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In vitro antibacterial effect of *Tinospora cordifolia* extracts against some selective bacterial pathogens

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Abstract

The petroleum spirit, methanol, dichloromethane and ethyl acetate extracts of the leaves of *Tinospora cordifolia* (Menispermaceae) were screened for their anti-microbial activity using disc-diffusion method on nutrient agar medium. This plant was tested against four bacteria; two Gram-positive bacteria (*Bacillus subtilis* and *Sarcina lutea*) and two Gram-negative bacteria (*Escherichia coli* and *Klebsiella pneumoniae*).All the organic solvent extract showed susceptibility against *Sarcina lutea*, *E.coli* and *Bacillus subtilis* whereas *Klebsiella pneumoniae* showed resistant against all the organic solvent extract. It was found that the antimicrobial activity of the methanol extract (11mm) showed the maximum zone of inhibition against *Escherichia coli.* On the other hand petroleum spirit extract showed the maximum inhibition against *Bacillus subtilis* (3mm) respectively. The minimum inhibitory concentration for petroleum spirit and dichloromethane extracts of *Tinospora cordifolia* were ranged between 32-512µg/mL for tested bacteria. The result of this study demonstrates the potentiality of *Tinospora cordifolia* as a source of antimicrobials that could be harness for use in the health care delivery process.

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Introduction

Plants have evolved a number of inducible defense mechanisms against pathogen attack by different chemical constituents. Some of the responses are constitutive and pathogen non-specific, but majority of them are induced after recognition of the pathogen. Scientific experiments on the antimicrobial properties of plant components were first documented in the 19th century (Penna *et al.*, 2001). Moreover, the use of current microbiological techniques demonstrates that medicinal plants normally exhibit significant strength against human bacterial and fungal pathogens (Palombo and Semple, 2001).

Tinospora cordifolia (Family: Menispermaceae) commonly known as "Guduchi" is an important drug of folk medicine system (Methew and Kuttan, 1999). It is widely used in general tonic, anti-cancer, anti-ulcer, anti-pyretic, anti-hepatitis, hypoglycemic, anti-neoplastic, cardiotonic, anti-microbial, anti-inflammatory, analgesic and diuretic properties (Jagetia and Rao, 2006; Methew and Kuttan, 1999; Osadebe *et al.*, 2012; Stanely *et al.*, 2000). It has been also indicated useful application in the treatment of heart disease, leprosy, helmenthiasis and rheumatoid arthritis (Kirtikar and Basu, 1933).

Tinospora cordifolia is a large, glabrous, deciduous climbing shrub. It is distributed throughout tropical Indian subcontinent. A large number of phytochemical compounds belonging to the different groups such as terpenoids, alkaloids, lignans and steroids have been investigated to treat different disease (Sinha *et al.*, 2004). In the view of medicinal importance of *Tinospora cordifolia* there remains adequate scope for further depth research to find out the antimicrobial effect on some vulnerable microbial strains. The main objective of this work was to identify the effect of different solvent extracts of *Tinospora cordifolia* against some disease causing human pathogens.

The present study was an attempt to investigate the antibacterial activity of *Tinospora cordifolia*leaf extracts against gram positive bacteria *Bacillus subtilis*, *Sarcinalutea*, and gram negative bacteria *Escherichia coli, Klebsiella pneumonia*. All the organic solvent extract showed susceptibility against *Sarcina lutea*, *E.coli* and *Bacillus subtilis* whereas *Klebsiella pneumonia* showed resistant against all the organic solvent extract. It was found that the antimicrobial activity of the methanol extract (11mm) showed the maximum zone of inhibition against *Escherichia coli.* On the other hand petroleum spirit extract showed the maximum inhibition against *Bacillus subtilis* (3mm).However, widespread exploration is desired to isolate the secondary metabolites from the extracts in order to test specific compounds for antimicrobial activity.

Materials and methods

Collection of plant leaves

Healthy, disease free and mature *Tinospora cordifolia* plant was collected directly from Islamic university campus, Kushtia, Bangladesh. This plant was then botanically identified and name of the plant, place and date of the collection were recorded.

Preparation of T. cordifolia leaves extracts

After collection of the *T. cordifolia* plant leaves was washed by running tap water, cleaned and then dried without sun. Then it was pulverized into a fine powder. Then 10 gm of the leaves powder was weighed with electric balance and 40 ml each of the solvent (methanol, petroleum spirit, ethyl acetate and dichloromethane) was added in each conical flask. The powder was extracted separately with Whitman No. 1 filter paper and the solvents were recovered by rotary evaporator.

