Isolation of Bactericidal Constituents from the
Stem Bark Extract of *Grewia tiliaefolia* Vahl.

1B. Mohamed Khadeer Ahamed, 2V. Krishna, 3Harish B. Gowdru, 4H. Rajanaika,
5H.M. Kumaraswamy, 6Sharath Rajshekarrappa, 7Chethan J. Dandin and 8K.M. Mahadevan
1Department of Biochemistry, Basaveshwara Medical College,
Rajiv Ghandhi University, Chitrnadurga-577 502, Karnataka, India
2P.G. Department of Studies and Research in Biotechnology,
Kuvempu University, Shankaraghatta-577 451, Karnataka, India
3Department of Microbiology, Gulberga University, Gulberga, Karnataka, India
4P.G. Department of Studies and Research in Chemistry, Kuvempu University,
Shankaraghatta-577 451, Karnataka, India

Abstract: *Grewia tiliaefolia* (Tiliaceae) is a subtropical medicinal tree, the stem bark is widely used in traditional Indian medicines to cure pneumonia, bronchitis and urinary infectious disorders. Antibacterial activity of sequential extracts of the stem bark and constituents isolated from petroleum ether extract were screened against eighteen clinical strains belonging to *Pseudomonas aeruginosa* and *Klebsiella pneumonia* collected from hospitalized patients suffering from different kinds of infectious ailments. Two steroids β-sitosterol, stigmastereol and a triterpenoid lupeol were isolated from petroleum ether extract. The compounds were characterized by UV, IR, 1H NMR, 13C NMR and mass spectral studies. Minimum inhibitory concentrations of petroleum ether, chloroform and methanolic extracts were 120, 150 and 210 μg per 100 μL, respectively. Minimum inhibitory concentrations for isolated constituents β-sitosterol, stigmasterol and lupeol were 80, 70 and 30 μg per 100 μL, respectively. Among the three crude extracts and the three bioactive constituents tested, petroleum ether extract and the lupeol showed significant zones of inhibition in the cultures of both bacterial strains. The activity was moderate in steroids loaded wells. Results of this investigation provide a supportive scientific evidence for the medicinal use of *Grewia tiliaefolia*.

Key words: Antibacterial activity, *Grewia tiliaefolia*, clinical strains

INTRODUCTION

In recent times, the search for potent antibacterial agents has been shifted to plants. However, the major part of the search has focused mainly on higher plants (Oluwole et al., 2003) while little attention is given to lower plants with possible antimicrobial properties. It is estimated that there are about 2,500,000 species of higher plants throughout the world and most of them have not been examined in detail for their pharmacological activities (Jeevan Ram et al., 2004). However, for some decades, there has been increasing interest in plant uses and the detection of their constituents with antibacterial activity. The most important reason for the latter was that infections represent one of the main causes of illness and mortality around the world (Hamill et al., 2003). Many investigators have evaluated the bioactivity of plant extracts and the isolated constituents against the serious infectious organisms (Parekh and Sumitra, 2006).

Corresponding Author: Dr. V. Krishna, Department of Biotechnology, Kuvempu University, Shankaraghatta, Karnataka, India Tel: +91-08252 256235, 9448 681856 Fax: +91-08252 256235
Infections due to multidrug-resistant gram-negative microorganisms pose an important clinical problem, resulting in significant morbidity and mortality worldwide (Hsueh et al., 2002; Livermore, 2002). *Pseudomonas aeruginosa* is an important gram-negative human pathogenic bacterium that causes nosocomial infections. In particular, the organism is a predominant respiratory pathogen among cystic fibrosis patients producing chronic pulmonary infection and progressive deterioration in lung infection (Van Delden, 2004; Van Delden and Iglewski, 2005). The increased prevalence of antibiotic resistant bacteria due to the extensive use of antibiotics may render the current antimicrobial agents insufficient to control the bacterial diseases (Cowan, 1999; Bax et al., 2000).

