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Full Length Research Paper

Screening of antimicrobial potential of extracts and pure compounds isolated from *Capparis decidua*

Ravi Kant Upadhyay[1]*, Shoeb Ahmad[1], Rajani Tripathi[1], Leena Rohtagi[2] and Subhash C. Jain[3]

[1]Department of Zoology, D. D. U. Gorakhpur University, Gorakhpur 273009, India.
[2]Department of Chemistry, I. P. University, Delhi, India.
[3]Department of Chemistry, University of Delhi, Delhi 110007, India.

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In the present investigation antimicrobial activity of solvent and aqueous extracts of *Capparis decidua* was determined against seven bacterial strains that is *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). From the experiments chloroform, acetone, methanol and ether extracts of *Capparis decidua* have shown very high susceptibility to the *Lactobacilus* that is MIC value was obtained in a range of 0.028 - 0.0625 µg/ml, while aqueous extracts have shown lowest MIC value in a case of *Klebsiella pneumoniae* and *Micrococcus luteus*. Similarly MBC values of various extracts were also determined in similar strains. Chloroform extract has shown lowest MBC value for *Lactobacilus acidophilus* i.e. 0.125 µg/ml followed by intermediate MBC values against *Klebsiella pneumoniae* and *Escherichia coli* (0.25 µg/ml). Further effectiveness of various plant extracts was determined in disc diffusion assays. The sizes of growth inhibition zone diameters were noted in presence of solvent and aqueous extracts. The strength of activity was presented as resistant; intermediate and susceptible. The highest growth inhibition zone diameter was obtained in methanolic extract that is 36 mm in *Bacillus cereus*, 35 in *Klebsiella pneumoniae,* 36 in *Escherichia coli* and 31 in *S. aureus* at 4 µg/disc. Pure compounds CS2, CS3, CS8 and CDF1 isolated from *C. decidua* have shown higher antimicrobial susceptibility in comparison to broad spectrum antibiotic that is tetracycline, ampicillin and ciprofloxacin. These have shown significantly very high inhibition zone diameters that is 22 - 40 mm at 4 µg/disc in agar disc diffusion assay.

Key words: Minimum inhibitory concentration, minimum bactericidal concentration, *Capparis decidua.*

INTRODUCTION

In the past various plant extracts have been extensively used to cure infectious diseases and healing of wounds. As literature reveals plants have great potential against infectious agents and can be used for therapeutic purposes. But with time new chemical formulations such as broad-spectrum antibiotics have been explored and are massively used for fast recovery from diseases. All such chemotherapeutic agents generate wider side effects and cross reactivity due to their residual presence in the tissues, cells and body fluids.

Due to indiscriminate use these synthetic chemicals also suppress the immune resistance of the body to fight against the diseases and failed to control further incidence of microorganisms. Besides this pathogens are also making immunological identification of antigens /antibiotics and have attained resistance to these antibiotics. Therefore it becomes highly important to explore new sources of more active and potential antibacterial compounds/components/extracts of plant origin, which might be safe alternative of these synthetic drugs. So far studies have been done a number of plant-origin extracts and compounds have been screened for their antibacterial activities. These newly discovered non-antibiotic substances have shown very good fighting potential

*Corresponding author. E-mail: rkupadhya@yahoo.com.

against different drug resistant pathogens (Cowan, 1999; Ahmed and Beg, 2001).

Few plant species such as *Caesalpinia pulcherima* (Maheswara et al., 2006), *Camellia sinensis,* (Simmonetti et al., 2004), *Hymendityon parvifolium* (Kariba, 2002), *Viola tricolor savolii* (Witkowska-Banaszezak, 2005). *Grassmans sealer*, *Solidago microglossa* (Morel et al., 2006), *Terminalia arjuna* (Chaudhari and Mengi, 2006) and *Struchiun sparganophora* (Oboh, 2006) have been explored for antibacterial activities. Besides crude extracts, some plant products such as homoisoflavinoids, (Maheswara et al., 2006), *isoflavones* (Hong et al., 2006), di-terpenes (Haridy et al., 2006), and gamma thionin (Franco et al., 2006) were evaluated for their antibacterial activities against pathogenic bacteria. In addition to it antibacterial activity of sulphur rich compounds from *Scoorodophelous zenkeri* (Kouokam et al., 2002), *Satureja species* (Azaz et al., 2002), *Artemisia asiatica* (Kalemba and Kunicka, 2003) and *Mentha piperita* (Iscan et al., 2002) were also evaluated against certain pathogenic bacterial strains.

