The Pharma Innovation Journal 2019; 8(3): 129-136

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(3): 129-136

www.thepharmajournal.com
Received: 01-01-2019
Accepted: 05-02-2019

Krishnadutt Pratihast
Department of Biochemistry, Patna University, Patna, Bihar, India

Rajneesh Kumar
Department of Chemistry, Patna University, Patna, Bihar, India

Sudhanshu Kumar Bharti
Department of Biochemistry, Patna University, Patna, Bihar, India

Correspondence
Sudhanshu Kumar Bharti
Department of Biochemistry, Patna University, Patna, Bihar, India

Comparative antimicrobial activity of ethanolic and aqueous extract of *Tinospora cordifolia*

Krishnadutt Pratihast, Rajneesh Kumar and Sudhanshu Kumar Bharti

Abstract
The present study was designed with the aim to evaluate and compare the effects of ethanolic (EtOH) and aqueous (H_2O) extracts of *Tinospora cardifolia* (*Menispermaceae*) plant extract according to phytochemical aspects for antibacterial efficacies. Phytochemical screening of the plant extract showed the presence of different percentage of active phytochemicals such as alkaloid, flavonoid, phenolic, saponin, tannin etc. The phenolic content of ethanolic extract was found to be 10.2 mg GAL/g while 8.2 mg GAL/g per gram dry weight basis for aqueous extract. The flavonoid content of ethanolic extract in terms of quercetin equivalent was found to be 0.32 mg/g while for aqueous extract it was found to be 1.15. The IC_{50} value of ethanolic extract was found to be 0.2498 µg/ml while that in water was found to be 0.2890 µg/ml. The alcoholic extracts at 50 µg/ml showed an antioxidant effect of 59.10% comparing with the high antioxidant effect of ascorbic acid. At 500 mg/ml concentration, the ethanolic extract showed maximum zone of inhibition of 10.5 mm, 12.5 mm, 15.5 mm and 14.5 mm on *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli* and *Salmonella typhi* respectively. The ethanolic extract and aqueous extracts in agar diffusion test at the concentrations of 250 mg/ml and 125 mg/ml also showed an effectual zone of inhibition against all tested micro-organisms. The minimum inhibitory concentration (MIC) as well as minimum bactericidal concentration (MBC) in µg/ml of investigated plant extract against bacterial strains of *E. coli* has been found to be 62.5 µg/ml.

Keywords: *Tinospora cardifolia*, minimum inhibitory concentration, minimum bactericidal concentration, Gram positive bacteria, Gram negative bacteria, antimicrobial

1. Introduction
Microbial infections have caused a big burden of diseases and bacteria are listed in the first position among common micro-organisms responsible for opportunistic diseases (Rathee *et al*., 2012) [49]. In developing countries, bacterial infections are prevalent due to factors such as poor hygiene, sanitation and overcrowding in the living conditions. On the other hand, amplified antibiotic resistance has become a global concern, in addition to the problem of microbial persistence, thus highlighting the need to develop novel microbial drugs that are not only active against drug resistant microbes, but more importantly, kill persistent micro-organisms and shorten the length of treatment (Mariita *et al*., 2010) [33]. In addition, antibiotics are associated with adverse effects on host, which include depletion of beneficial gut and mucosal microorganisms, immuno-suppression, hypersensitivity and allergic reactions (Kalayou *et al*., 2012) [29].

Bacterial infections are caused by a wide range of organisms resulting in mild infections to life threatening diseases. For instance, *Bacillus subtilis* is an aerobic, rod-shaped, motile, and endospore-forming Gram-positive bacterium. It is a soil inhabiting saprophytic organism (Sleigh and Timburg, 1998) [55]. Although harmless, it occasionally causes some opportunistic infections such as conjunctivitis (Murray *et al*., 1998) [35]. *Staphylococcus aureus*, a Gram-positive coccus bacterium, is part of the normal flora of human skin and mucous membranes (Murray *et al*., 1998) [55]. This organism is responsible for human staphylococcal skin infections (wounds and impetigo), soft tissues (septic arthritis) and pneumonia (Sleigh and Timburg, 1998) [55]. *Escherichia coli*, a Gram-negative rod-shaped bacterium is mostly found inhabiting the human gastrointestinal tract and commonly causes urinary tract infections (Najar *et al*., 2009) [36]. *Klebsiella pneumoniae* is a non-motile, rod-shaped Gram-negative bacterium. The organism is easily observed in culture as it forms large colonies. It is also part of the human intestinal and colon flora, having a prominent polysaccharide capsule that provides resistance against host defence mechanisms (Hugo, 1992) [27]. Common human *Klebsiella* infections include community acquired pneumonia, urinary tract infection, lower

And upper respiratory tract infections (Einstein, 2000) [20]. The great ancient Indian civilizations provided written evidence of man's utilization of plants for the treatment of a wide range of diseases in nearly all cultures (Bharti *et al*., 2018; Bharti *et al*., 2013) [4, 10]. Recently there has been a shift in universal trend from synthetic to herbal medicine, which can be said *"back to nature"* (Bharti *et al*., 2016) [6]. Phytochemical screening of plants has revealed the presence of numerous chemicals including phenolic compounds, alkaloids, tannins, flavanoids, steroids, glycosides and saponins etc (Bharti *et al*., 2012-17; Salazar *et al*., 2008) [4, 51]. The antimicrobial compounds of plants origin may inhibit bacterial growth by different mechanisms than those of synthetic antimicrobials and may have a significant clinical value in treatment of resistant microbial strains (Bharti *et al*., 2018; Houghton *et al*., 2007; Shankar *et al*., 1980) [4, 26, 53]. Therefore, the use of herbal products as antimicrobial agents may provide the better alternative of synthetic antibiotics (Berry *et al*., 2009; Falagas *et al*., 2009) [3, 22].