*Grewia tiliaefolia* is a subtropical tree belonging to the family Tiliaceae and is abundantly distributed in the Western and Eastern Ghats of India. The stem bark is acrid with a sharp and sweetish taste. Medicinally it is used to heal the infectious wounds, to cure the cough, throat complaints, dysentery and other infectious diseases (Kirthikar and Basu, 1975). The traditional medical practitioners residing in the vicinity of forests of Thritharameshwar range and Ubbrani range of the Western Ghats of Karnataka, India, are using the stem bark extracts and pastes for curing pneumonia, bronchial infections, urinary infections and to heal old wounds. The earlier investigators (Badami et al., 2003; Badami et al., 2004) isolated lupinel from the methanol extract of stem bark of *Grewia tiliaefolia* and evaluated the cytotoxic properties on in vitro cell lines.

The present investigation reports the isolation of two steroidal compounds β-sitosterol, stigmasterol and one pentacyclic triterpenoid lupinel from the petroleum ether extract of *Grewia tiliaefolia* stem bark. The antibacterial activity of the different extracts and isolated constituents was screened against clinical strains of *Pseudomonas aeruginosa* and *Klebsiella pneumonia*.

**MATERIALS AND METHODS**

**Plant Collection and Authentication**

The stem bark and flowering twigs of *Grewia tiliaefolia* were collected from the forests of Thritharameshwar range of the Davangere District of Karnataka, India, on 20th March 2005. The plant was identified by the senior author and authenticated by comparing with the Voucher specimen (No. FDD 929) deposited at the Botany Department, Kuvempu University (Manjunatha et al., 2004).

**Preparation of the Extracts**

Five hundred grams of shade dried and powdered stem bark was extracted successively with the solvents petroleum ether, chloroform and methanol for 24 h each by using Soxhlet apparatus. The extracts were concentrated under reduced pressure at 40±5°C using rotary flash evaporator (Büchi, Flawil, Switzerland). The yield of petroleum ether, chloroform and methanolic extracts was 5.40, 2.25 and 40.60 g, respectively.

**Phytochemical Studies**

The extracts were subjected to qualitative phytochemical tests for the screening of various secondary metabolites (The Ayurvedic Pharmacopoeia of India, 1990). The petroleum ether extract was subjected to column chromatography for the isolation of constituents. The extract (5 g) was dissolved in chloroform 10 mL and adsorbed onto neutral alumina (20 g). After evaporation of the solvent it was loaded onto a neutral alumina column (150 g) built in petroleum ether (60-80°C). The column was eluted with petroleum ether (60-80°C), 10, 25 and 50% benzene: petroleum ether (60-80°C), benzene: petroleum ether (60-80°C) graded mixture (50:50) then with C6H6. The elutions were monitored on TLC (Silica Gel G, visualization; vanillin-sulfuric acid, heated at 110°C).

The 25 and 50% benzene in petroleum ether eluates gave two identical spots on TLC (C6H6: EtoAc, 85:15). These were combined, concentrated to about 5 ml and left overnight, when it yielded
pearl white crystals which responded to Liebermann-Burchard test and Salkowski’s test for steroids. These were separated by preparative TLC using silver nitrate impregnated silica gel (C18, EtOAc, 85:15) into two compounds, recrystallized from benzene and designated as compound I (56 mg) and compound II (42 mg), respectively.

Elutions carried out with petroleum ether (60-80°C). benzene graded mixture (50:50) resulted in a single component. After removing the solvent, a residue resulted which was isolated as pure component by recrystallising with petroleum ether. The product was designated as compound III (38 mg).

The eluates were monitored by TLC and were characterized by spectral studies. The UV spectra were recorded on a Shimadzu UV-240 spectrophotometer. IR Spectra were recorded with KBr pellets on a Perkin-Elmer 1710 FT-IR spectrophotometer. The 1H and 13C NMR spectra were obtained on a Bruker AMX (400 MHZ) Spectrometer and the FAB mass spectra were recorded on a JEOL SX102 Mass Spectrometer.

**Bacterial Strains**

Eighteen bacterial strains belong to two Gram negative species *Pseudomonas aeruginosa* and *Klebsiella pneumonia* were collected from different infectious clinical specimens of the hospitalized patients, district health center Gulberga, Karnataka, India. The clinical isolates were cultured and identified following the standard method (Cowan and Steel, 1993) and were preserved in agar slants. In addition to these clinical strains *Pseudomonas aeruginosa-ATCC-20852* and *Klebsiella pneumonia-MTCC-618* were also used as test organisms.