Similarly antimicrobial activity of some ethno medicinal plant extracts was observed by Duraipandiyan (2006), against *B. subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli, Klebsiella pneumonia Pseudomonas aeruginosa, Ervimia species* Methanol extract of *Albizia procera, Cassia auriculata, Punica granatum* and *Syzygium cunumin* show strong antibacterial activity was supported by Rajakaruna (2002), Similarly essential oils from the leaves of *Toddalia asciatica*, (Saxena and Sharma, 1999), *S. microglossa* (Morel et al., 2006) and *Cassia alata* have shown inhibitory activity against both Gram negative and Gram positive bacteria.

C. decidua is an indigenous medicinal plant, belongs to the family *Capparidaceae* (Raina, 1987) and commonly known as 'Kurrel' in Hindi. It is a densely branching shrub with scanty, small, caducous leaves. Barks, leaves and roots of *C. decidua* have been claimed to relieve variety of ailments such as toothache, cough, asthma, intermittent fever and rheumatism (Dhar et al., 1972). The powdered fruit of *C. decidua* is used in anti-diabetic formulations (Yadav et al., 1997), while the bark of its leafless shrub is used for the treatment of asthma, cough, inflammation and acute pain (Ahmad et al., 1992). Seeds of *C. decidua* showed antibacterial activity against *Vibrio cholerae ogava*, *inaba* and *eltor* (Gaind et al., 1972). In the present study antibacterial activity of solvent and aqueous extracts of *Capparis decidua* was established against seven pathogenic bacterial strains that is *S. aureus, Bacillus cereus, Streptococcus pneumoniae, Escherichia coli, Klebsiella pneumoniae Lactobacilus acidophilus* and *Micrococcus luteus.*

For evaluation of antimicrobial potential growth inhibition bioassays were conducted to determine the MIC and MBC values in bacterial culture. Besides this, growth inhibition zone diameters were also determined in presence of each extract.

MATERIALS AND METHODS

Preparation of extracts

For preparation of solvent and aqueous extracts of *C. decidua*, was collected from Agra in western U.P. The stem of green plant is dried, cut in to small pieces, milled and powdered by using pestle and mortar. The acetone, methanol, chloroform and petroleum ether extracts were prepared by using 50 g of dried powder of *Capparis decidua* stem 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, the powdered material was dissolved in distilled water and kept for solubilization overnight at room temperature.

Bacterial cultures

Cultures of seven pathogenic bacterial strains each of *Escherichia coli* (ATCC 25922), *Bacillus cereus* (ATCC 11778), *Lactobacilus acidophilus* (ATCC 53103), *Micrococcus luteus* (ATCC 9341), *S. 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 8^{th} day in solid agar plates.

Extraction

Stem and flower of *Capparis decidua* were collected from Agra in Uttar Pradesh, India. These were separately dried and subsequently pulverized to obtain fine powder. The powdered stems (200 g) and flowers (40 g) were then extracted using $CHCl_3$/MeOH (1:1) to obtain dry extracts.

Isolation and identification of active components from methanol, chloroform (1:1) extract of Capparis decidua

The solvent free extract CD1 [Capparis decidua] (14 g) was found to be a mixture of several compounds on TLC, and was therefore fractionated by column chromatography to isolate the pure active compound(s). The column was prepared in petroleum ether using silica gel (60-80) as an adsorbent and eluted with petroleum ether/chloroform, chloroform, chloroform/methanol mixtures of increasing polarity. Twelve fractions were obtained, from which three compounds viz. CS2, CS3 and CS8 have been isolated and characterized.

Compounds CS3 [*Capparis* stem] (25 mg) and CS8 (50 mg) were earlier identified (40) as triacontanol and 2-carboxy-1,1-dimethylpyrrolidine, while compound CS2 has been now characterized as heneicosylhexadecanoate and this is the first report of it's isolation from the genus *Capparis*.