Based on the frequent usage in folk medicine and reported literature we selected *Tinospora cordifolia* plant (gudduchi; Family: *Menispermaceae*) acclaimed for antibiotic and blood purifying properties (Ghosh and Saha, 2012) [23]. *Tinospora cordifolia* is a genetically diverse, large, deciduous climbing shrub with greenish yellow typical flowers, found at higher altitude (Rana *et al*., 2012, Parthipan *et al*., 2011) [47]. The plant has been used for centuries for the treatment of various ailments (Patil and Malpathak, 2016) [41]. It has been reported to have anti-allergic, anti-arthritic, anti-diabetic, anti-inflammatory, anti-oxidant, anti-spasmodic, anti-periodic (Sharma *et al*., 2012; Upadhyay *et al*., 2011) [24], radio protective (Goel *et al*., 2014) [21] properties. Various compounds have been isolated and identified from the plant. These plants and their extracts have shown activity against pure culture of pathogenic bacteria such as *E. coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Staphylococcus aureus* (Sharma *et al*., 2012; Rana *et al*., 2012) [47, 12]. Thus, the present investigation has been carried out in order to study the possible antimicrobial effect of the two plants' extracts against isolated or procured microorganism's strains.

2. Materials and Methods

2.1 Characterization, preparation and phytochemical screening of plant extracts

The plant was collected from different regions in northern India in 2016 and identified according to the relevant monographs of Indian Pharmacopoeia (2012) [58]. The plants materials were botanically authenticated and were washed under running tap water, blotted with filter paper, were dried in the shade at room temperature. It was then grounded in a mortar. Fifty gram of each of the freshly prepared plant material was extracted with 500 ml of two solvents; distilled water and ethanol by soaking for 48 h. The extracts were filtered twice using Whatman filter paper No. 1, under strict aseptic conditions and the filtrate was centrifuged at 12000 rpm for 10 min. The collected filtrates were concentrated below 40^0 C using a rotary evaporator (Buchi, Switzerland) and were stored in freezer at -20^0 C until further test.

The screening of chemical constituents was carried out qualitatively and quantitatively with ethanolic (EtOH) and aqueous (H_2O) extracts by using various chemical methods. For qualitative tests, different solvent extracts of two plants were analyzed for the presence of alkaloid, saponin, flavonoid, fixed oils and fats, tannins and phenolic compounds according to standard methods. Flavonoids were determined by aluminium chloride colorimetric method (Chang *et al*., 2002) [15]. The absorbance of the reaction mixture was measured at 450 nm with a V-670 research grade UV-Vis spectrometer. Quercetin solutions at concentrations of 12.5–100 µg/ml in methanol were meant for calibration curve. The quantitative tests were performed for total phenol which was determined by Folin–Ciocalteu reagent and expressed as mg Gallic acid (GAL) equivalent/g dry weight. The standard curve was prepared using 0, 50, 100, 150, 200 and 250 mg/l solutions of Gallic acid in methanol: water mixture (50:50, v/v).

2.2 Selected micro-organisms

In the present study, the bacteria selected are described in Table 1. Microbial pure cultures were obtained from MTCC (Microbial type culture collection), Chandigarh. The bacterial cultures were grown on nutrient agar medium (Hi Media, pH 7.4) at 37°C and potato dextrose agar medium (Hi Media, pH 5.6) at 27°C respectively. Both the cultures were maintained at 4°C.

Table 1: Selected Gram positive and Gram Negative microorganisms

Types of microorganism	Micro-organism strains	Causes
Gram positive	*Bacillus subtilis* (MTCC 6038)	Food poisoning
	Bacillus cereus (MTCC 1765)	Food poisoning, vomiting, Diarrhoea
Gram negative	*Escherichia coli* (MTCC 5946)	Bloody diarrhoea, kidney diseases
	Salmonella typhi (MTCC 8345)	Typhoid, enteric fever

2.3 Inoculum preparation

A fresh microbial suspension was prepared by sub culturing the bacterial colonies in to the nutrient broth medium (Hi Media pH 7.4) and incubated at 37°C in order to maintain the uniform growth rate of each organism. The bacterial suspension of approximately 1×10^8 CFU/ml, which is equivalent to 0.5 Mc Farland turbidity standards to density (Perilla *et al*., 2003) [43] was used throughout the experimentation.

2.4 Bioassay of crude plant extracts

In the present study, the antimicrobial activity of various plant parts *i.e.* leaf, stem, fruit, inflorescence and whole plant extracts in different solvents (ethanol and distilled water) were screened by agar well diffusion method (Perez *et al*., 1990) [42]. The prepared agar plates were marked with organism and extract name. Fresh bacterial culture inoculum of 100 µl having 1×10^8 cfu/ml cell density was spread on agar plates with sterile glass spreader. A well of 8 mm diameter punched off at previously marked Petri plates into nutrient agar medium with sterile Cork borer and then filled with l00µl of each plant extract. Plates were placed in a refrigerator for 30 minutes for pre-diffusion of plant extracts and then incubated at 37 °C for bacteria until the appearance of inhibition zone. After incubation, plates were examined and zone of inhibition (excluding well diameter) was measured as a property of antimicrobial activity.

2.5 Assay of antibacterial activity

The antimicrobial activities of plant extracts were determined by disc diffusion test (Perez *et al*., 1990) [42] and micro-dilution assay (Okeke *et al*., 2001) [38]. Overnight grown culture of microorganisms was used for inoculums preparation. A loopful of isolated colony was inoculated in 4ml of Peptone water (HiMedia, Mumbai) at 37°C for 2 h. The turbidity of resulting suspension was compared to 0.5 McFarland turbidity standards. The level of turbidity was equivalent to approximately 3.0×10^5 cfu/ml. The Mueller Hinton Agar media (HiMedia, Mumbai) was prepared and poured into Petri dishes. Once the media solidifies it was then inoculated with micro-organism suspended in peptone water. The media was then punched with 6 mm diameter hole and filled with extract and control (positive and negative). Vancomycin (20µg/ml) was taken as positive control for bacterial strains and 100% DMSO was used as negative control. The experiment was performed at two different concentrations (8 and 10 mg). The bioassay was carried out in triplicates to eliminate any error. The Petri dishes were incubated for 24 h at 37±2°C for bacteria. The antimicrobial activity was calculated by measuring the diameter of zone of inhibition in millimetres around the well.