Tested bacteria

Antibacterial activity of *Tinospora cordifolia* leaves extracts were investigated against two Gram-positive (*Bacillus subtilis*and *Sarcina lutea*) and two Gram-negative (*Escherichia coli* and *Klebsiella pneumoniae*) bacterial isolates obtained from the Microbial Type Culture Collection (MTCC) of Microbiology Laboratory of the Biotechnology and Genetic Engineering Department, Islamic University Kushtia, Bangladesh. The tested bacteria were culture

on Nutrient agar (Hi Media, Mumbai, India) at 37 ° C for 24 h. The culture was sub cultured regularly (every 30 day) and stored at 4 ° C.

Inoculum preparation

Ten milliliter of distilled water was taken into the screw cap tube and pure colony of fresh culture bacteria was added into the tube and vortex vigorously. The OD (optical density) was measured with the colorimeter and bacterial population were confirmed to be within in 10^7-10^8 mL^{-1} and then plated out as inoculums (Gur *et al.*, 2006).

Antimicrobial bioassay

The *in vitro* antibacterial activities of the test samples were carried out by disc diffusion method according to Fritsche (2007). Dried and sterilized filter paper discs (6 mm diameter) were impregnated with known amount of the test substances (extracts) dissolved in solvent (400 µg disc-1) were placed on nutrient agar medium uniformly seeded with the test bacteria. Standard disc were used as positive and negative control, respectively. These plates were then kept at low temperature (4 ° C) for 24 h to allow maximum diffusion of test samples and then incubated at 37 ° C for 24 h to allow maximum growth of microorganisms. The test materials having antibacterial activity inhibited the growth of the bacteria and a clear, distinct zone of inhibition was visualized surrounding the disc. The antibacterial activity of the test agents was determined by measuring the diameter of zone of inhibition in millimeter.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) values were determined (Shahidi, 2004) by broth dilution assay. Petroleum spirit and ethyl acetate extracts were serially diluted to concentrations ranging from 512µg/ml to2 µg/ml in petroleum spirit and ethyl acetate solvent against three tested pathogenic microorganisms. Finally, the tested samples were incubated at 37 °C for 24 hour. The lowest concentration of all extracts that produced no visible bacterial growth was recorded as the MIC.

Statistical analysis

The antibacterial activity was determined by measuring the diameter of zone of inhibition of millimeter scale that was expressed as mean ± SD (Standard deviation of mean). ANOVA single factor was followed by Duncan's multiple ranges for multiple comparison tests. $P< 0.05$ was considered statistically significant.

Results

In the present study, the antimicrobial activity of four extracts against two gram positive and four gram negative bacterial strains and their potential activity were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and MIC values.

Antibacterial activity

All the extracts of the investigated plant species showed antimicrobial activities against all tested bacterial strains except *K. pheumoniae*. The antimicrobial activities of *Tinospora cordifolia* leaf extracts are compared with standard antibiotics such as amoxicillin, which was used as positive controls. Results of the antimicrobial activity obtained using the disc diffusion assay is summarized in Table 1and Figure 1. Diameter of the inhibition zone of different organic extract include 6 mm disc were tested at a concentrations of 400 µg/disc.

Measurement of zone of inhibition

According to concentration (400 µg/disc) the zone of inhibition for methanol extract was *B. subtilis* (7.10±0.14), *S. lutea* (7.33±0.33), *E. coli* (11.33±0.33); for ethyl acetate extract was *B. subtilis* (6.67±0.33), *S.lutea* (6.33±0.33), *E.coli* (8.33±1.33); for petroleum spirit extract was *B. subtilis* (10.67±0.33), *S. lutea* (3.67±0.33), *E. coli*(9.0±1.0) and for dichloromethane extract was *B. subtilis* (8.0±1.0), *S. lutea* (7.67±0.33), *E. coli* (6.67±0.33). The gram negative bacterium *K. pneumoniae* did not show any zone of inhibition against any organic extracts. The

highest zone of inhibition to *E. coli* for methanol extract at concentration of 400 µg/disc was 11.33±0.33mm ($p<0.005$). The lowest zone of inhibition to *S. lutea* for petroleum spirit extract at concentration of 400 µg/disc was 3.67±0.33mm ($p<0.005$).

Table 1. Antibacterial activity of different extract of *T. cordifolia* (400 µg disc^{-1}).