**Antibacterial Assays**

**Preparation of Media and Inoculation**

The agar well diffusion method was followed for the screening of antibacterial activities of the crude extracts and isolated constituents (Perez et al., 1990; Nair et al., 2005). All the clinical strains were incubated at 35±0.1°C for 24 h by inoculation into nutrient broth (Difco). The molten nutrient agar was inoculated with 100 μL of the inoculum (1×10^8 cfu mL^-1) and poured into the Petri plate (Hi-media). After the medium was solidified, a well was made in the plates with the help of a cup-borer (0.85 cm). The test compound was introduced into the well and the Petri plates were incubated at 35±0.1°C for 18-24 h. The crude extracts and purified bioactive constituents were dissolved in 10% aqueous dimethyl sulfoxide (DMSO; that enhances compound solubility) to get stock solutions. Commercial bactericide Ciprofloxacin was used as standard (100 μg per 100 μL of sterilized distilled water) concomitantly with the test samples. The diameters of inhibition zones (in mm) were determined and data was statistically evaluated by Tukey’s fair wise comparison test.

**Minimum Inhibitory Concentration (MIC) of Extracts and Bioactive Compounds**

The Minimum Inhibitory Concentrations (MIC) of the crude extracts and the isolated constituents were determined by micro-dilution techniques in nutrient broth, according to Clinical and Laboratory Standards Institute (CLSI), USA guidelines. The inoculates were prepared in the same medium at a density adjusted to a 0.5 McFarland turbidity standard colony forming units and diluted 1:10 for the broth micro dilution procedure. The microtiter plates were incubated at 37°C and MIC was determined after 24 h of incubation. MIC of the crude extracts was determined by dilution of petroleum ether, chloroform and methanolic extracts to 0.0-120, 0.0-150 and 0.0-210 μg per 100 μL, respectively. MIC of pure compounds isolated from petroleum ether extract was determined by dilution of β-sitosterol, stigmasterol and lupeol to 0.0-80, 0.0-70 and 0.0-30 μg per 100 μL, respectively (National Committee for Clinical Laboratory Standards, 1999).
RESULTS

Characterization and Structural Elucidation of Isolated Compounds

Analysis of Compound I

Pearl white crystalline compound gave red colour in Salkowski’s test and a green colour in Liebermann-Burchard test indicates the compound is sterol. Rf value is 0.38 (solvent system; C6H6: EtOAc, 85:15). The melting point (mp) of the compound is 138-140°C. The molecular formula and structure of compound was established by spectroscopic data.

Spectral Characteristics of Compound I

IR (KBr)
3430.1 cm⁻¹ (br, OH)
2938.9 cm⁻¹ (C-H str. in CH₃)
1637.8 cm⁻¹ (C = C str.)
1463.4 cm⁻¹ (C-H deformation in CH₃)
1377.5 cm⁻¹ (C-H deformation in gem dimethyl)
1051.7 cm⁻¹ (C-O str. of secondary alcohol)

1H NMR (CDCl₃)
δ 0.64 to 1.006 (18 H, 6 X CH₃)
δ 1.029 to 1.23 (22 H, 11XCH₂)
δ 1.44 to 2.28 (m, 8H, methine protons)
δ 3.5 (m, 1H,OH)
δ 5.4 (m, 1H, Vinylic proton).

13C NMR (CDCl₃)
δ 145 (C-5), δ 121 (C-6), δ 56 (C-17),
δ 50 (C-13), δ 45 (C-13), δ 40 (C-4), δ 36 (C-22), δ 37 (C-5), δ 33(C-1), δ 35 (C-8),
δ 34 (C-23), δ 35.1 (C-10), δ 32 (C-16),
δ 33 (C-7), δ 29.12 (C-25), δ 30.25 (C-24), δ 30 (C-14), δ 28.24 (C-27),
δ 28 (C-29), δ 31.6 (C-26), δ 78.7 (C-28).

Mass spectra (EI-MS)
Molecular formula : C₅₀H₆₀O
Molecular weight : 414
EI-MS (m/z) : 414 (M⁺, C₅₀H₆₀O, 54%), 397 (18%), 329 (12%),
303 (10%), 288 (4%), 273 (10%), 255 (M⁺-Side chain + H₂O, 6%), 231 (10%), 199 (20%), 161 (30%), 147 (34%), 133 (24%),
105 (50%), 91 (76%), 71 (44%), 57 (100%).

