



## Antibacterial activity of secondary metabolites from *Fumaria parviflora* Lam. (Fumariaceae)

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### ABSTRACT

We report the antibacterial activity of the plant extracts and three previously isolated known compounds viz., nonacosane-10-ol (alcohol), 23a-homostigmast-5-en-3 $\beta$ -ol (homolog of  $\beta$ -sterol) and *cis*- and *trans*-protopinim (alkaloid) of *Fumaria parviflora* Lam (Fumariaceae) for the first time. Plant extracts and pure compounds were *in vitro* assessed against seven clinical Gram (-) and Gram (+) bacteria viz. *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Salmonella typhi*. Bacterial culture was maintained on Mueller-Hinton agar nutrient slants and the tested strains were evaluated following well diffusion test, agar dilution method and minimum inhibitory concentration. The zone of inhibition (IZ) and the activity index (AI) were maximum for Gram (-) *Escherichia coli* (IZ = 28  $\pm$  0.9; AI = 0.93  $\pm$  0.3), *Klebsiella pneumonia* (IZ = 22  $\pm$  0.4; AI = 0.73  $\pm$  0.4) and *Salmonella typhi* (IZ = 22  $\pm$  0.4; AI = 0.73  $\pm$  0.9) and these bacteria were strongly sensitive (SS) to the *n*-hexane root extracts. The minimum inhibitory concentration value for the plant extracts was found to be in the range of 3.12 to 50.0 mg ml<sup>-1</sup>. The three compounds displayed strong antibacterial activity at a conc. of 100, 200 and 300  $\mu$ g ml<sup>-1</sup> against the tested strains. The zone of inhibition of these compounds ranged from minimum (9  $\pm$  0.9, AI = 0.3  $\pm$  0.5) for *Salmonella typhimurium* to maximum (46  $\pm$  0.9, AI = 1.53  $\pm$  0.3) for *E. coli*. The *cis*- and *trans*-protopinim was the most potent antibacterial compound against all the strains tested at the highest conc. of 300  $\mu$ g ml<sup>-1</sup>. The three compounds were completely bactericidal as measured by the viable cell count studies. *Fumaria parviflora* derived extracts and the phytochemicals particularly the *cis*- and *trans*-protopinim possess antibiotic properties and these compounds could be used in the development of novel chemotherapeutic agents.

**Keywords:** Antibacterial activity, Gram (+), *Escherichia coli*, *Fumaria parviflora*, modified well diffusion test (MWDT), minimum inhibitory concentration (MIC).

### INTRODUCTION

The use of herbs as complementary and alternative medicine has increased dramatically in the last 20-25 years.<sup>1</sup> Medicinal plants are genuinely useful for primary health care and they represent a rich source of antimicrobial agents.<sup>2</sup> The emergence of multiple drug resistance in microorganisms due to indiscriminate use of antibiotics has greatly generated the interest in herbal medicines. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs.<sup>3</sup> A wide range of medicinal plants have been used for extracts as raw drugs. A large number of plant extracts on bacteria have been reported with antibacterial activities.<sup>4</sup> Phytochemical and pharmacological investigations of a number of plants have already led to the isolation of some of the natural antimicrobials agents.<sup>5</sup> Although a large number of plant species have been evaluated for their antimicrobial properties, the vast majority of it has not been amply explored.<sup>2</sup>

The family Fumariaceae consists of 18 genera and 450 species globally, mainly in North America, Europe, Asia and Africa.<sup>6</sup> *Fumaria parviflora* Lam (Fumariaceae) locally known as 'Papra' or 'Pitpapra' in Pakistan, is a small, annual medicinal weed growing wild in agricultural wheat fields and lower hills.<sup>7</sup> The anthelmintic, antipyretic,

hepatoprotective and hypoglycemic properties of the medicinal plant are well documented.<sup>8</sup> Locally the plant has been used as an antidiarrhetic, blood purifier, cholagogue, diaphoretic, diuretic, laxative, stomachic and sedative tonic and is also considered useful to treat abdominal cramps, diarrhea, fever, jaundice, leprosy and syphilis.<sup>9</sup>