2.6 Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

In the present study, minimum inhibitory concentration (MIC) was evaluated by serial broth dilution method (Subramanian *et al*., 2002) [56] for the plant extracts showing more than 7mm 30ml of inhibition. Density of bacterial suspension was maintained uniformly throughout the experiment at 1×10^8 CFU/ml by comparing with 0.5 Mc-Farland turbidity standards. About 40µl of plant extract from stock solution (l00mg/ml) was taken into the first dilution tube and added 960µl of nutrient broth and mixed well. About 500µl of solution from first dilution tube was taken and added 500µl of nutrient broth into second tube, this step was repeated 5 times and from last tube 500µl solution was discarded. Final volume was made upto 1ml by adding 500µl of test organism in each tube. The MIC was tested in the concentration range between 8mg/ml to 0.250mg/ml. Tubes were incubated at 37°C for 24 hours in an incubator. About l00µl (0.1%) 2,3,5-triphenyl tetrazolium chloride solution as a growth indicator was incorporated in each tube to find out the bacterial inhibition and tubes were further incubated for 30 minutes at 37°C. Bacterial growth was visualized when colourless 2, 3, 5-triphenyl tetrazolium chloride was converted into red color formation in the presence of live bacteria. MIC assay was repeated thrice by using DMSO and nutrient broth as controls. To determine the minimum bacterial concentration (MBC), l00µl of broth was collected from those tubes tested for determination of MIC which did not show any growth and spread on sterile nutrient agar plate for any bacterial growth. Plates were incubated at 37°C for 24 hours. After incubation the concentration at which no visible bacterial growth was observed considered as the minimum bactericidal concentration (Doughari, 2008) [19].

2.7 Statistical analysis

Data were expressed as the mean ± S.E.M. For statistical analysis of the data, group means were compared by one-way ANOVA with *Post Hoc* analysis. The Tukey–Karmer *Post Hoc* test was applied to identify significance among groups. Graphs are plotted using MATLAB version 7.8.0 R2009a, Natick, Massachusetts: The Mathworks Inc. 2009. The *p*-value 0.001 was considered to be statistically significant.

3. Results

3.1 Phytochemical screening of plant extracts

Phytochemical screening of the selected plant extract showed the presence of different percentage of active phytochemicals such as alkaloid, flavonoid, phenolic, saponin, tannin etc. (Table 2). The phenolic content of the ethanolic extract of *Tinospora cardifolia* was found to be 10.2 mg GAL/g while 8.2 mg GAL/g per gram dry weight basis for aqueous extract (Table 3). The flavonoid content of the ethanolic extract of *Tinospora cardifolia* in terms of quercetin equivalent was found to be 0.32 mg/g while for aqueous extract it was found to be 1.15 (Table 3). The difference in total phenolic and flavonoid content of EtOH, and aqueous extracts between two plants was found statistically significant ($p< 0.01$). The IC_{50} value of *Tinospora cardifolia* in ethanol was found to be 0.2498 µg/ml while that in water was found to be 0.2890 µg/ml. The alcoholic extracts of *Tinospora cardifolia* at 50 µg/ml showed an impending antioxidant effect of 59.10% comparing with the high antioxidant effect of ascorbic acid (Table 6 and Figure 2).

Table 2: Qualitative determinations of active ingredients in alcoholic and water extracts of *Tinospora cardifolia* plants

Plant constituents	Extract of *Tinospora cardifolia*	
	Ethanolic	Aquous
Alkaloids	+	+
Saponins	+	+
Flavonoids	+	+
Fixed oils and fats	+	+
Tannins and phenolic compounds	+	+

Table 3: Total phenolic content (expressed as mg Gallic acid (GAL) equivalent/g dry weight) and flavonoid content (expressed as mg Quercetin solution equivalent/g dry weight) of *Tinospora cardifolia* plant extracts.

Plant	Extracts	Total phenol	Total flavonoid
Tinospora cardifolia	Ethanol	10.2	0.32
	Water	8.2	1.15

3.2 Antimicrobial activity of plant extracts

The plant extracts showed different degrees of antimicrobial activity depending on the concentration of extracts, type of solvent used for extraction and the bacterial strains tested for susceptibility assay (Table 4 and Figure 1). The collective analysis of antimicrobial activity of extract indicated that among the two medicinal plants used in the study the ethanolic extracts exhibited better antibacterial activities than aqueous extracts. The ethanolic extract of *Tinospora cardifolia* showed maximum antibacterial activity with maximum diameter of zone of inhibition against the four strains (Table 4 and Figure 1). At 500 mg/ml concentration, the ethanolic extract of *Tinospora cardiofolia* on *Bacillus subtilis, Bacillus cereus, Escherichia coli* and *Salmonella typhi* showed maximum zone of inhibition of 10.5 mm, 12.5 mm, 15.5 mm and 14.5 mm respectively (Table 4 and Figure 1). Again the aqueous extracts of the plant showed different degrees of antimicrobial activity depending on the concentration of extracts and the bacterial strains tested for susceptibility assay (Table 4 and Figure 1). The aqueous extract of *T. cardifolia* showed maximum antibacterial

activity with maximum diameter of zone of inhibition against the four strains (Table 4 and Figure 1). At 500 mg/ml concentration, the ethanolic extract of *T. cardiofolia* on *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli* and *Salmonella typhi* showed maximum zone of inhibition of 10.5 mm, 12.5 mm, 14.5 mm and 14.5 mm respectively (Table 4 and Figure 1). The result also showed that both the ethanolic extract and aqueous extracts of *T. cardifolia* in agar diffusion test at the concentrations of 250 mg/ml and 125 mg/ml also showed an effectual zone of inhibition of all the tested micro-organisms.