From mp, IR, 1H NMR, 13C NMR and mass spectral data, compound-I was identified as β-sitosterol (Fig. 1). It was further confirmed by co-chromatography with an authentic sample (sigma chemical company, USA) on silica gel, solvent system C₆H₆:EtOAc, 85:15, spraying reagent; vanillin-sulphuric acid, heated at 110°C.

Analysis of Compound II

Pearl white crystalline compound gave red colour in Salkowski’s test and a green colour in Liebermann-Burchard test indicates the compound is sterol. Rf value is 0.8 (solvent system; C₆H₆: EtOAc, 85:15). The melting point of the compound is 167-170°C. The molecular formula and structure of compound was established by spectroscopic data.
Fig. 1: Structure of β-sitosterol

**Spectral characteristics of compound II**

**IR(KBr)**
- 3351.63 cm⁻¹ (br, OH)
- 2919.38 cm⁻¹, 2863.81 cm⁻¹ (C-H str. in CH₃ and CH₂)
- 1665.87 cm⁻¹ and 1634.99 cm⁻¹ (C=C str.)
- 1462.09 cm⁻¹ (C-H deformation in gem dimethyl)
- 1054.55 cm⁻¹ (C-O str. of secondary alcohol).

**¹H NMR (CDCl₃)**
- δ 0.714 to δ 1.046 (m, 18H, 6xCH₃)
- δ 1.14 to δ 2.28 (m, 18H, 9 x CH₃ and 8 H, CH protons)
- δ 3.53 (br, 1H, CHOCH)
- δ 5.36 (s, 1H, vinylic proton)
- δ 5.05 (J = 27 Hz) and δ 5.14 (J = 27Hz) (2H, br, olefinic proton).

**¹³C NMR (CDCl₃)**
- δ145 (C-5), δ138 (C-22), δ128 (C-23),
- δ121 (C-6), δ78 (C-3), δ56 (C-17),
- δ50 (C-20), δ51 (C-13), δ40 (C-4),
- δ38 (C-12), δ37 (C-15), δ33 (C-1),
- δ35 (C-8), δ35.1 (C-10), δ32 (C-18),
- δ32.5 (C-7), δ29.12 (C-25), δ30.25 (C-24), δ30 (C-14), δ28.24 (C-27),
- δ28 (C-29), δ31.6 (C-26), δ19.8 (C-19), δ18.7 (C-21), δ18.24 (C-18),
- δ26 (C-2), δ18.8 (C-28).

**Mass spectra (EI-MS)**
- Molecular formula: C₂₉H₄₄O
- Molecular weight: 412
- EIMS (m/z): 412 (M⁺, C₂₉H₄₄O, 100%), 397 (12%), 369 (10%),
  351 (18%), 329 (4%), 300 (38%), 299 (1.4%),
  273 (M⁺-side chain, 10%), 255 (M⁺-side chain + H₂O, 30%).

From mp, IR, ¹H NMR, ¹³C NMR and mass spectral data, compound-II was identified as Stigmasterol (Fig. 2). It was further confirmed by co-chromatography with an authentic sample (sigma
Chemical Company, USA) on silica gel, solvent system C₆H₆:EtOAc, 85:15, spraying reagent, vanillin-sulphuric acid, heated at 110°C.

**Analysis of Compound-III**

White crystals isolated from petroleum ether extract showed positive result for Liebermann-Burchard test and indicates the compound is triterpenoid. Rₓ value is 0.71 (solvent system; petroleum ether: benzene 50:50). The melting point of the compound is 213-215°C. The molecular formula and structure of compound was established by spectroscopic data.