Characterization of compound CS2

Compound CS2 was obtained as colorless oil. Its ^{1}H NMR spectrum revealed its aliphatic nature. Its IR spectrum showed absorptions at 1735 and 1175 cm^{-1} which were characteristic of C = O and C - O

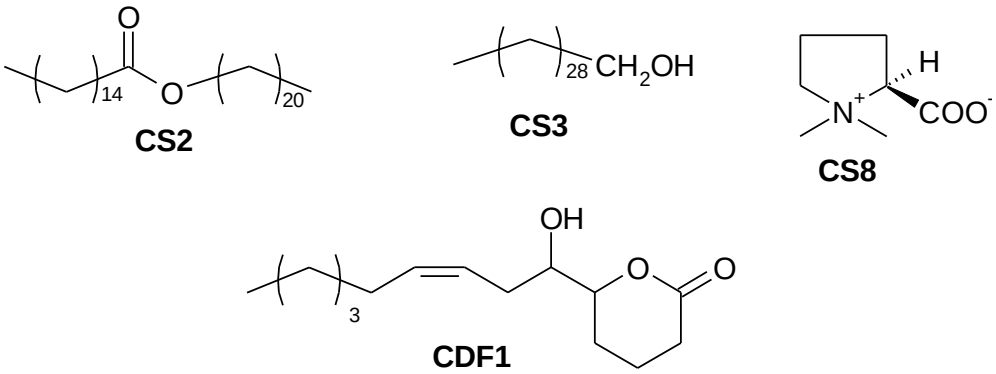

Figure 1. Chemical structures of CS2, CS3, CS8 and CDF1.

stretching of an ester linkage. Its ^{1}H NMR spectrum exhibited a triplet at δ 2.29 integrating for two protons of α-methylene linked to the carbonyl group. At δ 4.05, another triplet was observed which was assigned to the methylene directly attached to the oxygen atom. Apart from this, ^{1}H NMR spectrum exhibited a triplet at δ 0.88 corresponding to two terminal methyl groups indicating it to be a middle ester. Compound CS2 displayed a molecular ion peak at m/z 550 in EIMS and its elemental analysis suggested $C_{37}H_{74}O_2$ to be its molecular formula. Its mass spectrum also showed prominent peaks for McLafferty rearrangement at m/z 355 and 257 thereby revealing the presence of hexadecanoate as the acid moiety and heneicosane as the alcohol moiety in the molecule (Figure 1).

On the basis of above mentioned spectral data, compound CS2 was identified as heneicosylhexadecanoate. Heneicosylhexadecanoate (CS2) Compound CS2 was obtained as colorless oil (20 mg). It showed a single spot on TLC using petroleum ether as the developing solvent, R_f = 0.3 IR v_{max} (KBr): 2918, 2850, 1735, 1463, 1175, 758, 719 cm^{-1}. ^{1}H NMR (δ, $CDCl_3$, 300MHz): 0.88 (t, 6H, 2x-CH_3), 1.25 (m, -CH_2), 1.58 (m, 4H, -CH_2CH_2-C=O and -CH_2CH_2-O), 2.29 (t, 2H, -CH_2-C=O), 4.05 (t, 2H, -O-CH_2-). Elemental analysis: C, 80.34%; H, 13.38% (required C, 80.72%; H, 13.45%. Mass Spectral data, EIMS m/z(%): 550 (10, M^+), 453 (5), 355 (24), 257 (31), 239 (8, M^+-$O(CH_2)_{20}CH_3$), 202 (15), 174 (10), 111 (20), 97 (35), 71 (48), 57 (100), 41 (80).

Extraction of CD7

Extract CD7 (3 g) from *Capparis decidua* flowers, when checked on TLC, gave one major spot. It was subjected to column chromatography using silica gel (60-80) as an adsorbent and eluted with petroleum ether/chloroform, chloroform, chloroform /methanol mixtures of increasing polarity.

Isolation of CDF1

One major compound CDF1 (15 mg) was isolated from the chloroform fraction of the extract. It was earlier assigned constitution as 6-(1-hydroxy-non-3-enyl)-tetrahydropyran-2-one (Upadhyay et al., 2006).

