysis of Variance (ANOVA) followed by LSD's test. $P<0.05$ was considered to indicate statistical significance. All the results were expressed as mean ± S.E. for the 3 replicate in each treatment.

3. RESULTS AND DISCUSSION

The medicinal plant selected for investigation in this study form part of the traditional medicinal plants used in India. The growing demand and popularity of medicinal plants in rural as well as urban communities [23] has placed many medicinal plants under the threat of extinction. The growing threat and spread of antibiotic resistance by a wide range of common pathogens has led to increased investigations into traditional medicinal plants as alternatives [24]. Antibiotic resistances are not selective in that antibiotic resistance with the same consequences may affect people living in urban and rural communities around the world. Antibiotics that once readily cured a wide range of infections are becoming less useful mainly due to the misuse of antibiotics and the development of antibiotic resistance [25]. The antimicrobial activities of *T. cordifolia* extracts against the pathogens causing urinary tract infections, examined in this study were determined by the presence of inhibition zones. Antibacterial activity of plant extract was more susceptible to *E. coli* whereas S*. aureus less* susceptible to the extract. Antibacterial antibiotics belonging to different classes Penicillin and Gentamicin were used as the standard antibiotics evaluated to profile the susceptible pattern of the test uropathogens, *Escherichia coli and Staphylococcus aureus.* The *E. coli* and *S. aureus* were sensitive towards Gentamicin and Penicillin respectively. The plant (*Tinospora cordifolia*) extracts viz., aqueous (TA), methanol (TM), and ethanols (TE) were investigated against these two test uropathogens. In over all study ethanol extract showed relatively wide spectrum of antibacterial activity via ZOIs (1.40 ±0.058cm to 1.77 ±0.30cm) against the test bacterial strains compared to methanol extract (1.20 ±0.100cm to 1.23 ±0.067cm) (Fig. 1). The relatively wider spectrum of activity of the ethanol extracts over the methanol extract is difficult to explain since all the extracts contained same types of photochemical. Perhaps, the active principles were more soluble in ethanol than the methanol solvents.

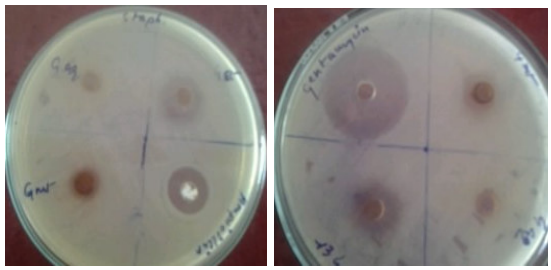
3.1 Escherichia coli

One way analysis of variance was used to determine whether levels of significant with plant extracts treated in E. coli bacterial strain differed among control, antibiotic Gentamycin. The analysis showed significant difference among the treatments ($F_{4, 10}=65.524$ $P<0.05$). The treatment T_M T_E showed significant result with other treatment somewhat treatment T_A showed greatest significant in this treatment. Which indicate *T. cordifolia* ethanolic showing very efficient antimicrobial result against *E.coli* (Table 1).

3.2 Staphylococcucs aureus

One way analysis of variance was used to determine whether levels of significant with plant extract treated in *S. aureus* bacterial strain different among control, antibiotic Penicillin. The analysis showed significant difference among the treatment ($F_{4, 10}=45.231$ $P<0.05$). The treatment T_M T_E showed significant result with other treatment somewhat treatment T_A showed greatest significant in this treatment. Which indicate *T. cordifolia* ethanolic showing very efficient antimicrobial result against *S. aureus* (Fig. 2). These evaluated results are far below than the standard antibiotics. Based on the results, it is possible to conclude that ethanol extract has stronger and broader spectrum of antibacterial activity as compared to the others.

The present study also implied that the ethanolic extracts have significant potential of antibacterial activity over the water extracts (Fig.3). This information confirmed the evidence in previous study reported that ethanol is a better solvent for extraction of antimicrobial substances from medicinal plants than water and methanol [26].