**Spectral Characteristics of compound-III**

**IR(KBr)**

- 3326.49 cm⁻¹ (br, OH)
- 2937.42 cm⁻¹, 2869.48 cm⁻¹ (C-H str. in CH₃ and CH₂)
- 1640.51 cm⁻¹ (C=O str.)
- 1449.07 cm⁻¹ (C-H deformation in CH₃/CH₂)
- 1374.96 cm⁻¹ (C-H deformation in gem dimethyl)
- 1041.47 cm⁻¹ (C-O str. of secondary alcohol)
- 890 cm⁻¹ (Exocyclic CH₂)

**¹H NMR (CDCl₃)**

- δ 0.7 to δ 1.026 (m, 18 H, 6X CH₃)
- δ 1.76 (s, 3H, -CH₃-C=CH₂) OH, 1 H)
- δ 3.322 (s, 1H, OH)
- δ 4.581 (s, 1 H, H-3)
- δ 4.700 (s, 1H, β-C-24H)
- δ 4.88 (s, 1H, α-C-24 H)
- δ 0.8 to δ 1.74 (m, 25H for CH₃ and CH protons)

**Mass spectra (EI-MS)**

- Molecular formula : C₉₆H₁₅₂O
- Molecular weight : 426

Mass spectrum shows molecular ion peak at m/z 426(15.2%). The other peaks appeared at 409(28.5%), 395(34.9%), 318(47.6%), 218(40%), 207(52.3%), 187(67.6%), 175(26.6%), 161(31.4%), 147(36.1%), 125(65.7%), 121(65.7%) and 81 (100%).
From the mp, IR, 1H NMR and mass spectral data, compound III was identified as lupeol (Fig. 3). It was further confirmed by co-chromatography with an authentic sample (sigma chemical company, USA) on silica gel, solvent system petroleum ether: benzene 50:50; spraying reagent; vanillin-sulphuric acid, heated at 110°C.

Antibacterial Evaluation

The profile of two bacterial species and their strains of different clinical origin are shown in Table 1. The zones of inhibition of the bacterial colonies are depicted in Table 2 and 3.

Among the crude extracts, the petroleum ether extract demonstrated good antibacterial activity against all the clinical strains of bacteria having inhibition zones of 8.7-17.8 mm for the strains of Pseudomonas aeruginosa and 6.5-11.2 mm for the strains of Klebsiella pneumonia. The chloroform and methanolic extracts were moderately effective against both the species of bacteria and their clinical isolates.

Fig. 3: Structure of lupeol

Table 1: Profile of the clinical strains used

<table>
<thead>
<tr>
<th>Clinical strains</th>
<th>Clinical condition</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P. aeruginosa</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pa1</td>
<td>Bronchitis</td>
<td>Sputum</td>
</tr>
<tr>
<td>Pa2</td>
<td>Otitis media</td>
<td>Ear swab</td>
</tr>
<tr>
<td>Pa3</td>
<td>Burns</td>
<td>Pus</td>
</tr>
<tr>
<td>Pa4</td>
<td>Upper UTI</td>
<td>Urine</td>
</tr>
<tr>
<td>Pa5</td>
<td>Suppurative infection in middle ear</td>
<td>Ear swab</td>
</tr>
<tr>
<td>Pa6</td>
<td>Food poisoning</td>
<td>Stool</td>
</tr>
<tr>
<td>Pa7</td>
<td>Cross infections in UTI</td>
<td>Urine</td>
</tr>
<tr>
<td>Pa8</td>
<td>Septicemia</td>
<td>Sputum</td>
</tr>
<tr>
<td>Pa9</td>
<td>Unknown</td>
<td>Hospital effluent</td>
</tr>
<tr>
<td><strong>K. pneumonia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kp1</td>
<td>Pneumonia</td>
<td>Mucus</td>
</tr>
<tr>
<td>Kp2</td>
<td>Gram negative Folliculitis</td>
<td>Stipules</td>
</tr>
<tr>
<td>Kp3</td>
<td>Burns</td>
<td>Pus</td>
</tr>
<tr>
<td>Kp4</td>
<td>UTI</td>
<td>Urine</td>
</tr>
<tr>
<td>Kp5</td>
<td>Septicemia</td>
<td>Sputum</td>
</tr>
<tr>
<td>Kp6</td>
<td>Cross infections in UTI</td>
<td>Urine</td>
</tr>
<tr>
<td>Kp7</td>
<td>Abscess in immunodeficiency</td>
<td>Wounds</td>
</tr>
<tr>
<td>Kp8</td>
<td>Upper UTI</td>
<td>Urine</td>
</tr>
<tr>
<td>Kp9</td>
<td>Unknown</td>
<td>Hospital effluent</td>
</tr>
</tbody>
</table>