Table 4: Antimicrobial activities of the investigated plant *Tinospora cardifolia* in agar diffusion test.

Plant	Extracts	Concentration in mg/ml	Zone of inhibition (mm)			
			B. s.[a]	B. c.[b]	E. c.[c]	S. t.[d]
Tinospora cardifolia	Ethanol	500	10.5	12.5	16.5	14.5
		250	8.0	9.0	14.0	11.5
		125	7.0	7.5	12.5	8.0
		60	4.0	3.5	9.0	4.0
		30	–	–	4.0	–
		15	–	–	–	–
	Water	500	10.5	12.5	14.5	14.5
		250	8.0	10.5	12.5	12.5
		125	4.0	9.0	11.5	11.0
		60	3.5	4.0	9.0	10.0
		30	–	–	4.0	4.0
		15	–	–	–	–

Note: [a] B. s. *Bacillus subtilis;* [b] B. c., *Bacillus cereus*; [c] E. c., *Escherichia coli;* [d] S. t., *Salmonella typhi*.

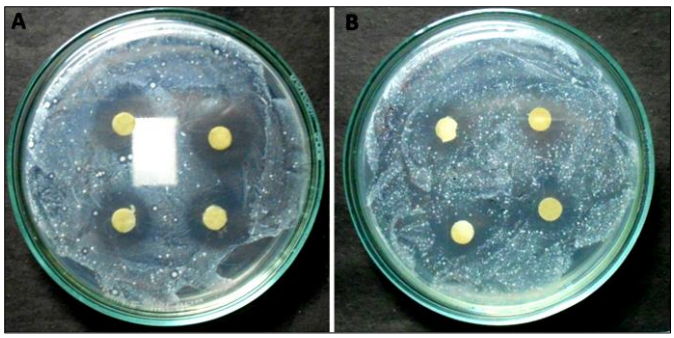

Fig 1: Antimicrobial activities of the investigated plant Tinospora cardifolia in agar diffusion test at 500 mg/ml concentration of ethanol (A) and water (B).

3.3 Minimum inhibitory concentration (MIC)/ Minimum bactericidal concentration (MBC)

The minimum inhibitory concentration (MIC) in µg/ml minimum bactericidal concentration (MBC) in µg/ml of the plant extract on four bacterial strains was carried out by Broth dilution method (Table 5). The statistically significant results were obtained with *p* value of 0.001 ($p<0.05$). Data obtained from the MIC indicated that plant extracts in ethanolic medium had the same effects as of aqueous extract on the solid medium (disc diffusion agar) and were observed to be concentration dependent (Table 5). The minimum inhibitory concentration (MIC) as well as minimum bactericidal concentration (MBC) in case of the investigated plant extract of *T. cardifolia* against bacterial strains of *E. coli* had been found to be 62.5 µg/ml.

Discussions

Plants produce a diverse range of bioactive molecules, making them rich source of different types of medicines. It has been reported that between the years 1983 and 2016 (Cragg *et al*., 2017) [17], the systematic screening of antibacterial plant extracts represents a continuous effort to find new compounds with the potential to act against multi-resistant bacteria. Despite the presence of various approaches to drug discovery, plants still remain the main reservoir of natural medicines (Bharti *et al*., 2018) [4]. Phytochemical constituents are responsible for medicinal and antimicrobial activity of plant species (Parveen and Sharma, 2014) [24]. Secondary metabolites found in plants are the main reason for their antimicrobial potential. Several workers throughout the world have carried out antimicrobial studies on some medicinal plants including *Tinospora cardifolia* (*Menispermaceae*) (Ghosh and Saha, 2012) [23]. This plant and its extracts have shown activity against pure culture of pathogenic bacteria such as *E. coli*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* (Rao *et al*., 2006) [48]. The plant has been reported to have anti-allergic, anti-arthritic, anti-diabetic, anti-inflammatory, anti-oxidant, anti-spasmodic, anti-periodic (Sharma *et al*., 2012) [14], radio protective (Goel *et al*., 2004; Patil and Malpathak, 2016) [41, 21].

Table 5: Minimum inhibitory concentration (MIC) (µg/ml) and minimum bactericidal concentration (MBC) (µg/ml) of the investigated plant extract of *Tinospora cardifolia* against bacterial strains.

Plant	Extract	B. s.[a]		B. c.[b]		E. c.[c]		S. t.[d]	
		MIC[i]	MBC[j]	MIC[i]	MBC[j]	MIC[i]	MBC[j]	MIC[i]	MBC[j]
Tinospora cardifolia	Ethanol	500	500	500	250	62.5	62.5	250	250
	Water	500	500	250	250	62.5	62.5	250	250

Note: [a] B. s. *Bacillus subtilis;* [b] B. c., *Bacillus cereus*; [c] E. c., *Escherichia coli;* [d] S. t., *Salmonella typhi*; [i] MIC, minimum inhibitory concentration; [j] MBC, minimum bactericidal concentration.

Table 6: IC_{50} values (µg/ml) for DPPH radical scavenging activity of two selected plant extract of *Tinospora cardifolia* and phytochemical screening.

Plant	Extracts	10 µg/ml	50 µg/ml	100 µg/ml	500 µg/ml	1000 µg/ml	IC_{50}
Tinospora cardifolia	Ethanol	50.65	59.10	62.28	63.76	70.90	0.2016
	Water	52.34	52.88	55.26	56.14	60.96	0.2248

Note: IC, inhibitory concentration

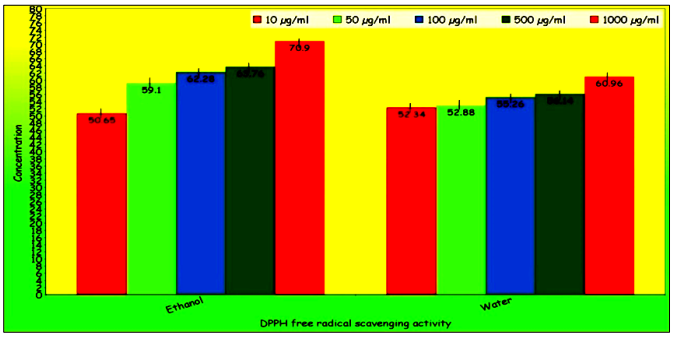

Fig 2: DPPH radical scavenging activity of the selected plant extract of Tinospora cardifolia.