Screening of antibacterial activity

Antimicrobial activity of *C decidua* extracts and pure compounds was evaluated by agar disc diffusion method. 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. These extract and ingredient coated discs dried under laminar flow cabinet. Before experiment inoculum size was determined and adjusted to prepare a final colony number as 10^8 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 parallel. All treated and untreated plates were incubated for 24 h at 37°C. The antibacterial activity was assessed on the size of the inhibition zone diameter obtained surrounding the filter paper discs.

Similar method was followed for testing antibacterial activity of pure compounds CS2, CS3, CS8 and CDF1. These pure compounds were solubilized in dichloromethane and their increasing concentrations were used for microbial testing. The antibacterial activity of these compounds was assessed both in broth dilution and agar disc diffusion assays.

Determination of MIC and MBC values

For determination of antimicrobial activity, bacterial growth inhibition was accessed in the presence of different increasing concentrations of *C decidua* extracts and pure compounds. For this purpose both crude extracts and pure compounds were separately diluted by using serial micro dilution method with Luria Broth culture medium at a final concentration range from 32.0 - 0.0078 µg/ml. The tested crude extracts and pure compounds were added to fresh suspension after following the serial dilutions up to $10^{-10.}$ 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 (Prescott et al., 1990). 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 h 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 inoculum size was adjusted to prepare a final colony number as10^8 colony forming units (CFU/ml in sterile agar plates. The incubation of test and control cultures was performed at 37°C for 24 h. 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

Table 1. Determination of MIC (µg/ml) of aqueous and solvent extracts of *Capparis decidua* against certain bacterial strains.

Fractions	*K. pneumoniae*	*E. coli*	*M. luteus*	*S. pneumoniae*	*S. aureus*	*B. cereus*	*L. acidophillus*
Chloroform	0.125	0.0625	0.25	0.5	0.125	0.25	0.125
Acetone	0.083	0.083	0.166	0.083	0.083	0.041	0.028
Methanol	0.05	0.25	0.20	0.20	0.0625	0.0625	0.0625
Ether	0.125	0.25	0.0625	0.0625	0.0156	0.25	0.0312
Aqueous	0.0156	0.0312	0.0156	0.0625	0.0625	0.125	0.0625
CS2	0.05	0.025	0.125	0.0125	0.010	0.05	0.0125
CS3	0.062	0.031	0.135	0.015	0.062	0.312	0.135
CS8	0.025	0.0125	0.0125	0.010	0.05	0.010	0.05
CDF1	0.032	0.062	0.016	0.065	0.025	0.010	0.012
Tetracycline	0.458	0.458	0.458	0.0458	0.458	0.915	0.458
Ampicillin	0.229	0.458	0.915	0.114	0.458	0.458	0.229
Ciprofloxacin	0.915	0.915	0.229	0.458	0.458	0.458	0.229

antibiotics and extracts) parallel controls were set up for each and every test extract. Bacterial growth was obtained in presence and absence of various concentrations of solvent and aqueous extracts of *C. decidua*.

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, various extracts and pure compounds. The related effectiveness was also tested by applying linear correlation between control and tests.

RESULTS

Determination of MIC and MBC values

In the present investigation MIC values of solvent and aqueous extracts of *C. decidua* were determined against seven pathogenic bacterial strains. Both solvent and aqueous extracts have shown very high anti-microbial susceptibility against all seven pathogenic bacterial strains. The lowest MIC value was recorded in presence of chloroform extract against *L. acidophilus* and *E. coli* that is 0.0312 and 0.0625 µg/ml. Similar extract was also found highly susceptible against *M. luteus, B. cereus, S. aureus, S. pneumoniae* and *K. pneumoniae* (Table 1). Similar results were obtained in presence of acetone extracts as the bacterial strains *E. coli* and *B. cereus* have shown MIC value i.e. 0.083 and 0.041 µg/ml respectively, while *K. pneumoniae* has shown 0.083 µg/ml. Highest MIC value was found against *M. luteus* i.e.0.166 which is less susceptible to the acetone extract of *C. decidua* (Table 1). Methanol extract of *C. decidua* has shown 0.25 µg/ml MIC value against *E. coli, M. luteus* and *S. pneumoniae* while it has shown 0.0625 µg/ml against *S. aureus, B. cereus* and *L. acidophilus* bacterial strains (Table 1).

