Antimicrobial activity of two Indian medicinal plants Tinospora cordifolia (Family: Menispermaceae) and Cassia fistula (Family: Caesalpinaceae) against human pathogenic bacteria

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ABSTRACT

In the present investigation antimicrobial activity of two Indian medicinal plants i.e. Tinospora cordifolia and Cassia fistula were evaluated against seven human pathogenic bacterial strains. Both were tested by serial micro-dilution method. Antimicrobial susceptibility of aqueous and solvent extracts was assessed. For this purpose, both positive and negative controls were set to determine MIC and MBC values. After bioassays, aqueous and solvent extracts of Tinospora cordifolia and Cassia fistula exhibited significant antimicrobial susceptibility against bacteria both Gram negative and Gram positive i.e. Klebsiella pneumoniae (ATCC 15380), Escherichia coli (ATCC 25922), Micrococcus luteus (ATCC 9341), Streptococcus pneumoniae (ATCC 12755), Staphylococcus aureus (ATCC 25923), Bacillus cereus (ATCC 11778) and Lactobacilus acidophilus (ATCC 53103) at a very low concentration. More specifically, higher percent growth inhibition was obtained in presence of aqueous extracts in comparison to solvent extracts, which was much higher than synthetic antibiotics. Further, different extracts have shown very low MIC value, which was obtained in a range of 0.0078-0.125 mg/ml. It was much lower than the MIC values (0.223-0.892 mg/ml) obtained in presence of standard antibiotics i.e. tetracycline, ampicillin and ciprofloxacin. More specifically, aqueous extracts of both plants have shown lower MIC (0.00975 mg/ml in E. coli and K. pneumoniae) and MBC values (0.078 mg/ml in E. coli and K. pneumoniae) than broad-spectrum antibiotics (0.223-0.892 mg/ml). Further, aqueous extracts of both plant species have shown significantly much higher inhibition zone diameters (20-30mm) against all seven bacterial strains than the synthetic antibiotics (6-18).

Key words: MIC, MBC, antibacterial activity, plant extracts, Tinospora cordifolia and Cassia fistula

INTRODUCTION

In the present time multiple drug resistance in microbial pathogens become a serious health problem to humankind worldwide (Peng et al., 2006). It is aroused due to indiscriminate and repetitive use of antimicrobial drugs by inadequate disease treatment (Shariff, 2001). To acquire drug resistance microbes have developed new enzyme system to cleave the drug and make it useless for control of infection (Ritch-Kro et al., 1999). Hence, plant origin herbal medicines are considered as safe alternatives of synthetic drugs. There are varied methods of medicines like Ayurveda, Homeopathy and Unani, which utilize plant materials for drug production. Currently, Ayurveda considered as a vital system of medicine and governed the worldwide recognition and having non-toxic substances. Hence, in the last few decades, so many plant species were explored for obtaining potential antimicrobials for therapeutic purposes (Rios and Recio, 2005; Liu, 1987), which later on become an integral part of primary health care in many parts of world (Desta, 1993; Anesini and Perez, 1993; Cown, 1999). Hence, plants, which possess strong antimicrobial potential against pathogens are considered as valuable source of medicinal compounds and show lesser side effects.

Plants are rich source of wide variety of secondary metabolites viz. tannins, terpenoids, alkaloids, and flavonoids, which possess enormous antimicrobial properties (Suresh et al., 1992). Approximately 25 to 50 % of current pharmaceuticals are derived from plants. Most of them were found effective against many pathogenic bacteria (Bilgrami et al., 1992), fungi (Pacheco et al.,1993), viruses (Nascimento et al., 2000) and even found active against drug-resistant microorganisms (Mohana et al., 2008). Besides this, few antimicrobials such as essential oils (Yang et al., 2010; Nannapaneni et al., 2008), plant extracts (Mohana et al., 2008) and pure compounds have shown broad-spectrum antimicrobial activity against pathogens (Acharya et al., 2009; Serrentino, 1991). Some of the plant products are used as nutraceuticals (Adwan, 2006; Tiwari et al., 2009) and in food preservation (Rege et al., 1999). In the present investigation, two indigenous plant species from India have been screened for their antimicrobial activities. Tinospora cordifolia is a large glabrous ascending shrub belongs to family Menispermaceae and known as Giloy in Hindi. The leaves are membranous and cordate. It is used as a blood purifier and anti-infectious agent. It is also used for the treatment of jaundice, rheumatoid arthritis, diabetes, gout, viral hepatitis, arthropathies and general weakness. Cassia fistula is a tree belongs to family Caesalpinaceae and is an indigenous medicinal plant, commonly known as Amaltash. Cassia fistula is a medium size tree and having yellow flowers and green leaves. It is an ornamental flowering plant, its seeds and legumes are used for gastric and respiratory problems. Long bunches of flowers are used for decoration purposes. Seeds are used for stomach ailments while bark and leaves for burns. The pulp of the fruit is used as purgative and laxative.