In addition, Rahman *et al*., (2009) [46] proposed that the aqueous and ethanolic extracts of *Moringa oleifera* have antimicrobial activity against *Microsporum canis, Aspergillus fumigatus*, *Candida albicans*, *Escherichia coli* and *Staphylococcus aureus* by disc diffusion method. The leaves and roots of the aqueous extract of *Moringa oleifera* inhibit the growth of *Microsporum canis*. There was no zone of inhibition of ethanol and aqueous extract of leaves, seeds roots and stem of *Acalypha indica* against *Staphylococcus aureus* and *Escherichia coli*. The data pertaining to the antibacterial and antifungal potential of the plant extracts and the inhibition zone formed by extracts and Minimum inhibitory concentration (MIC) and Minimum Bactericidal concentration (MBC) values against bacteria are presented in Table 4 The results obtained reveal that the medicinal plant shows different degree of inhibition against the selected micro-organisms in different solvent *viz.*, ethanol and aqueous. The diameter of zone of inhibition produced depends on several factors broadly classified as extrinsic and intrinsic parameters. However, intrinsic factors such as nature of medicinal and aromatic plants including its components, solubility and diffusing property are predetermined. Due to variable infusibility, the antibacterial activity with very high potency may not be demonstrated a zone of inhibition commensurate to its efficacy (Prasai *et al*., 2004) [45].

In classifying the antimicrobial activity of gram-positive and gram-negative micro-organisms, it would generally be expected that a much greater number of antimicrobial agents would be active against Gram-positive than Gram-negative bacteria. In case of bacteria the basis for their differences in susceptibility might be due to the differences in the cell wall composition of gram-positive and gram-negative bacteria (Saranraj *et al*., 2011) [52]. The Gram negative bacteria having an outer phospholipid membrane carrying the structural lipopolysaccharide components, makes the cell wall impermeable to lipophilic solutes, while protein constitutes a selective barrier to the hydrophilic solutes (Nikaido and Vaara, 1985) [37]. Gram-positive bacteria should be more susceptible having only an outer permeability barrier. In this study, the extract showed some degree of activity against one or more of the bacterial and fungal strains extracts. The antimicrobial activities of plant extracts against different selected organisms are broadly classified in to five categories as: a) better activity with more than 20 mm of zone of inhibition, b) good activity with 15 mm to 20 mm of zone of inhibition, c) moderate activity with 6 mm to 15 mm of zone of inhibition, d) least activity with 1 mm to 5 mm of zone of inhibition, e) no activity with zero zone of inhibition. The zone of inhibition (mm) of various plant extracts against the tested organisms was shown in Table 4 as well as in Figure 1. The antibacterial activity of ethanol and chloroform extracts against a number of pathogenic micro-organisms was found to be more active than chloroform extract. In our study ethanolic extracts have shown better activity against selected gram positive bacteria than gram negative bacteria. However, aqueous extract has shown moderate activity against pathogenic micro-organisms.

In the present study different solvent extract (ethanolic and aqueous) of *Tinospora cardifolia* was evaluated according to phytochemical aspects showed the presence of different percentage of active phytochemicals such as alkaloid, favonoid, phenolic, saponin, tannin etc. The correlation between antibacterial and antioxidant activity and chemical composition: (phenols, quinones, flavones, tannins, terpenoids, and alkaloids) has been well documented (Akgul and Gulshen, 2005, Doughari and Manzara, 2008; Cowan, 1999) [1, 19, 16]. Plant's phenolics and flavonoids have also been documented as free radical scavengers and antioxidants (Amal *et al*., 2009; Pourmorad *et al*., 2006) [2, 44]. The phenolic content of the ethanolic extract of *T. cardifolia* was found to be 10.2 mg GAL/g while 8.2 mg GAL/g per gram dry weight basis for aqueous extract (Table 3). The flavonoid content of the ethanolic extract of *T. cardifolia* in terms of quercetin equivalent was found to be 0.32 mg/g while for aqueous extract it was found to be 1.15 (Table 3). The difference in total phenolic and flavonoid content of EtOH, and aqueous extracts between two plants was found statistically significant ($p< 0.01$). The alcoholic extracts of the plant showed an effectual free radical scavenging in the DPPH assay in comparison with aqueous extracts (Table 6). The IC_{50} value of *T. cardifolia* in ethanol was found to be 0.2498 µg/ml while that in water was found to be 0.2890 µg/ml. The alcoholic extracts of *T. cardifolia* at 50 µg/ml showed an impending antioxidant effect of 59.10% comparing with the high antioxidant effect of ascorbic acid (Table 6). The alcoholic extracts of the plant showed an effectual free radical scavenging in the DPPH assay in comparison with aqueous extracts in line with the outcome of Akgul and Gulshen 2005 [1]. The minimum inhibitory concentration of the leaf extract was observed in the range of 25 µg to 100 µg for the bacterial strains. In this study ethanolic extracts followed by aqueous extracts of the plant extracts have shown considerable high antibacterial activity against most of the test organisms in line with Datta *et al*., (2011) [18]. Based on the results the extracts which showed better to good activity contain antibacterial principles which can be evaluated further.