In the presence of ether extract MIC value obtained was 0.0625 µg/ml against *M. luteus* and *S. pneumoniae,* while it was 0.0156 against *S. aureus*, 0.25 for *B. cereus*, 0.0312 µg/ml for *L. acidophilus*. Among all these bacterial strains *S. aureus* has shown high susceptibility to the ether extract. Among solvent extracts lowest MIC value was obtained in acetone extract against *B. cereus,* that is, 0.041 µg/ml. Higher MIC value in presence of aqueous extracts was obtained in *B. cereus* 0.125 µg/ml. In aqueous extract the MIC value of *K. pneumoniae* and *M. luteus* was similar, that is, 0.0156 µg/ml, while for *E. coli* it was 0.0312 µg/ml. In case of *L. acidophilus* and *S. aureus,* the MIC value obtained was similar that is 0.0625 µg/ml.

Besides MIC values, MBC values of solvent and aqueous extracts were also determined. The MBC value for chloroform extract was higher for *S. pneumoniae, S. aureus* and *B. cereus* that is 1.0 µg/ml. The lowest MBC value was obtained for *L. acidophilus,* that is, 0.125 µg/ml while for *K. pneumoniae, M. luteus* and *E. coli* it is obtained in a range of 0.25 - 0.5 µg/ml. MBC value of acetone extract was similar for *K. pneumoniae, M. luteus, S. aureus* and *B. cereus* that is 0.665 µg/ml. In methanol extract the lowest MBC value was obtained. For ether extract higher MBC value was obtained in *B. cereus* and *E. coli* that is 0.5 µg/ml, while lowest MBC value was obtained against 0.0312 µg/ml against *S. aureus* (Table 2). Aqueous extract has shown lowest MBC value for *E. coli* that is 0.0625 µg/ml, while it was obtained higher in *M. luteus, B. cereus,* and *L. acidophilus* that is 0.25 µg/ml. MBC value of *K. pneumoniae, S. pneumoniae* and *S. aureus* was similar that is 0.125 µg/ml.

Besides this, growth inhibition zone diameters in presence of solvent and aqueous extracts of *C. decidua* were also determined by agar disc diffusion method. In presence of chloroform extract growth inhibition zone diameter was obtained 25 mm for *K. pneumoniae*, 24 mm

Table 2. Determination of MBC (µg/ml) values of aqueous and solvent extracts of *Capparis decidua* against certain bacterial strains.

Fractions	*K. pneumoniae*	*E. coli*	*M. luteus*	*S. pneumoniae*	*S. aureus*	*B. cereus*	*L. acidophillus*
Chloroform	0.25	0.25	0.5	1.0	1.0	1.0	0.312
Acetone	0.665	0.083	0.665	0.332	0.665	0.665	0.332
Methanol	0.10	0.010	0.25	0.25	0.20	0.125	0.25
Ether	0.25	0.5	0.125	0.25	0.0312	0.5	0.0625
Aqueous	0.125	0.0625	0.25	0.125	0.125	0.25	0.25
CS2	0.15	0.20	0.25	0.10	0.12	0.20	0.80
CS3	0.10	0.50	0.15	0.16	0.12	0.25	0.62
CS8	0.20	0.25	0.25	0.10	0.20	0.30	0.45
CDF1	0.12	0.20	0.12	0.20	0.32	0.20	0.25
Tetracycline	0.915	0.915	0.732	0.915	0.915	1.83	0.915
Ampicillin	1.83	1.83	0.372	0.915	0.915	1.83	0.915
Ciprofloxacin	1.83	1.83	0.915	0.915	0.915	1.83	3.66

Table 3. The quantitations of microbial activity of different aqueous extracts of *Capparis decidua* were measured by agar diffusion method. The effectiveness of different extracts is demonstrated by the size of the micro-organism growth inhibition zone around the filter paper disc, which is typically expressed as the diameter of the zone in mm.