Tested bacteria	Diameter of zone of inhibition (mm)				
	A-10 (Control)	Methanol extracts	Ethyl acetate extracts	Petroleum spirit extracts	Dichloromethane extracts
B. subtilis	8.90±0.42	7.10±0.14	6.67±0.33	10.67±0.33	8.0±1.0
S. lutea	8.10±0.28	7.33±0.33	6.33±0.33	3.67±0.33*	7.67±0.33
E.coli	10.00±0.14	11.33±0.33*	8.33±1.33	9.0±1.0	6.67±0.33
K.pneumoniae	16.10±0.14	-	-	-	-

Data were measured in mm and represented as mean ± SD of triplicate. (A-10): Amoxicillin (30 µg/disc); (*) indicates significance value $P<0.005$."-" indicates no zone formation.

Table 2. Minimum Inhibitory concentration (MIC) value of petroleum spirit and dichloromethane extract of *T.cardifolia*.

Tested bacteria	Petroleum spirit extract of *T. cardifolia* (µg/ml)										Dichloromethane spirit extract of *T. cardifolia*(µg/ml)									
	512	256	128	64	32	16	8	4	2	0	512	256	128	64	32	16	8	4	2	0
B.subtilis	-	-	-	-	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+
S.lutea	-	-	-	-	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+
E.coli	-	-	-	-	+	+	+	+	+	+	-	-	-	-	+	+	+	+	+	+

"+" indicates no zone formation and "-" indicates formation of inhibition zone

Minimum inhibitory concentration (MIC)

Minimum inhibitory concentration was observed with all bacterial strains against three different leaf extracts of *Tinospora cordifolia* (Table 2). Almost all organic extracts showed MIC value at a concentration of 64µg/ml.

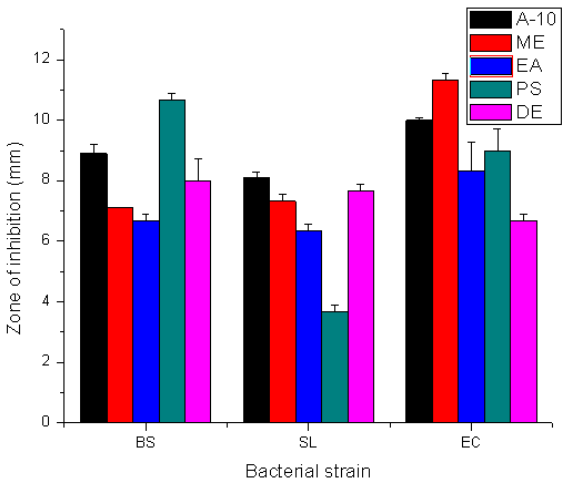

Fig. 1. The comparison of antibacterial potential of *T. cordifolia* leaf extracts and standard antibiotics against some pathogenic bacteria. (A-10): Amoxicillin (30 µg/disc); ME: Methanol, EA: Ethyl acetate, PS: Petroleum spirit, DE: Dichloromethane extract. The diagram of leaf extracts were revealed at the concentrations of 400 µg/disc. BS: *B. subtilis*, SL: *S. leutia*, EC: *E. coli*.

Discussion

Our present investigation was designed to perform the study on comparative antibacterial activity of different leaf extracts of *Tinospora cordifolia* against some selective gram positive and gram negative pathogenic bacterial strains. All of the organic extracts of *Tinospora cordifolia* exhibited greater extend of antibacterial activities against all bacterial strain except *K. pneumoniae*.

For a long period of time, plants have been a valuable source of natural products for maintaining human health (Tschesche, 1971). The phytochemical screening of the extracts of *Tinospora cordifolia* showed the presence of alkaloids, flavonoids,

terpenoids, sterols and tannins (Sinha *et al.*, 2004). Presence of these chemical constituent in different extract of *Tinospora cordifolia* confirm its potential against three selected pathogens. The plant extracts were justified with standard antibiotics i.e. amoxicillin (30 µg/disc), and negative control (only solvent absorbing disc). The negative control showed no activity against all tested bacteria. The standard antibiotics showed significant antimicrobial activity against all tested gram positive and gram negative bacterial strains.

Antimicrobial activity of *Tinospora cordifolia* extracts of using different extraction solvents was investigated against some selective bacterial strains. The result of this work indicates that the various soluble extracts of *Tinospora cordifolia* have antibacterial properties. When the extracts were tested on all the above mentioned bacterial strains different zones of inhibition was produced by each of the strain. These differences in the zones of inhibition may be directly related to the susceptibility of each test organisms to the *Tinospora cordifolia* extracts. The factors responsible for this high susceptibility to the extracts are not exactly known but may be attributed to the presence of secondary plant metabolites. The growth inhibitory effect is usually depends on concentration (Achi, 2006). This is important to identify the dosage and rate at which the extract inhibits the growth of organism (Egwari, 1999). Statistical analysis showed that there is no significant difference between the amoxicillin and different extracts of *Tinospora cordifolia*. This demonstrates that the extracts were as effective as standard antibiotic used.