The plant extracts showed different degrees of antimicrobial activity depending on the concentration of extracts, type of solvent used for extraction and the bacterial strains tested for

susceptibility assay (Table 4 and Figure 1). The collective analysis of antimicrobial activity of extract indicated that the ethanolic extracts exhibited better antibacterial activities than aqueous extracts. The ethanolic extract of *T. cardifolia* showed maximum antibacterial activity with maximum diameter of zone of inhibition against the four strains (Table 4 and Figure 1). At 500 mg/ml concentration, the ethanolic extract of *T. cardiofolia* on *Bacillus subtilis, Bacillus cereus, Escherichia coli* and *Salmonella typhi* showed maximum zone of inhibition of 10.5 mm, 12.5 mm, 15.5 mm and 14.5 mm respectively (Table 4 and Figure 1). Accordingly, the result also showed that both the ethanolic extract and aqueous extracts of *T. cardifolia* in agar diffusion test at the concentrations of 250 mg/ml and 125 mg/ml also showed an effectual zone of inhibition of all the tested micro-organisms. The ethanol extracts exhibited better antibacterial activities than aqueous extracts. The differences in bacterial susceptibility to the extracts is perhaps due to the differences in cell wall and/or genetic composition (Karaman *et al*., 2003) [30] or due to the differences in the composition, concentrations and the mechanism of action of the bioactive compounds.

Determination of minimum inhibitory concentration (MIC) involves exposing the test organism to serially diluted extract and determining the minimum concentration that inhibits growth (Bharti *et al*., 2018) [4]. The four micro-organisms were tested for their ability to produce visible growth in presence of serially diluted antimicrobial agents. The samples were removed, serially diluted and the numbers of surviving bacteria were determined by plating on agar media. The results are almost comparable with the reference antibiotics tested in this study. Data obtained from the MIC indicated that plant extracts in liquid medium had the same effects on the solid medium (disc diffusion agar) and were observed to be concentration dependent (Table 5). Comparison of minimum inhibitory concentration (MIC) in µg/ml minimum bactericidal concentration (MBC) in µg/ml of the plant extracts on four bacterial strains was carried out by Broth dilution method (Table 5). The statistically significant results were obtained with p value of 0.001 (p<0.05). Data obtained from the MIC indicated that plant extracts in ethanolic medium had the same effects as of aqueous extract on the solid medium (disc diffusion agar) and were observed to be concentration dependent (Table 5). The minimum inhibitory concentration (MIC) as well as minimum bactericidal concentration (MBC) in case of the investigated plant extract of *T. cardifolia* against bacterial strains of *E. coli* had been found to be 62.5 µg/ml. The inhibitory activity of *Tinospora cardifolia* extract compared to other extracts against the pathogenic bacteria confirms the anti-infective potential of this plant.

Masola *et al*., (2009) [34] obtained MIC values *A. digitata* stem bark extracts have shown varied sensitivity (1.5 mg/ml to 12 mg/ml) against various pathogenic microorganisms. In this study the ethanol and aqueous extracts have shown sensitivity between 2- 8 mg/ml which appeared to be significant. No antimicrobial activity was seen in lower concentration of aqueous extracts against tested bacterial strains. Thankamani *et al*., (2011) [57] found no antimicrobial activity in hexane flower extracts of *Alstonia schlorais* against *Salmonella typhi* and *Lactobacillus* sp. as well as in water extract against *Pseudomonas aerouginosa* and *S. typhi*. In this study also no activity was noticed against the tested organisms for all the extracts. Kumar *et al*., (2012) [11] evaluated antimicrobial activity against twenty pathogenic microorganisms and tested leaf extracts prepared in various solvents i.e. benzene, chloroform, acetone, methanol and distilled water and found solvent extracts were more effective at variable inhibition zone against tested organisms than distilled water extracts. The MIC values of ethanolic extract revealed significant activity and lowest MIC values were obtained against the tested microorganisms. In this study, both gram positive and gram negative bacteria appeared to be more susceptible to ethanolic extracts of plants in comparison with other organisms.

Conclusion

Thus, the present investigation report systematically and validate that the investigated plant extracts put forth the antibacterial effect. Such a method may provide a treatment that is simple, relatively inexpensive and could be incorporated into the normal diet of the patient, which is highly favorable. Clinical data validate can be used as a kind of validation of the ethno pharmacological and the clinical phyto therapeutic use while more advanced computational techniques can explicate the antimicrobial property of their phytochemicals. Further studies on pharmacokinetics and clinical efficacy have to be performed to prove the antimicrobial effects under *in vitro* and *in vivo* conditions.

References

1. Akgul C, Gulshen S. Antibacterial activity of crude methanolic extract and its fractions of aerial parts of *Anthemis tinctoria*. Ind J Biotech Biophys 2005; 42:395-397.
2. Amal A, Ashraf A, Hossam ES. Chemical compositions and antioxidant/ antimicrobial activities of kaffmaryam (*Anastatica hierochuntica*) and doum palm (hyphaenethebaica) cultivated in Egypt. J Bio Bilimleri Arastirma Dergisi. 2009; 2(2):71-079.
3. Berry RE, Klumpp DJ, Schaeffer AJ. Urothelial cultures support intracellular-bacterial community formation by uropathogenic *Escherichia coli*. Infect Immun 2009; 77(7):2762-72.
4. Bharti SK, Krishnan S, Kumar A, Kumar A. Antidiabetic phytoconstituents and their mode of action on metabolic pathways. Therapeutic Advances in Endocrinology and Metabolism. 2018; 9(3):81-100.
5. Murari K, Sharma NK, Bharti SK. The coming age of future of medicine: Next Frontier. Biochemistry & Analytical Biochemistry, 2016, 5(297). Doi: 10. 4172/2161-1009.1000297.
6. Bharti SK, Krishnan S, Kumar A. Phytotherapy for diabetes mellitus: back to nature. Minerva Endocrinologica. 2016; 41(1):143-6.
7. Bharti A, Krishnan S, Bharti SK. Personalized Diabetes Clinics Revisited. Biochemistry & Analytical Biochemistry. 2015; 4(4):1000e159.
8. Bharti SK, Krishnan S, Kumar A, Gupta AK, Kumar A. Mechanism-based antidiabetic activity of Fructo- and isomalto-oligosaccharides: Validation by *in vivo, in silico* and *in vitro* interaction potential. Process Biochemistry. 2015; 50(2):317–327.
9. Bharti SK, Krishnan S, Sharma NK, Kumar A, Prakash O, Gupta AK *et al*. *In vivo* and *in silico* investigation of antidiabetic activity of fruit of *Withania coagulans* Dunal. Current Hypertension Reviews. 2015; 11(2):143-58.