The plant has been reported to possess alkaloids, glycosides, flavonoids, tannins, saponins and phenols.<sup>10</sup> Many alkaloids such as, adlumidicine, coptisine, fumariline, parfumine, protopine,<sup>11</sup> fumaranine, fumaritine, paprafumicin, paprarine,<sup>12</sup> fumarophycine, cryptopine, sinactine, stylopinine, bicuculline, adlumine, perfumidine and dihydrosanguirine<sup>13</sup> have been reported from the plant. There is only couple of reports available on the anthelmintic.<sup>8</sup> Previously we reported the nematocidal activity of *F. parviflora* against the Southern root knot nematodes *Meloidogyne incognita* affecting tomato plants and detected seven bioactive classes of compounds.<sup>10</sup> However, reports on the antibacterial activity of *F. parviflora* are very scanty. The present study was undertaken to evaluate antibacterial activity of the extracts and the compounds of *Fumaria parviflora* against clinical pathogens.



## MATERIALS AND METHODS

All the chemicals (n-hexane, chloroform, methanol, ethyl acetate and dimethyl sulfoxide, standard antibiotics (Streptomycin) and thin layer chromatography (TLC) plates were purchased from Biosciences, Merck, Germany and all the solvents used were of analytical grade. All extracts were filtered through whatman No. 11  $\mu\text{m}$  filter paper (GE Health care, UK) and concentrated using a rotary evaporator (BUCHI, Rotavapor®, UK) under reduced pressure.

### Bacterial cultures and inoculums preparation

Seven reference bacteria *viz.* *Staphylococcus aureus*, *Staphylococcus epidermis*, *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumonia*, *Bacillus subtilis*, *Salmonella typhi* were obtained from Phytopharmaceutical and Nutraceutical Research Laboratory (the Institute of Chemical Sciences, University of Peshawar). All bacterial isolates were maintained on Mueller-Hinton agar nutrient slants and stored at 4 °C prior to subculture. A loopful of isolated bacterial colonies was pre cultured into 5 ml of nutrient broth at 37 °C overnight on a rotary and centrifuged at 10,000 rpm for 5 min. The pellet obtained was suspended in double distilled water and the cell density of the actively growing bacterial suspension was spectrophotometrically ( $A_{610\text{ nm}}$ ) standardized equivalent to approximately  $1-2 \times 10^8$  colony forming units per ml (CFU/ml).<sup>14</sup>

### Preparation of the organic extracts of different plant parts of *Fumaria parviflora*

Mature *F. parviflora* plants collected in the months of March-April 2009 from wheat fields of new Agricultural Research Farm, Malakandher, The University of Agriculture, Peshawar, Khyber Pakhtunkhwa, Pakistan were authenticated and voucher specimen (No. ISH-1732) deposited in the herbarium of the department of Botany, University of Peshawar, Pakistan.<sup>10</sup> Plants were washed thoroughly in running tap water and once with sterile water. The dried plant-parts (stem, roots, leaves and flowers) were crushed in a grinder to obtain particles approximately 1 mm long. The powdered plant material (350 g each) was subjected to Soxhlet extraction according to the standard procedure.<sup>10</sup> The extracts obtained *viz.* n-hexane, chloroform ( $\text{CHCl}_3$ ), ethyl acetate (EtOAc) and methanol (MeOH) were filtered and evaporated to dryness at 60 °C under reduced pressure using a rotary evaporator. The yields of all extracts were recorded. The extracts were stored at 4 °C in brown bottles for use in antimicrobial activities. Further, three compounds *viz.*, nonacosane-10-ol (alcohol; Retention factor ( $R_f$ ) = 0.65), 23a-homostigmast-5-en-3 $\beta$ -ol ( $\beta$ -sitosterol;  $R_f$  = 0.32)<sup>15</sup> and *cis*- and *trans*-prtopinium (alkaloid;  $R_f$  = 0.36) previously isolated from the n-hexane and methanol roots fractions of *F. parviflora* were *in vitro* evaluated against the reference bacteria.