Our results indicates the potential usefulness of *Tinospora cordifolia* in the treatment of various pathogenic diseases as it may help in the discovery of new chemical classes of antibiotics that could serve as selective agents for the maintenance of human health and may provide biochemical tools for the study of bacterial diseases.

Conclusion

The extracts of *Tinospora cordifolia* leaf were found to be effective antibacterial agent**.** This study revealed the way for further attention and research to identify the active compounds responsible for the plant biological activity with the required minimum inhibitory concentration (MIC).

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References

Achi OK. 2006. Composition and antibacterial activities of *Tetrapleura tetraptera* Taub. pod extracts. Research Journal of Microbiology **5**, 416 – 422.
http://dx.doi.org/103923/jm.2006. 416.422

Egwari LO. 1999. Antibacterial activity of crude extracts of *Nauclea latifolia* and *Eugenia aromatica*. West African Journal of Pharmacology and drug Resistance **15**, 55 – 59.

Fritsche TR, McDermott PF, Shryock TR, Walker RD. 2007. Agar dilution and disk diffusion susceptibility testing of *Campylobacter* spp. Journal of Clinical Microbiology, **45**, 2758-2759.
http://dx.doi.org/10.1128/JCM.00569-07

Gur S, Turgut-Balik D, Gur N. 2006. Antimicrobial activities and some fatty acids of turmeric, ginger root and linseed used in treatment of infectious diseases. World Journal of Agricultural Sciences **2**, 439-442.

Jagetia GC, Rao SK. 2006. Evaluation of the antineoplastic activity of guduchi (Tinospora cordifolia) in Ehrlich ascites carcinoma bearing mice. Biological and Pharmaceutical Buletins **29**, 460-466.
http://dx.doi.org/10.1248/bpb.29460

Kirtikar KR, Basu BD. 1933. Indian medicinal plants **2**, 77.

Methew S, Kuttan G. 1999. Immunomodulatory and antitumour activities of *Tinospora cordifolia*. Fitoterapia **70**, 35-43. http://dx.doi.org/10.1016/S0367-326X(98)00017-3

Osadebe PO, Okoye FB, Uzor PF, Nnamani NR, Adiele IE, Obiano NC. 2012. Phytochemical analysis, hepatoprotective and antioxidant activity of Alchornea cordifolia methanol leaf extract on carbon tetrachloride-induced hepatic damage in rats. Asian Pacific Journal of tropical Medicine **5,** 289-293. http://dx.doi.org/10.1016/S1995-7645(12)60041-8

Palombo EA, Semple SJ. 2001. Antibacterial activity of traditional Australian medicinal plants. Journal of Ethnopharmacology 77, 151-157. http://dx.doi.org/10.1016/ S0378-8741(01)00290-2

Penna C, Marino S, Vivot E, Cruanes MC, de D Munoz J, Cruanes J, Ferraro G, Gutkind G, Martino V. 2001. Antimicrobial activity of Argentine plants used in the treatment of infectious diseases. Isolation of active compounds from *Sebastiania brasiliensis*. Journal of Ethnopharmacology **77**, 37-40. http://dx.doi.org/10.1016/S0378-8741(01)00266-5

Stanely P, Prince M, Menon VP. 2000. Hypoglycaemic and other related actions of Tinospora cordifolia roots in alloxan-induced diabetic rats. Journal of Ethnopharma - cology **70**, 9-15. http://dx.doi.org/10.1016/S0378-8741(99)00136-1

Sinha K, Mishra NP, Singh J, Khanuja SPS. 2004. *Tinospora cordifolia* (Guduchi), a reservoir plant for therapeutic applications: A review. Indian Journal of Traditional knowledge **3**, 257-270.

Shahidi GA. 2004. Evaluation of antimicrobial properties of Iranian medicinal plants against *Micrococcus luteus, Serratiamarceceans, Klebsiella pneumonia and Bordetellabranchoseptica*. Asian Journal of Plant Sciences **3**, 82-86.

Tschesche R. 1971. Advances in chemistry of Antibiotics substance from higher plant; pharmacognosy and phytochemistry proceeding of the 1st international congress. Verlong, Berlin, Heidelbeg, New York, 274 - 276.