10. Bharti SK, Krishnan S, Kumar A, Razak KK, Murari K, Bharti BK *et al*. Antidiabetic activity and molecular docking of Fructooligosaccharides produced by *Auridobasidium pullulans* in poloxamer-407 induced T2DM rats. Food Chemistry. 2013; 136(1):813-821.
11. Bharti SK, Krishnan S, Kumar A, Razak KK, Murari K, Bharti BK *et al*. Antihyperglycemic Activity with DPP-IV Inhibition of Alkaloids from seed extract of *Castanospermum australe*: Investigation by experimental validation and molecular docking. Phytomedicine. 2012; 20(1):24–31.
12. Bharti SK, Kumar A, Sharma NK, Prakash O, Krishnan S, Gupta AK *et al*. Tocopherol from seeds of *Cucurbita pepo* against diabetes: Validation by *in vivo* experiments supported by computational docking. Journal of the Formosan Medical Association. 2013; 112(11):676–690.
13. Bharti SK, Kumar A, Prakash O, Krishnan S, Gupta AK. Essential oil of *Cymbopogon citratus* against diabetes: Validation by *in vivo* experiments and computational studies. Journal of Bioanalysis and Biomedicine. 2013; 5(5):194-203.
14. Bharti SK, Sharma NK, Gupta AK, Padamdeo SR. Antidiabetic effect of aqueous extract of *Withania coagulans* flower in Poloxamer-407 induced type 2 diabetic rats. Journal of Medicinal Plants Research, (Academic Journals publishing). 2012; 6(45):5706-5713.
15. Chang C, Yang M, Wen H, Chern J. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Analaysis. 2002; 10:178-182.
16. Cowan MM. Plant Products as Antimicrobial Agents. Clinical Microbiology Reviews. 1999; 12:564-582.
17. Cragg GM, Boyd MR, Khanna R, Kneller R, Mays TD, Mazan KD *et al*. International collaboration in drug discovery and development: the NCl experience. Pure Appl. Chem. 2017; 71:1619-1633.
18. Datta A, Ghosh S, Singh M. Antimicrobial property of *Piper betel* leaf against clinical isolates of bacteria. International Journal of Pharma Sciences and Research. 2011; 2(3):104-109.
19. Doughari J, Manzara S. *In vitro* antibacterial activity of crude leaf extracts of *Mangifera indica* Linn. Afr J Microbiol Res. 2008; 2:67-72.
20. Einstein BI, Mandell GL, Bennett JE, Dolin E. (Eds.) Douglas and Bennett's Principles and Practice of Infectious Diseases. Churchill Livingstone, New York, USA, 2000.
21. El-Mahmood AM, Doughari JH, Kiman HS. *In vitro* antimicrobial activity of crude leaf and stem bark extracts of *Gmelina arborea* (Roxb) against some pathogenic species of Enterobacteriaceae. African Journal of Pharmacy and Pharmacology. 2010; 4(6):355-361.
22. Falagas ME, Kotsantis IK, Vouloumanou EK, Rafailidis PI. Antibiotics versus placebo in the treatment of women with uncomplicated cystitis: a meta-analysis of randomized controlled trials. J Infect. 2009; 58:91-102.
23. Ghosh S, Saha S. Tinospora cordifolia. One plant, many roles. Anc. Sci. Life. 2012; 31(4):151-9.
24. Goel A, Sharma K. Plant Extracts and Phytochemicals as Herbal Medicines and Antimicrobials. International Journal of Biological & Medical Research. 2014; 5(1):3940-3946.
25. Harborne JB. Phytochemical methods**.** Chapman and Hall, London, 1991.
26. Houghton PJ, Howes MJ, Lee CC, Steventon G. Uses and abuses of *in vitro* tests in ethnopharmacology: visualizing an elephant. Journal of Ethnopharmacology. 2007; 110:91-400.
27. Hugo WB. Biology of microorganisms. IN Hugo, W.B. and Russell, A.D. (Eds.) *Pharmaceutical Microbiology*. Blackwell Scientific Publications, Oxford, UK, 1992.
28. Ishaku A, Ishakeku D, Agwale S. Phytochemical screening and antibacterial activity of *Gmelina arborea* fruit extracts. International Journal of Microbiology and Immunology Research. 2013; 1 (2) 026-031.
29. Kalayou S, Haileselassie M, Gebre-egziabher G, Tiku T, Sahle S, Taddele H *et al*. *In-vitro* antimicrobial activity screening of some ethnoveterinary medicinal plants traditionally used against mastitis, wound and gastrointestinal tract complication in Tigray Region, Ethiopia. Asian Pacific Journal of Tropical Biomedicine. 2012; 2(7):512-522.
30. Karaman I, Shahin F, Gulluce M, Ogutsu H, Sngul M, Adiguzel A. Antimicrobial activity of aqueous and methanol extracts of Juniperus oxycedrus L. J Ethnopharmacol. 2003; 85:231-235.
31. Kumar A, Jha KK, Kumar D, Agrawal A, Gupta A. Preliminary phytochemical analysis of leaf and bark (mixture) extract of *Ficus Infectoria* plant. The Pharma Innovation. 2012; 1(5):71-76.
32. Kumar M, Nehra K, Duhan JS. Phytochemical analysis and antimicrobial efficacy of leaf extracts of *Pithecellobium Dulce.* Asian Journal of Pharmaceutical and Clinical Research. 2013; 6(1):70-76.
33. Mariita RM, Ogol CKP, Oguge NO, Okemo PO. Antitubercular and Phytochemical Investigation of Methanol Extracts of Medicinal Plants used by the Samburu Community in Kenya. Tropical Journal of Pharmaceutical Research. 2010; 9(4):379-385.
34. Masola SN, Mosha RD, Wambura PN. Assessment of antimicrobial activity of crude extracts of stem and root barks from *Adansonia digitata* (Bombacaceae) (African baobab). African Journal of Biotechnology. 2009; 8(19):5076-5083.
35. Murray PR, Rosenthal KS, Kobayashi GS, Pfaller MA. Medical Microbiology. Mosby Inc., Missouri, USA, 1998.
36. Najar MS, Saldanha CL, Banday KA. Approach to urinary tract infections. Indian J Nephrol. 2009; 19:129-39
37. Nikaido H, Vaara M. Molecular basis of bacterial outer membrane permeability. Microbiology Reviews. 1985; 49(1):1-32.
38. Okeke MI, Iroegbu CU, Eze EN, Okoli AS, Esimone CO. Evaluation of extracts of the root of *Landolphia owerrience* for antibacterial activity. J Ethnopharmacol 2001; 78: 119-127.
39. Parthipan M, Aravindhan V, Rajendran A. Medico-botanical study of Yercaud hills in the Eastern Ghats of Tamil Nadu, India. Anc Sci. Life. 2011; 30:104-9.
40. Parveen T, Sharma K. Phytochemical Profiling of Leaves and Stem Bark of *Terminalia arjuna* and Tecomella undulate. International Journal of Pharmacy & Bioscience. 2014; 1(1):1-7.
41. Patil VS, Malpathak NP. Micro-morphoanatomical approach for comparative analysis of Tinospora cordifolia (Willd.) Miers and its adulterant plant using