### Determination of antibacterial activity by well diffusion method

Sensitivity of different bacteria to various extracts and the three compounds was measured in terms of zone of inhibition using Modified-Well Diffusion Assay (MWDA).<sup>16</sup> The plates containing Mueller-Hinton (nutrient) agar were spread with 0.2 ml of the inoculum. Wells (6 mm diameter) were cut out from agar plates using a sterilized stainless steel borer and filled with 200  $\mu\text{l}$  of the plant extracts each at concentration of 20 mg/ml. Three compounds *viz.*, nonacosane-10-ol, 23a-homostigmast-5-en-3 $\beta$ -ol, and *cis*- and *trans*-prtopinium were initially reconstituted in DMSO (less than 1% v/v). About 200  $\mu\text{l}$  of the three compounds each at a concentration of 100, 200 and 300  $\mu\text{g ml}^{-1}$  were *in vitro* tested against the reference strains in separate simultaneous experiment. The antibacterial activity of these extracts and the compounds were compared with Streptomycin at a concentration of 2 mg  $\text{ml}^{-1}$  (for plant extracts) and 100, 200 and 300  $\mu\text{g ml}^{-1}$  (for the compounds). In either experiment the standard was added as 200  $\mu\text{l/well}$  whereas DMSO dissolved in distilled water was utilized as negative control (200  $\mu\text{l/well}$ ). Final concentration of DMSO did not exceed 1% v/v. The plates inoculated with different bacteria were incubated at 37 °C up to 48 h and diameter (mm) of zone of inhibition (IZ) was measured. For each combination of extracts/compounds and the bacterial strain, the experiment was performed in a completely randomized design with five replications per treatment and was repeated twice. The microbial growth of bacteria with a clear zone of inhibition of more than 30 mm or above were considered very strongly sensitive (VSS), 20 mm were considered to be strongly sensitive (SS) whereas those with more than 10 mm and 5 mm were regarded as sensitive (S) and moderately sensitive (MS). Bacterial strain exhibiting no activity was placed in a non-sensitive group (NS) (17). Activity index (AI) of all the extracts was calculated as follow; AI= Activity Index (IZ developed by extract/IZ developed by standard).<sup>18</sup>

### Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using each of the active extracts (extracts which inhibited growth of the majority of the tested bacteria) following an agar-dilution method.<sup>1</sup> Each species of bacteria was cultured overnight on agar slant and then suspended in sterile saline to give  $10^4$  cfu  $\text{ml}^{-1}$ . Each dried plant extract (roots, stem and leaves extracts) was reconstituted first in DMSO (less than 1 %) and then serially diluted with sterile water before mixed with sterile molten Mueller-Hinton agar to give final concentration of 3.12 to 50.0 mg  $\text{ml}^{-1}$  (3.12, 6.24, 12.5, 25.0 and 50 mg  $\text{ml}^{-1}$ ). Each agar solution was vortexed and then poured immediately in the petri dish. Finally a suspension of each of the test specie ( $10^4$  cfu  $\text{ml}^{-1}$ ) was gently spotted on the cold agar with a micropipette under aseptic conditions and plates were incubated at 37 °C for 24 hr. The plates were evaluated for the presence or



absence of microbial growth. The MIC was recorded as the lowest concentration of an extract at which there was no visible growth for the extract-microbe combination under consideration. The MIC of the standard (positive control) was also determined in a similar way and streptomycin was serially diluted with distilled water to give final concentrations of 3.12 to 50.0  $\mu\text{g ml}^{-1}$ . The experiment was performed in duplicate and repeated three times.

### Bactericidal activity and viable cell count (VCC)

Bactericidal activity of the three compounds *viz.*, nonacosane-10-ol, 23a-homostigmast-5-en-3 $\beta$ -ol, and *cis*- and *trans*-prtopinium prepared at 27°C at a concentration of 100  $\mu\text{g ml}^{-1}$  was measured by viable cell count (VCC). Bacterial inoculums of seven mentioned species (5 ml each) grown for 24 h were serially diluted ( $10^{-3}$ ) with nutrient broth. Equal volumes of the diluted inoculum and each compound to be tested were mixed and incubated at 37°C. About 0.5 ml of the mixed suspension at different time intervals (i.e 0, 1, 2, 3, ..., 24 h) was gently spread on two separate nutrient agar plates and incubated for 24 h at 37 °C. Similar procedure was applied to control plates in which compounds were replaced with sterilized distilled water. Mean number of colonies were counted and compared with control. The experiments were repeated three times. The results were expressed as number of viable cells as a percentage of control.<sup>18</sup>