EXPERIMENTAL

PREPARATION OF EXTRACTS

Plant parts of Tinospora cordifolia and Cassia fistula were collected from forest areas of Gorakhpur in eastern U.P. The stem of Tinospora cordifolia and legumes of Cassia fistula were chopped into small pieces, dried, milled and powdered by using pestle and mortar. The acetone, methanol, chloroform and petroleum ether extracts were prepared by using 50g of dried powder of C. fistula legumes and leaves of Tinospora cordifolia in 250 ml of solvent separately. Each solvent extract was fractionated to get different serial fractions in different solvent. The extract was dried; residue was weighed and dissolved in known quantity of fresh solvent. In the preparation of aqueous extract, 50 mg the powdered material was dissolved in 250 ml of distilled water and kept for solubilization overnight at room temperature. For quantization plant extracts were evaporated and re-dissolved in known volume of each solvent separately.

MICRO-ORGANISMS

Cultures of seven pathogenic bacterial strains each of Escherichia coli (ATCC 25922), Bacillus cereus (ATCC 11778), Lactobacilus acidophilus (ATCC 53103), Micrococcus luteus (ATCC 9341), Staphylococcus aureus (ATCC 25923), Klebsiella pneumoniae (ATCC 15380) and Streptococcus pneumoniae (ATCC 12755) were maintained in the laboratory in Luria Broth (2% w/v) regularly for four days at 37°C before use in experiments. For experiments a portion (100 μl) of the overnight culture was mixed in the tests and control for inoculation. For activity testing bacterial cultures were stored at 4°C and sub cultured after every 8th day in solid agar plates.
Table 1. MIC values obtained in presence of solvent and aqueous extracts of Tinospora cordifolia and Cassia fistula against seven pathogenic bacterial strains (mg/ml).

<table>
<thead>
<tr>
<th>Tinospora cordifolia</th>
<th>Plant extracts</th>
<th>K.pneumoniae</th>
<th>E.coli</th>
<th>B.cereus</th>
<th>M.luteus</th>
<th>L.acidophilus</th>
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Antibiotics

| Tetracycline*       | 0.446         | 0.446        | 0.446  | 0.223    | 0.446     | 0.223         | 0.446    |              |
| Ampicillin*         | 0.223         | 0.446        | 0.446  | 0.892    | 0.223     | 0.446         | 0.223    |              |
| Ciprofloxacin*      | 0.892         | 0.446        | 0.892  | 0.223    | 0.446     | 0.223         | 0.446    |              |

MIC—Minimum Inhibitory Concentration

Solubility of each plant extract was determined before treatment

No turbidity was found visible after 24 hrs incubation

*Antibiotics was used for comparison

Table 2. MBC values obtained in presence of solvent and aqueous extracts of Tinospora cordifolia and Cassia fistula against seven pathogenic bacterial strains (mg/ml).

<table>
<thead>
<tr>
<th>Tinospora cordifolia</th>
<th>Plant extracts</th>
<th>K.pneumoniae</th>
<th>E.coli</th>
<th>B.cereus</th>
<th>M.luteus</th>
<th>L.acidophilus</th>
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Antibiotics

| Tetracycline*       | 0.892         | 0.892        | 1.78   | 3.57     | 0.892     | 0.892         | 0.892    |              |
| Ampicillin*         | 1.78          | 1.78         | 1.78   | 1.49     | 0.892     | 0.892         | 0.892    |              |
| Ciprofloxacin*      | 1.78          | 1.78         | 0.892  | 3.57     | 0.892     | 0.892         | 0.892    |              |

MBC—Minimum Bacterial Concentration

For Comparison both negative and positive controls were set

*Antibiotics were used for comparison

MEDIA PREPARATION AND ITS STERILIZATION

For antimicrobial susceptibility testing both solid (Agar-agar) and liquid agar (Luria broth) media were used. It has shown a good bacterial growth and reproducibility. For suspension culture of bacterial cells 1% Lauria Broth (w/v) was prepared by dissolving 1 gm of broth media in 100 ml of distilled water, while 2% agar was used for developing surface colony growth.

