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EVALUATION OF PHYTOCHEMICALS AND ANTIMICROBIAL PROPERTIES OF *Tinospora cordifolia* (Thunb.) Miers AGAINST URINARY TRACT INFECTION CAUSING MICROBES

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ABSTRACT

Various phytochemicals and bioactive compounds are sourced from medicinal plants. The aim of this study was to evaluate and compare the phytochemicals and antimicrobial properties of *Tinospora cordifolia* against urinary tract infection causing microbes. The phytochemical screening of this ethanolic and aqueous extract revealed the presence of tannins, saponins, flavonoids, phenolic and coumarins. Quantitative analysis clearly showed the *Tinospora cordifolia* leaves contains notable amount of phenolics and flavonoids. Ethanolic extract of *Tinospora cordifolia* leaves was potential antibacterial activity confirmed against UTI strains.

Keywords: *Tinospora cordifolia*, qualitative, quantitative and antibacterial activity.

INTRODUCTION

Medicinal plants have always been considered as a source for healthy life for people. Therapeutical properties of medical plants are very useful in healing various diseases and the advantage of these medicinal plants are natural. In many parts of the world, medicinal plants have been used for its antibacterial, antifungal and antiviral activities for hundreds of years (Ali *et al*., 2008; Yasunaka *et al*., 2005). India is the largest producer of medicinal herbs and is appropriately called the botanical garden of the world. These natural compounds formed the foundation of modern prescription drug as we know today (Chopra and Nayar Chopra, 1986).

Urinary tract infections (UTIs) are some of the most common bacterial infections, affecting 150 million people each year worldwide (Stamm and Norrby, 2001). UTIs are caused by both Gram-negative and Gram positive bacteria, as well as by certain fungi. The most common causative agent for both uncomplicated and complicated UTIs is uropathogenic *Escherichia coli* (UPEC). For the agents involved in uncomplicated UTIs, UPEC is followed in prevalence by *Klebsiella pneumoniae*, *Staphylococcus saprophyticus*, *Enterococcus faecalis*, group B *Streptococcus (GBS)*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida spp.* (Foxman, 2014; Nielubowicz and Mobley, 2010; Kline *et al*., 2011). In our present study preliminary phytochemical screening and anti-microbial activity of *Tinospora cordifolia* leaves were evaluated using standard procedure.

MATERIALS AND METHODS

Collection of plant materials

The leaves of *Tinospora cordifolia* were purchased in March 2022 from Siddha Medicinal Shop, Thanjavur, Thanjavur district, Tamil Nadu, India.

Preparation for extract

1 gram of the powder of *Tinospora cordifolia* leaves were transferred in to each conical flask (250ml). The conical flask containing 50ml of ethanol and aqueous solvent. The conical flask containing *Tinospora cordifolia* leaves were shake it well for 30 minutes by free hand. After 24 hrs, the extracts were filtered using whatman filter paper No.1 and filtrate used for further analysis.

Phytochemical screening

Chemical tests were carried out on the extract using standard procedures to identify the constituents as described by Sofowara (1993), Trease and Evans (1989) and Harborne (1973 and 1984). Total phenols estimated by the method of Edeoga *et al.*, (2005). Flavonoid determine by the method of Bohm and Kocipai-Abyazan (1994).

Determination of antimicrobial activity

The antimicrobial activity was performed by disc diffusion method (NCCLS, 1993; Awoyinka *et al.*, 2007).

Antimicrobial assay

Petri plates were prepared by pouring 30 ml of NA /PDA medium for bacteria/fungi. The test organism was inoculated on solidified agar plate with the help of micropipette and spread and allowed to dry for 10 mints. The surfaces of media were inoculated with bacteria/fungi from a broth culture. A sterile cotton swab is dipped into a standardized bacterial/ fungi test suspension and used to evenly inoculate the entire surface of the Nutrient agar/PDA plate. Briefly, inoculums containing specie of bacteria were spread on Nutrient agar plates for bacteria were spread on potato dextrose agar for fungus strains. Using sterile forceps, the sterile filter papers (6 mm diameter) containing the crude extracts (50µl, 100µl and 150µl) were laid down on the surface of inoculated agar plate. The plates were incubated at 37ºC for 24 h for the bacteria and at room temperature (30±1ºC) for 24-48 hr. for yeasts strains. Each sample was tested in triplicate. The antimicrobial potential of test compounds was determined on the basis of mean diameter of zone of inhibition around the disc in millimeters. The zones of inhibition of the tested microorganisms by the samples were measured using a millimeter scale.

RESULTS AND DISCUSSION

Phytochemicals are playing vital role for the treatment of different types of diseases and still are use in, both traditional and modern system of medication. In the present study was carried out on the *Tinospora cordifolia* leaves extract revealed the presence of medicinally active constituents. The phytochemical characters of *Tinospora cordifolia* leaves investigated and summarized in Table 1. *Tinospora cordifolia* leaves showed that the presence of tannin, saponins, flavonoids, steroids, terpenoids, triterpenoids, polyphenol, and coumarins while anthraquinone, glycoside and alkaloids were absent in ethanolic extract. Aqueous extract of *Tinospora cordifolia* leaves showed that the presence of tannin, saponin, flavonoids, anthraquinone, polyphenol, glycoside and coumarins while alkaloids, terpenoids, triterpenoids and steroids were absent. Significant amount of flavonoids (40.87±5.31 mg/gm) and total phenol (209.00±11.09 mg/gm) were found in *Tinospora cordifolia* leaves extract (Table 2).