(a) (b)

Figure 1

Antimicrobial action of T. cordifolia different plant extracts with (a) S. aureus and (b) E. coli their comparison with the antibiotics.

Table 1

Antibacterial Activity of T. cordifolia extract against E. coli determined by Disc Diffusion method (Data are expressed as mean ± SE, n=3)

Treatment	Code	Details	ZOI cm
T1	CONTROL	Control	0.16 ±0.033
T2	TA	Aqueous extract *T. cordifolia*	0.67 ±0.065
T3	TM	Methanolic extract *T. cordifolia*	1.23 ±0.067
T4	TE	Ethanolic extract *T. cordifolia*	1.77 ±0.30
T5	ANBT	Antibiotic Gentamicin	3.43 ±0.060

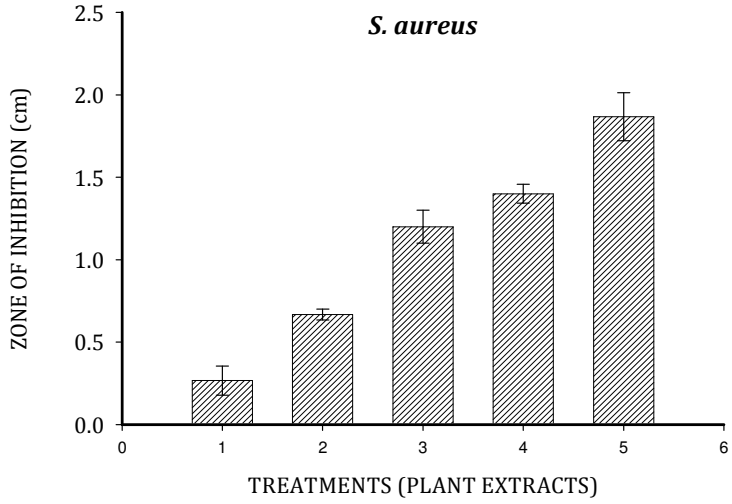


Figure 2

Antibacterial activity of T. cordifolia plant extracts against S aureus showing zone of inhibition determined by disc diffusion methods. Abbreviations are given in Table 1

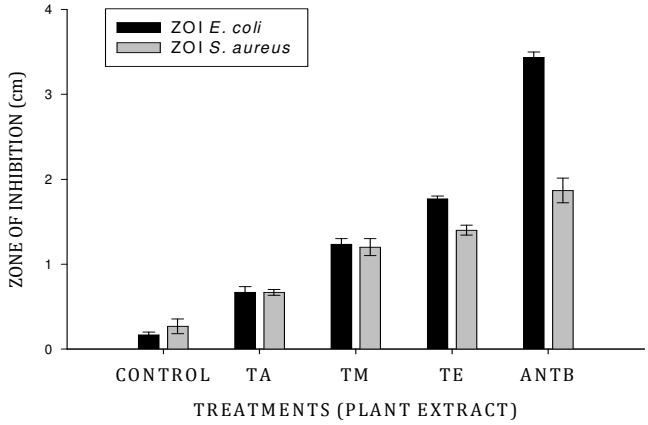


Figure 3

Comparison of antibacterial activity of T. cordifolia plant extracts against S. aureus and E. coli showing zone of inhibition determined by disc diffusion methods. Abbreviations are given in Table 1

CONCLUSION

Based on the above research work it can be concluded that *T. cordifolia* can be a good and potential source for herbal drug preparations for treating UTIs. The above research, evaluated results are far below than the standard antibiotics. This suggests that it may be possible that by increasing the concentration of extracts its activity can be increased further. Solvents including water and methanol can be explored further for the extraction of antimicrobials by more sophisticated procedures for extraction in order to enhance the solubility of active principle and increase yield. Future work also includes the further phytochemical groups wise screening and isolation of bio-active compounds from *T. cordilfolia* will be the focus of future work. The medicinal plant, *T. cordilfolia* can also be further investigated to develop as a new therapeutic bio-weapon to combat UTIs causing infectious diseases.

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