DETERMINATION OF MIC AND MBC VALUES

For determination of antimicrobial activity, bacterial growth inhibition was accessed in the presence of different increasing concentrations of Tinospora cordifolia and Cassia fistula extracts. For this purpose both crude extracts were separately diluted by using serial micro dilution method with Luria Broth culture medium at a final concentration range from 8.0 to 0.00975 mg/ml. The tested crude extracts and pure compounds were added to fresh suspension after following the serial dilutions up to 10-6 Each extract was assayed in triplicate. Before conducting experiments all the conditions for in vitro anti-microbial activity were standardized to determine MIC and MBC values. The MIC values considered as the lowest concentration of crude extract and pure compounds, which have shown no turbidity in the culture flask after 24 hrs of incubation at 37°C. The turbidity in the culture flasks was considered as visible growth of microorganisms. Further, it was standardized in terms of absorbance at 600 nm in a visible spectrophotometer. For determination of minimum bactericidal concentration (MBC) growth inhibitory assays were performed. For this purpose inoculum size was adjusted to prepare a final colony number as 106 colony forming units (CFU/ml in sterile agar plate). The incubation of test and controls cultures was performed at 37°C for 24 hours. The least concentration at which no visible growth was obtained in agar plates was considered as MBC value. For evaluation of inhibition, both negative (with antibiotics) and positive (without antibiotics and extracts) parallel controls were set for each and every test. Bacterial growth was obtained in presence and absence of various concentrations of solvent and aqueous extracts of T. cordifolia and C. fistula separately.

MEASUREMENT OF INHIBITION ZONE DIAMETER

Antimicrobial susceptibility of Tinospora cordifolia and Cassia fistula extracts was evaluated by agar disc diffusion method of Kirby et al. (1966). Molten agar was used as media for bacteria. For this purpose six different concentrations of each extract and pure compounds were coated on sterile filter paper discs (Whatman No. 1) of 6 mm in sizes. Extract coated discs were dried under laminar flow cabinet. Before experiment inoculum size was determined and adjusted to prepare a final colony number as 105 CFU/ml ( Colony Forming Unit) in sterile agar plates. Bacterial inoculum was spread evenly on to the surface of agar plate with sterile rubber pad spreader before the coated discs were positioned on the inoculated agar surface. Each extract and pure compound was assayed in triplicate. For comparison three broad spectrum antibiotics i.e. tetracycline, ampicillin and ciprofloxacin were also used as parallel controls. All treated and untreated plates were incubated for 24 hrs at 37°C. The antibacterial activity was assessed and size of inhibition zone diameter surrounding the filter paper discs was measured.

STATISTICAL ANALYSIS

The results were interpreted with the standard deviation. Student t-test was

applied to know significant differences between the antimicrobial effectiveness of each extract and antibiotics. The data was also statistically analyzed by applying ANOVA to know the significant difference in antimicrobial susceptibility of broad-spectrum antibiotics and various plant extracts. The related effectiveness was also tested by applying linear correlation between control and tests.

RESULTS

DETERMINATION OF MIC AND MBC VALUES

Chloroform extract of *T. cordifolia* showed least MIC value i.e. 0.146 mg/ml against *E. coli* while acetone extract 0.0156 mg/ml against *S. pneumoniae*. Aqueous extract of *T. cordifolia* showed lowest MIC values i.e. 0.00975 mg/ml against *E. coli*, while methanol extract has shown highest activity against *M. luteus* and *S. pneumoniae* at 0.0156 mg/ml concentration (Table 1). Petroleum ether extract of *T. cordifolia* showed high activity against *E. coli*, *B. cereus*, and *L. acidophilus* at 0.312 mg/ml (Table 1).

Similarly, *Cassia fistula* chloroform extract was found to be highly susceptible, as it has shown very low MIC value i.e. 0.0078 mg/ml against *K. pneumoniae*. Acetone extract has shown 0.0156 mg/ml MIC value against *K. pneumoniae*, *E. coli*, *B. cereus* and *S. pneumoniae*. *Cassia fistula* aqueous extract has shown MIC value i.e. 0.0075 mg/ml in *K. pneumoniae* and 0.048 mg/ml in *E. coli*, *B. cereus*, *M. luteus* and *L. acidophilus*. Besides this, antibiotics such as tetracycline, ampicillin and ciprofloxacin have shown higher MIC values which were obtained in a range of 0.892-3.57 mg/ml (Table 1).