Table 1: Qualitative analysis of Phytochemicals in *Tinospora cordifolia* extract

S. No	Phytochemicals	Aqueous	Ethanol
1	Tannin	+	+
2	Saponin	++	++
3	Flavonoids	+	+
4	Steroids	-	+
5	Terpenoids	-	+
6	Triterpenoids	-	+
7	Alkaloids	-	-
8	Anthraquinone	+	-
9	Polyphenol	++	++
10	Glycoside	++	-
11	Coumarins	+	++

(+) Presence, (++) High concentrations and (–) Absences

Table 2: Quantitative phytochemical analysis of *Tinospora cordifolia* leaves

S. No	Secondary Metabolites	Result (mg/gm)
1	Flavonoids	40.87±5.31
2	Total phenol	209.00±11.09

Values are expressed as mean ± SD for triplicates

According to Aziman *et al*. (2012), several phenolic compounds like tannins present in cells of plant are potent inhibitors of many hydrolytic enzymes such as proteolytic macerating enzymes used by plant pathogens. In addition, herbs that has tannins as their main components are astringent in nature (Ikhane *et al.*, 2015; Vedhanarayanan *et al.*, 2013). According to Baljeet *et al*., (2015) the phytochemical screening of different spices extracts demonstrated the presence of flavonoids and saponins which supported these findings. The presence of these metabolites probably explains the various uses of this plant in traditional medicine.

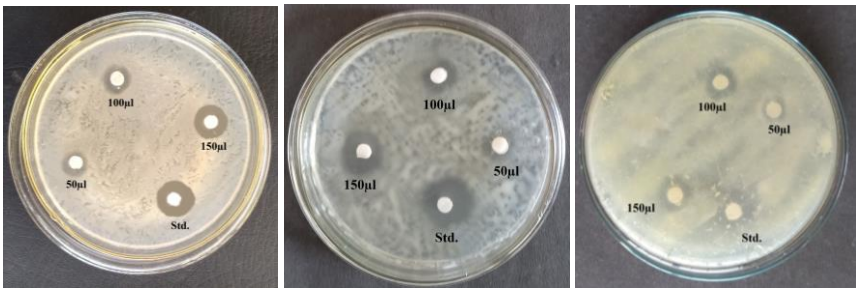
Anti-bacterial activity

The *in vitro* antimicrobial activity of the *Tinospora cordifolia* ethanolic extract against these bacteria and fungi were qualitatively assessed by the presence of inhibition zones represented in the photographic Plate 1. The inhibitory activities in culture media of the *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* for bacterial strains while fungal strains were *Candida albicans* and *Candida tropicalis* species strains reported in table 5 were comparable with standard antimicrobiotics. The ethanolic extract of *Tinospora cordifolia* was potential antibacterial activity was confirmed against UTI strains followed by disc diffusion method.

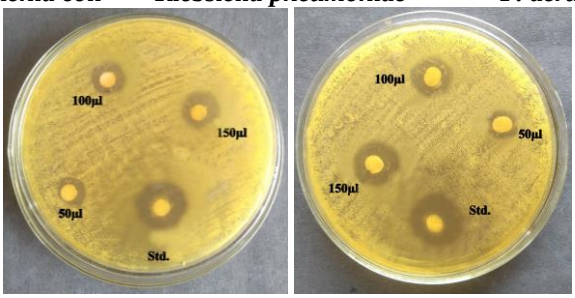
Table 5: Anti-microbial activity of *Tinospora cordifolia* leaves ethanolic extract

Microbial Strains	Concentration (µl)			Std. (30µl)
	50	100	150	
Bacterial strains	**Zone of inhibition (mm)**			
Escherichia coli	3.60	5.90	7.50	10.00
Pseudomonas aeruginosa	2.50	4.50	7.50	10.80
Klebsiella pneumoniae	3.50	5.00	6.00	12.50
Fungi strains	**Zone of inhibition (mm)**			
Candida albicans	2.50	4.00	6.10	10.05
Candida tropicalis	2.65	4.10	6.30	10.80

Standard: Chloramphenicol for bacteria and Fluconazole for fungi mm: Millimeter



Escherichia coli ***Klebsiella pneumoniae*** ***P. aeruginosa***



Candida albicans ***Candida tropicalis***

Plate 1: Anti-microbial activity of *Tinospora cordifolia* leaves ethanolic extract

Plants produce a diverse array of secondary metabolites. Some of these metabolites have antifungal activity and are considered as a potential source of active principles in the formulation of new pesticides (Vyvyan 2002). Plant extracts with antifungal properties were used for controlling phytopathogens at laboratory, greenhouse and field level (Bergeron *et al.* 1995). More than 2000 plant species have been reported to have antimicrobial and antifungal properties.

CONCLUSION

As evident from the above discussion phytochemicals and possess potential antibacterial activity against urinary tract causing microbes. The study concluded that *Tinospora cordifolia* leaves may be used for the treatment of microbial infections. They are alternative to chemical drugs. This would suggest the use of the plant for the treatment of disorders related to microbes.

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