*Pa = Clinical strain of Pseudomonas aeruginosa, Kp = Clinical strain of Klebsiella pneumonia*
Table 2: Antibacterial activity of the crude extracts and the isolated pure compounds of *Grewia tiliaefolia* bark against *Pseudomonas aeruginosa*  

<table>
<thead>
<tr>
<th>Clinical strains</th>
<th>P</th>
<th>C</th>
<th>M</th>
<th>L</th>
<th>BS</th>
<th>SS</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pw-1</td>
<td>17.8±0.2</td>
<td>10.5±0.3</td>
<td>10.5±0.1</td>
<td>33.0±0.9</td>
<td>25.5±0.5</td>
<td>12.3±0.5</td>
<td>26.6±1.7</td>
</tr>
<tr>
<td>Pw-2</td>
<td>10.6±0.3</td>
<td>6.1±0.1</td>
<td>10.1±0.3</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>31.4±0.6</td>
</tr>
<tr>
<td>Pw-3</td>
<td>8.7±0.3</td>
<td>4.7±0.2</td>
<td>12.2±0.1</td>
<td>ND</td>
<td>8.6±0.3</td>
<td>8.4±0.4</td>
<td>7.0±0.6</td>
</tr>
<tr>
<td>Pw-4</td>
<td>10.4±0.1</td>
<td>4.3±0.1</td>
<td>8.6±0.2</td>
<td>20.5±0.5</td>
<td>ND</td>
<td>ND</td>
<td>20.2±0.2</td>
</tr>
<tr>
<td>Pw-5</td>
<td>12.3±0.2</td>
<td>6.6±0.1</td>
<td>10.4±0.6</td>
<td>26.6±1.2</td>
<td>ND</td>
<td>11.6±0.2</td>
<td>18.2±0.9</td>
</tr>
<tr>
<td>Pw-6</td>
<td>12.8±0.2</td>
<td>6.8±0.2</td>
<td>11.7±0.3</td>
<td>24.6±1.2</td>
<td>11.9±0.2</td>
<td>ND</td>
<td>16.7±0.3</td>
</tr>
<tr>
<td>Pw-7</td>
<td>12.0±0.4</td>
<td>8.7±0.3</td>
<td>10.1±0.2</td>
<td>23.0±0.8</td>
<td>11.1±0.4</td>
<td>8.4±0.3</td>
<td>17.6±0.4</td>
</tr>
<tr>
<td>Pw-8</td>
<td>12.0±0.1</td>
<td>7.1±0.3</td>
<td>10.7±0.2</td>
<td>ND</td>
<td>10.5±0.4</td>
<td>9.2±0.3</td>
<td>18.4±0.5</td>
</tr>
<tr>
<td>Pw-9</td>
<td>10.3±0.5</td>
<td>7.4±0.2</td>
<td>9.4±0.2</td>
<td>25.7±0.4</td>
<td>9.2±0.3</td>
<td>9.2±0.8</td>
<td>18.2±0.5</td>
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<tr>
<td>E-value</td>
<td>63.1</td>
<td>69.0</td>
<td>10.6</td>
<td>248.7</td>
<td>60.2</td>
<td>431.1</td>
<td>101.6</td>
</tr>
</tbody>
</table>

*a* Clinical strains of *Pseudomonas aeruginosa* from different clinical sources. *b* The values are the mean of three experiments±S.E. of the mean. ND = Represents not done, P = Petroleum ether extract (5 mg mL⁻¹), C = Chloroform extract (5 mg mL⁻¹), M = Methanolic extract (5 mg mL⁻¹), L = Lupeol (1 mg mL⁻¹), BS = β-sitosterol (1 mg mL⁻¹), SS = Stigmasterol (1 mg mL⁻¹) and S = standard reference drug Ciprofloxacin (1 mg mL⁻¹)

Table 3: Antibacterial activity of the crude extracts and the isolated pure compounds of *Grewia tiliaefolia* bark against *Klebsiella pneumonia*  