SEM and Cryostat. Pharmacogn. J. 2014-2016; 9(1):39-45.
42. Perez C, Pauli M, Bezevque P. An antibiotic assay by agar well diffusion method. Act. Biol. Med. Exp. 1990; 15:113-115.
43. Perilla MJ, Ajello MS, Bopp C, Elliott J, Facklam R, Popovic T, Wells J. Manual for the Laboratory Identification and Antimicrobial Susceptibility Testing of Bacterial Pathogens of Public Health Importance in the Developing World. Centre for Disease Control and Prevention: National Centre for Infectious Diseases, Atlanta, Georgia, USA, 2003.
44. Pourmorad F, Hosseinimehr SJ, Shahabimajd N. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr J Biotechnol. 2006; 5(11):1142–1145.
45. Prasai, T, Lekhak B, Baral MP. Antibacterial property of medicinal plants against gram negative bacteria isolated from water. Proceedings of IV National Conference on Science and Technology. 2004; 2:410-415.
46. Rahman MM, Mominul M, Sheikh L, Sharmin SA, Islam SM, Rahman AM *et al*. Antibacterial Activity of Leaf Juice and Extracts of *Moringa oleifera* Lam. against some human pathogenic bacteria. Journal of Natural Sciences. 2009; 8(2):219-227.
47. Rana V, Thakur K, Sood R, Sharma V, Sharma TR. Genetic diversity analysis of *Tinospora cordifolia* germplasm collected from northwestern Himalayan region of India. J Genet. 2012; 91:99-103.
48. Rao MR, Reddy IB, Ramana T. Antimicrobial activity of some Indian medicinal plants, Ind J Microbiol 2006; 46: 259-262.
49. Rathee D, Rathee D, Rathee P, Rathee S. Phytochemical screening and antimicrobial activity of Picrorrhiza kurroa, an Indian traditional plant used to treat chronic diarrhea. Arabian Journal of Chemistry. In press, 2012.
50. Reddy LJ, Jose B. Evaluation of antibacterial activity of the bark, flower and leaf extracts of *Gliricidia Sepium* from South India. International Journal of Current Pharmaceutical Research. 2010; 2(3):18-20.
51. Salazar R, Pozos ME, Cordero P, Perez J, Salinas MC, Waksman N. Determination of the antioxidant activity of plants from Northeast Mexico. Pharmaceutical Biology. 2008; 465:166-170.
52. Saranraj P, Siva Sakthi S, Geetha M. Pharmacological screening of Datura metel and Acalypha indica for its Antifungal activity against fungal pathogens, International Journal of Pharmaceutical Science and Health Care. 2011; 1(2):15-30.
53. Shankar TB, Shantha NV, Ramesh HP, Murthy IA, Murthy VS. Toxicity studies on turmeric (Curcuma longa): acute toxicity studies in rats, guineapigs and monkeys. Indian journal of experimental biology. 1980; 18(1):73- 75.
54. Sharma U, Bala M, Kumar N, Singh B, Munshi RK, Bhalerao S. Immunomodulatory active compounds from *Tinospora cordifolia*. J Ethnopharmacol. 2012; 141:918-26.
55. Sleigh DJ, Timburg MC. Notes on Medical Bacteriology. Churchill Livingstone, Edinburgh, UK, 1998.
56. Subramanian M, Chintalwar GJ, Chattopadhyay S. Antioxidant properties of a *Tinospora cordifolia* polysaccharide against iron-mediated lipid damage and gamma-ray induced protein damage. Redox Rep. 2002; 7:137-43.
57. Thankamani, V, James J, Veettil AKT, Sagadevan LDM. Phytochemical screening and anti microbial activity of *Alstonia scholaris* flowers (L) R.BR. Fam: Apocynaceae. International Journal of Pharmaceutical Research and Development. 2011b; 3(3):172-178.
58. The Indian Pharmacopoeia, 2012. http://ipc.nic.in
59. Upadhyaya RPR, Sharma V, Anita KV. Assessment of the multifaceted immunomodulatory potential of the aqueous extract of *Tinospora cordifolia*. Res J Chem Sci. 2011; 1:71-9.
60. Yagoub, SO. Anti-microbial activity of Tamarindus indica and *Adansonia digitata* extracts against *E. coli* isolated from urine and water specimens. Research Journal of Microbiology. 2008; 3(3):193.