Fractions	Conc. µg /disc	*K. pneumoniae*	*E. coli*	*M. luteus*	*S. pneumoniae*	*S. aureus*	*B. cereus*	*L. acidophillus*
Chloroform	4	25	24	20	23	20	22	17
Acetone	4	24	25	20	22	20	20	26
Methanol	4	31	46	35	25	22	36	26
Ether	4	22	21	19	20	20	19	17
Aqueous	4	31	27	25	23	24	22	25
CS2	4	34	27	29	33	30	36	39
CS3	4	38	26	30	31	25	32	27
CS8	4	33	36	32	30	22	28	27
CDF1	4	40	32	36	32	36	33	31
Tetracycline	8.0	20	19	24	23	24	25	24
Ampicillin	8.0	15	15	20	23	16	18	20
Ciprofloxacin	8.0	24	23	22	24	26	23	24

Susceptibility 12 represent resistant, 13-22-represent intermediate and ≥ 23 represent susceptibility.

for *E. coli*, 20 mm for *M. luteus*, 23 mm for *S. pneumoniae*, 20 mm for *S. aureus*, 22 mm for *B. cereus* and 17mm for *L. acidophilus.* In acetone extract the highest inhibition zone diameter obtained was 20 mm in *M. luteus, S. aureus* and *B. cereus* while rest of the bacterial strains have shown inhibition zone diameter between 20 - 25 mm. Methanol extract has shown higher growth inhibition as zone diameter obtained was 36 mm against *B. cereus*, *K. pneumoniae* 31 mm, *E. coli* 46 mm, *S. aureus* 22 mm, *S. pneumoniae* 25mm *L. acidophilus* 26 mm respectively. In presence of ether extract inhibition zone diameter was obtained between 17 - 22 mm against above pathogenic bacterial strains (Table 3). Similarly aqueous extract has shown growth inhibition zone between 22 - 27 mm against other bacterial strains. From the results aqueous extracts exhibited high susceptibility to these bacterial strains (significant at $p < 0.05$). Susceptibility was tested by applying ANOVA and most significant variance was obtained in methanol extract when compared with susceptibility obtained in presence of broad spectrum antibiotics (variance 48.55 - 83.97). Similar variance was obtained in CS2, CS3, CS8 and CDF1 compounds. When antimicrobial susceptibility of extracts and pure compounds was applied on Student t-test, again methanol extract has shown higher susceptibility in comparison to broad spectrum antibiotics ($p = 0.0074$). Besides this, pure organic compounds CS3 and CS8 have shown significant antimicrobial susceptibility. When data was applied for linear correlation it shows dose and time dependent susceptibility as the correlation was obtained in a range of 0.151 - 0.842.

In comparison to solvent extracts, pure compounds

CS2, CS3 CS8 and CDF1 have shown lower MIC that is 0.05, 0.06, 0.025 and 0.032 µg/ml respectively (Table 1). Similarly MBC values also shown lower values in comparison to above compounds. Results show MBC values in different pure compounds that is 0.15, 0.10, 0.20 and 0.12 µg/ml in CS2, CS3, CS8 and CDF1 respectively. When the above fractions and compounds were tested for their antimicrobial activity by agar disc diffusion method it have shown higher inhibition zone diameter in comparison to broad-spectrum antibiotics. Synthetic antibiotics such as tetracyclin, ampicilin and ciprofloxacin have shown lower disc diffusion diameters at 8 µg concentration in agar disc diffusion assays.

DISCUSSION

Drug resistance in microbes is a very serious problem. It is induced due to indiscriminate use of antibiotics. Therefore conventional drugs become failed to control pathogenic infection. However, new alternatives of synthetic drugs are highly recommended *in vitro* and have high demand. Synthetic drugs such as antibiotics after entering inside the body, generated so many biochemical aberrations and side effects in patients. Therefore, effective drug dose level of these broad spectrum antibiotics has been increased manifold. Besides this, antibiotics put un-favorable impact on the body and show cross reactivity inside the body fluid, and widely inhibit bio-membrane functioning. Normally during their life cycle, plants encounter various infections caused by viruses, bacteria, fungi and other parasites and synthesize a variety of secondary metabolites capable of providing them protection against these infectious agents.

Hence, plants are considered as valuable source of medicinal compounds with proven potential of treating infectious diseases and show lesser side effects than the synthetic drugs.