<table>
<thead>
<tr>
<th>Clinical strains</th>
<th>P</th>
<th>C</th>
<th>M</th>
<th>L</th>
<th>BS</th>
<th>SS</th>
<th>S</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kp-1</td>
<td>6.5±0.1</td>
<td>4.5±0.18</td>
<td>8.6±0.3</td>
<td>16.6±0.4</td>
<td>8.4±0.3</td>
<td>9.1±0.4</td>
<td>15.6±0.3</td>
</tr>
<tr>
<td>Kp-2</td>
<td>8.7±0.2</td>
<td>4.3±0.1</td>
<td>6.5±0.1</td>
<td>9.3±0.5</td>
<td>5.8±0.8</td>
<td>9.1±0.4</td>
<td>16.8±0.2</td>
</tr>
<tr>
<td>Kp-3</td>
<td>ND</td>
<td>3.0±0.1</td>
<td>8.6±0.1</td>
<td>8.4±0.4</td>
<td>ND</td>
<td>8.5±0.4</td>
<td>18.9±0.2</td>
</tr>
<tr>
<td>Kp-4</td>
<td>6.8±0.2</td>
<td>6.2±0.3</td>
<td>8.2±0.0</td>
<td>22.4±0.7</td>
<td>8.7±0.4</td>
<td>12.8±0.3</td>
<td>17.7±0.2</td>
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<tr>
<td>Kp-5</td>
<td>7.4±0.2</td>
<td>6.0±0.1</td>
<td>9.7±0.2</td>
<td>17.2±0.5</td>
<td>8.8±0.5</td>
<td>11.4±0.3</td>
<td>16.0±0.1</td>
</tr>
<tr>
<td>Kp-6</td>
<td>8.3±0.0</td>
<td>5.3±0.1</td>
<td>9.6±0.3</td>
<td>19.7±0.4</td>
<td>ND</td>
<td>10.8±0.3</td>
<td>17.4±0.0</td>
</tr>
<tr>
<td>Kp-7</td>
<td>11.2±0.3</td>
<td>5.0±0.1</td>
<td>8.5±0.2</td>
<td>8.5±0.4</td>
<td>8.7±0.5</td>
<td>9.1±0.5</td>
<td>16.1±0.1</td>
</tr>
<tr>
<td>Kp-8</td>
<td>9.3±0.1</td>
<td>5.1±0.1</td>
<td>6.9±0.2</td>
<td>13.6±0.5</td>
<td>7.0±0.4</td>
<td>8.3±0.4</td>
<td>18.3±0.2</td>
</tr>
<tr>
<td>Kp-9</td>
<td>16.6±0.2</td>
<td>5.3±0.1</td>
<td>7.7±0.2</td>
<td>14.8±0.5</td>
<td>8.4±0.4</td>
<td>10.3±0.3</td>
<td>16.8±0.2</td>
</tr>
<tr>
<td>E-value</td>
<td>238.9</td>
<td>34.5</td>
<td>20.1</td>
<td>91.7</td>
<td>60.0</td>
<td>13.8</td>
<td>22.2</td>
</tr>
</tbody>
</table>

*a* Clinical strains of *Klebsiella pneumonia* from different clinical sources. *b* The values are the mean of three experiments±S.E. of the mean. ND = Represents not done, P = Petroleum ether extract (5 mg mL⁻¹), C = Chloroform extract (5 mg mL⁻¹), M = Methanolic extract (5 mg mL⁻¹), L = Lupeol (1 mg mL⁻¹), BS = β-sitosterol (1 mg mL⁻¹), SS = Stigmasterol (1 mg mL⁻¹) and S = standard reference drug Ciprofloxacin (1 mg mL⁻¹)

![Fig. 4: Bactericidal effect of pure compounds and Ciprofloxacin on *Pseudomonas aeruginosa* (ATCC-20852) and *Klebsiella pneumonia* (MTCC-618). L = Lupeol (1 mg mL⁻¹), BS = β-sitosterol (1 mg mL⁻¹), SS = Stigmasterol (1 mg mL⁻¹) and S = Standard reference drug Ciprofloxacin (1 mg mL⁻¹) (79)](image-url)
Among the purified bioactive components (Fig. 4), lupeol a triterpenoid proved to be potent bactericidal agent against both species of bacteria (24.1 mm for Pseudomonas aeruginosa and 18.1 mm for Klebsiella pneumonia) and their clinical strains, since it showed efficient activity as compare to that of reference drug Ciprofloxacain (22.7 mm Pseudomonas aeruginosa for and 18.9 mm for Klebsiella pneumonia). The other isolated constituents demonstrated significant inhibitory activity than the crude extract.