Lowest MBC value was found in *Tinospora cordifolia* methanol extract i.e. 0.0312 mg/ml in *M. luteus* and *S. pneumoniae* (Table 2). Similarly, *Cassia fistula* ethyl acetate extract has shown very low MBC values i.e. 0.156 mg/ml in *S. pneumoniae* (Table 2), while chloroform extract has shown MBC values i.e. 0.0312 mg/ml in *K. pneumoniae*, *E. coli* and *M. luteus*, while it’s aqueous extract has shown very low MBC values i.e. 0.39 mg/ml in *K. pneumoniae*, *E. coli*, *S. aureus* and *B. cereus* also than antibiotics (Table 2). Antibiotics tetracycline and ampicillin have shown very high MBC values i.e. 3.57 mg/ml and 7.14 mg/ml in *M. luteus*, while ciprofloxacin has shown very high MBC value i.e. 3.57 mg/ml in *L. acidophilus* (Table 2).

MEASUREMENT OF INHIBITION ZONE DIAMETER

Antimicrobial activity of crude extract was also confirmed by agar disc diffusion method and inhibition zone diameters were measured in presence and absence of each solvent and aqueous extract of *Tinospora cordifolia* and *Cassia fistula*. Results are presented in Table 3. In presence of *Tinospora cordifolia* chloroform extract maximum inhibition zone diameter was obtained i.e. 25 mm in *K. pneumoniae*, 26 mm in *E. coli* and *S. aureus*, 27 mm in *M. luteus* at 320µg concentration. In *Tinospora cordifolia* acetone extract maximum inhibition zone diameter was obtained i.e. 25 mm in *K. pneumoniae*, *M. luteus* and *L. acidophilus*. Similarly, *Tinospora cordifolia* methanol, ether and aqueous extracts have shown maximum inhibition zone diameter at 320µg concentration (Table 3).

In presence of *Cassia fistula* chloroform extract inhibition zone diameter was obtained 15mm in *K. pneumoniae* and *E. coli*, 16mm in *S. aureus* and *L. acidophilus*, 20mm in *M. luteus* and 21mm in *S. pneumoniae* at 320µg concentration (Table 3). *Cassia fistula* acetone extract has shown highest inhibition zone diameter i.e. 27mm in *E. coli*, 26 mm in *S. aureus* and *L. acidophilus*, while rest of the bacterial strains have shown inhibition zone diameter between 20-22mm. *Cassia fistula* methanol extract has shown higher inhibition zone diameter in *S. aureus* 32mm and 25mm in *E. coli*. It has shown 16-22mm inhibition zone diameter in other bacterial strains. In presence of *Cassia fistula* ether extract, lowest inhibition zone diameter was obtained between 17-19 mm against all pathogenic bacterial strains at 20µg concentration. Similarly, *Cassia fistula* aqueous extract has shown highest inhibition zone diameter i.e. 30 mm in *M. luteus*, 27 mm in *K. pneumoniae*, 25 mm in *E. coli* and *S. pneumoniae*, 22 mm in *S. aureus*. Moreover, aqueous extract represented higher susceptibility to bacterial strains (Table 3). Antibiotics tetracycline, ampicillin and ciprofloxacin have shown significantly smaller inhibition zone diameter than that of plant extracts. It was obtained in a range of 7-17 mm (Table 3).

DISCUSSION

Both herbs and herbal products are known to have antibacterial potential (De Boer et al., 2005; Adwan et al., 2006). Various ethnic groups and local population use these, as medicine worldwide. Herbal treatments become very popular because it is easily available, cheaper and less toxic than synthetic drugs. In the present investigation, solvent and aqueous extracts of *Tinospora cordifolia* and *Cassia fistula* were evaluated for exploration of their antimicrobial activity against certain Gram negative and Gram positive human pathogenic bacteria. Susceptibility of each plant extract was tested by serial microdilution method and MIC and MBC values were determined. Aqueous and solvent extracts of both plant species have shown very high antimicrobial susceptibility against all seven bacterial strains at a very low concentration. More specifically, higher percent growth inhibition was obtained in presence of plant extracts in comparison to synthetic antibiotics. All different extracts have shown very low MIC value, which was found to be lower (0.0078-0.125 mg/ml) than the MIC values (0.223-0.892 mg/ml) obtained in presence of standard antibiotic drugs i.e. tetracycline, ampicillin and ciprofloxacin. It proves antimicrobial susceptibility of plant extracts. *Tinospora cordifolia* aqueous extract has shown lowest MIC value i.e. 0.00975 mg/ml in *E. coli*. Similar antimicrobial activity was reported by (Shashidharan et al., 2007) in leaves of *Stachyophytae majoralensis* against *E. coli*, *S. epidermidis* and *Pseudomonas aeruginosa* with an MIC value of 5.00 mg/ml. *Bixa orellana* (L) exhibited an MIC of 0.2 µg/ml against *B. cereus* (Rastogi and Mehrotra, 1999). Similarly *Cassia fistula* aqueous extract exhibited a significant antimicrobial activity against *Gram-ve* and *Gram+ve* bacteria. The results are summarized in Table 1-3. Such inhibitory effects of *C. fistula* can be attributed to the chemical substances present in the fruits/legumes (Rojas et al., 2006). It might be tannins and propyl gallate which are formed in ripening fruits and inhibit the growth of infectious microorganisms (Chung et al., 1998). Further, antimicrobial activity of plant extracts is strengthened by MIC and MBC values obtained against the bacterial strains. However, MBC values obtained in solvent and aqueous extracts of *Tinospora cordifolia* were in range of 0.0292-0.39 mg/ml while these were 0.0156-0.39 mg/ml in *Cassia fistula* in same extracts. Further MBC values obtained in plant extracts were significantly much lower than broad spectrum antibiotics 0.892-7.17 mg/ml Again it proves antimicrobial potential of plant extracts.