In the present study both solvent and aqueous extracts of *C. deciduas* have shown very low MIC and MBC values which are presented in Tables 1 and 2. Chloroform extract of *C. decidua* has shown least MIC value against *E. coli*, that is, 0.0625 µg/ml. More remarkable results were obtained in presence of pure compounds isolated from *C. decidua.* Pure compounds CS2, CS3 and CS8 from stem and CDF1 from flower have shown significantly lower MIC value (0.05, 0.0625, 0.025 and 0.032 µg/ml). Similarly few natural products such as gerontoxanthone isolated from *Calophyllum* (Sakagami et al., 2002) and terpenoids, enterosone and labdane isolated from *Sagittaria pygmaea* showed antimicrobial activity against *S. mutans* and *Actinomyces naeslundii* with MIC values between 62.5 and 125 mg /ml (Liu et al., 2007). Similar broad spectrum antimicrobial activity was obtained in *Trigonella foenum graecum* against pathogenic bacterial strains (Upadhyay et al., 2008).

Similarly very low MBC values were obtained in chloroform, acetone methanol, ether and aqueous extracts of *C. decidua* against *K. pneumoniae, E. coli. M. luteus, S. pneumoniae, S. aureus, B. cereus* and *L. acidophilus* (Table 2), MBC values obtained from each *C. decidua* extract were found 2 - 4 times higher than MIC values against all the bacterial strains. This suggests that the active components CS2, CS3, CS8 and CDf1 in *C. decidua* possess potent antimicrobial activity against all such bacterial strains. High amount of triacontanol present in *C. decidua stem* is also responsible for antibacterial activity. From the results it is much clear that both extracts and pure compounds have shown significantly much higher antimicrobial activity against all seven bacterial strains in comparison to synthetic antibiotics. Aqueous extract showed great anti-microbial potential which may be due to diffusion of toxic components through the bacterial cell wall after fludification.

Further, antimicrobial potential was noted in disc diffusion assays. When disc loaded with phytochemical extracts and pure compounds isolated from *C. decidua* were exposed to various bacterial strains in solid agar medium; these have shown significantly much larger inhibition zone diameters in comparison to synthetic antibiotics. However, all the tested bacterial strains in presence of pure compounds (4 µg/disc) gave significantly larger inhibition zone diameters in a range 22 - 40 mm (Table 3). It shows very high susceptibility of *C. decidua* natural products to Gram-positive and Gram-negative bacteria. Similar results were obtained in presence of solvent and aqueous extract of *C. decidua* (Table 3). Similar MIC values were obtained in *A. procera, C. auriculata, P. granatum, Syzgium cumin* (Duraipandiyan et al., 2006), *Indigofera scrutifosa* (Leite et al., 2006), *T. asciatica* (Saxena and Sharma, 1999), *S. microglossa* (Morel et al., 2006), *Cassia alata* and *H. parvifolium* (Hong et al., 2006). Similarly few medicinal plants such as *Terminalia avicenioides, Phylantus discoideu, Bridella ferruginea, Ageratum conyzoides, Ocimum gratissimum* and *Acalypha wilkesiana* have shown very high antimicrobial activity against menthicilin resistant *S. aureus* (Akinyemi et al., 2005). Similar antimicrobial activity was also found in bignoniaceae plant's extracts against Gram negative and Gram positive bacteria (Rsadah and Houghton, 1998).

It is finally confirmed by the results that *C. decidua* posses active components which have shown very low MIC and MBC values against microbes. Both pure compounds, aqueous and solvent extracts of *C. decidua* have shown significantly higher inhibition zone diameter (22 - 40 mm) in comparison to synthetic antibiotics (5 - 25 mm).

This anti-microbial potential is due to presence of mixed functional groups found in bio-organic com-pounds which are too complex in their structure and show diverse action mechanisms. More specifically, these natural

products may dislodge bacterial cell membrane and make it more permeable for water (Knobloch et al., 1986). It is also possible that these might coagulate some cell constituents and cause denaturation by ionic release.

In conclusion *C. decidua* extracts and active components have shown better bactericidal potential in comparison to antibiotics as it is proved by significantly very high growth inhibition obtained in various bioassays at a very low concentration. Therefore, it concluded that these active components can be used in formulation of new antimicrobials for therapeutic purposes.

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