DISCUSSION

For a long period of time, plants have been a valuable source of products to treat a wide range of medical problems, including ailments caused by microbial infection. Numerous studies have been carried out in different parts of the globe to extract plant products for screening antibacterial activity (Ravikumar et al., 2005; Essawi and Stour, 2000). Plants produce highly bioactive molecules that allow them to interact with other organisms in their environment. Many of these substances are important in the defense against herbivores and contribute to the resistance to disease (Cowan, 1999).

Antibacterial activity of different plant extracts against Pseudomonas aeruginosa and Klebsiella pneumonia has been reported earlier by several authors who did not include Grewia tiliaefolia in their studies. The earlier investigators (Badami et al., 2003; Badami et al., 2004) isolated two triterpenoids; betulin and lupeol from the Grewia tiliaefolia stem bark by HPTLC method. They evaluated the cytotoxic properties of constituents on various types of cell lines under in vitro condition. Nonetheless, most general cytotoxins can be legitimately called antimicrobial under conditions of in vitro testing. The constituent lupeol and some of its derivatives lupeol long chain fatty acid esters isolated from Holarrhena floribunda exhibited an excellent antimalarial activity (Fotie et al., 2006). In the present investigation a triterpenoid lupeol and two steroids β-sitosterol and stigmasterol were isolated from petroleum ether extract of stem bark of Grewia tiliaefolia. For the first time we reported the isolation of β-sitosterol and stigmasterol from the stem bark of Grewia tiliaefolia. The compounds were characterized with the help of mechanistic spectral data. In the present study the petroleum ether, chloroform, methanolic extracts and the constituents isolated from petroleum ether extract were concurrently screened for antibacterial activity against human pathogenic medical isolates. The Pseudomonas aeruginosa has been implicated in infections of respiratory and urinary tract, suppurative infections in sinuses and middle ear; Septicemia etc., Klebsiella pneumonia has been associated with a range of clinical conditions, infections of wounds, infections of urinary tract and eyes; Septicemia etc.

Generally the Gram-positive bacteria should be more susceptible having only an outer peptidoglycan layer which is not an effective permeability barrier (Scherer and Gerhardt, 1971). Where as, the Gram-negative bacteria possess an outer phospholipidic membrane carrying the structural lipopolysaccharide components. This makes the cell wall impermeable to drug constituents. Because of the presence of multilayered peptidoglycan and a phospholipidic bilayer wall most of the Gram-negative bacteria showed multi drug resistant characteristics. In spite of these barriers the constituents of Grewia tiliaefolia were effective in controlling the growth of these pathogenic strains.

A good activity of the isolated compounds indicates that those compounds alone are solely responsible for antibacterial activity of the stem bark of Grewia tiliaefolia. Among the crude extracts tested against Gram-negative bacteria, the least polar one showed the greatest activity, suggesting a correlation between polarity and antibacterial activity of Gram-negative bacteria. One of the antimalarial studies of lupeol (Fotie et al., 2006; Ziegler et al., 2004) concluded that the antimalarial activity of this type of compounds was indirect, exclusively due to stomatocytic transformation of the host cell membrane and not to toxic effects via action on a drug target within the parasite (Ziegler et al., 2004).
Usually the targets of antibiotics would be DNA replication, protein synthesis or the rate limiting enzymes. To accomplish this task the antibiotics should be hydrophilic in nature in order to invade the bacterial cell. However, in case of hydrophobic drugs, a certain degree of lipophilicity might determine the toxicity by the interaction with the membrane constituents and their arrangement (Tomas-Barberan et al., 1990).

However, we cannot be certain of the reason for the efficient activity of the isolated compounds, since the drug-pathogen interaction studies are still to be confirmed by profound investigation.

CONCLUSION

Nearly all bioactive constituents isolated from petroleum extract of stem bark in our study demonstrated significant antibacterial activity against Gram-negative bacteria Pseudomonas aeruginosa and Klebsiella pneumoniae. Among all, lupeol exhibited more pronounced activity. Our results therefore offer a scientific basis for the traditional use of the stem bark of Grewia tiliaefolia for several infectious diseases.

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