Further, effectiveness of each plant extract was determined by agar disc diffusion method and inhibition zone diameters obtained in presence and absence of each extract. Based on growth inhibition zone diameter obtained bacterial strains were divided into three categories i.e. resistant (> 7 mm), intermediate (≥ 12 mm) and susceptible (> 18 mm). However, at a very low concentration (20-320 µg/disc) 15-32 mm inhibition zone diameters were obtained in chloroform, acetone, methanol, petroleum ether and aqueous extracts of both plant species (Table 3). These were significantly much larger than the antibiotic drugs i.e. tetracycline, ampicillin and ciprofloxacin which have shown inhibition zone diameters in a range of 6-18 mm against *K. pneumoniae*, *E. coli*, *B. cereus*, *M. luteus*, *L. acidophilus*, *S. aureus* and *S. pneumoniae* at the same concentration. Highest inhibition zone diameter 30 mm was obtained in aqueous extract of *Cassia fistula* against *M. luteus* (Table 3) at 320 µg/disc. Similar inhibition zone diameter (22 mm) was obtained in methanolic extract of *R. coraria* (Abdelrahim et al., 2002). Results clearly indicate that aqueous and solvent extracts of both plant species were found to be more effective against both Gram-ve and Gram+ve bacteria. Similarly *Psidium guajava* plant showed very strong antimicrobial activity against *Staphylococcus*, *Shigella*, *Salmonella*, *Bacillus*, *E. coli*, *Clostridium* and *Pseudomonas* (Abere et al., 2007; Kass et al., 2004) due to presence of tannins, phenols, lectins, saponins and flavonoids (Adenyi et al., 2008; Aijyegoro et al., 2008). Similar antimicrobial susceptibility was reported in leaf extract of *Mitracarpus scaber* (Azu and Onyeagba, 2007), *Capparis decidua* (Upadhyay et al., 2010a), bark extract of *Distemonanthus benthamianus* (Baill) (Zhao et al., 1991), *Allium cepa* and *Zingiber officinals* (Kumar et al., 2000) *Parthenium hysterophorus* *Linn* (Serremonte, 1991), and volatile components of *Phyllanthus emblica* (Yang et al., 2010), *Fortunella japonica* and *Citrus sunki* essential oils against skin pathogens (Nannapaneni et al., 2008).

From the present work, it can be stated that aqueous extracts of above plant species were found to be more potent which can efficiently reduce the contamination of pathogenic bacteria. There are two possible explanations for the observed antimicrobial activity. First, the components are water soluble and bioactive, second these are less soluble in organic solvents. It is the main reason behind their antimicrobial activity. In the present study, both plant species have shown nearly equal antimicrobial effects on both gram positive and gram-negative bacteria in suspension culture. It is further proved by inhibition zone diameters obtained in filter paper disc diffusion assays, which have shown better effectiveness of aqueous extracts against gram-positive bacteria. It shows the presence of some strong antimicrobial constituents belonging to the different groups (Batista et al., 1994; Scuzzochinio et al., 2001; Lambert et al., 2001), which might have very high permeability across the bacterial cell wall (Walsh et al., 2003; Innouye et al., 2001) due to ion leakage (Delequis et al., 2002) There might be another possibility that plant extract may successfully inhibit microbial respiration and increase the plasma membrane permeability, which results in to death of bacterial cells.
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REFERENCES


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