### RESULTS

Results in table 1 showed that the yields of the extracts ranged from 20.0 mg for EtOAC flowers extracts to 61.0 mg for n-hexane roots extracts. Different plant extracts were blackish in color and their texture was gummy, oily or sticky (Table 1). Both Gram (+) and Gram (-) bacteria showed varied sensitivity levels to the extracts of *F. parviflora* in a modified well diffusion test (Table 2). The *n*-hexane extracts of the roots showed the highest zone of inhibition (IZ) and activity index (AI) against the Gram (-) *E. coli* (IZ = 28  $\pm$  0.9; AI = 0.93  $\pm$  0.3), *S. typhi* (22  $\pm$  0.4; AI = 0.73  $\pm$  0.9) and *K. pneumonia* (22  $\pm$  0.4; AI = 0.73  $\pm$  0.4) and three were graded as strongly sensitive (SS) strains. All bacterial strains were graded either as sensitive (S) or moderately sensitive (MS) based on the zone of inhibition and the activity index when exposed to the four stem extracts. Most of the tested strains were non-sensitive (NS) to the *n*-hexane, EtOAC and  $\text{CHCl}_3$  extracts of the flowers except the MeOH, which grouped the tested strains in a moderately sensitive (MS) category. The three compounds *viz.*, nonacosane-10-ol, 23a-homostigmast-5-en-3 $\beta$ -ol, and *cis*- and *trans*-prtopinium showed the highest IZ and AI at a conc. of 100, 200 and 300  $\mu\text{g ml}^{-1}$  compared with the standard streptomycin (Table 3). Irrespective of the Gram (+) or Gram (-), the IZ and AI were directly related to increase in the concentrations of these compounds under reference. The strains for example *S. aureus* (IZ = 33  $\pm$  0.3; AI = 1.1  $\pm$  0.3), *E. coli* (IZ = 37  $\pm$  0.6; AI = 1.23  $\pm$  0.3), *S. typhimurium* (IZ = 31  $\pm$  0.1; AI = 1.03  $\pm$  0.2) and *S. typhi* (IZ = 37  $\pm$  0.8; AI =

1.23  $\pm$  0.2) were very strongly sensitive (VSS) to the highest concentration (300  $\mu\text{g ml}^{-1}$ ) of nonacosane-10-ol. Except *E. coli*, which was strongly sensitive to 23a-homostigmast-5-en-3 $\beta$ -ol, all other bacterial strains showed very strong sensitivity at the highest conc. of 300  $\mu\text{g ml}^{-1}$  (Table 3). The alkaloid *cis*- and *trans*-prtopinium was more potent than the former two compounds and the IZ and AI were the highest for Gram (-) *E. coli* (IZ = 39  $\pm$  0.9; AI = 1.3  $\pm$  0.1), *K. pneumonia* (IZ = 30  $\pm$  0.4; AI = 1.33  $\pm$  0.3) and *S. typhi* (IZ = 43  $\pm$  0.1; AI = 1.4  $\pm$  0.3) followed by Gram (+) strains. All strains were very strongly sensitivity to a concentration of 300  $\mu\text{g ml}^{-1}$  (Table 3).

**Table 1:** Colour, texture and weight of the extracts from different plant parts of *F. parviflora*.

Extracts of <i>F. parviflora</i>	Weight (mg)	Texture	Colour
<b>Root</b>			
n-hexane	61.0	Sticky	Blackish
EtOAC	23.0	Gummy	Black
$\text{CHCl}_3$	55.0	Oily	Greenish
MeOH	59.0	Gummy	Reddish-Black
<b>Stem</b>			
n-hexane	58.0	Sticky	Greenish-black
EtOAC	28.0	Gummy	Reddish-black
$\text{CHCl}_3$	31.0	Oily	Black
MeOH	49.0	Gummy	Black
<b>Leaves</b>			
n-Hexane	67.0	Sticky	Greenish-black
EtOAC	45.0	Gummy	Black
$\text{CHCl}_3$	33.0	Oily	Greenish
MeOH	45.0	Gummy	Reddish-black
<b>Flowers</b>			
n-hexane	22.0	Sticky	Black
EtOAC	20.0	Gummy	Reddish-black
$\text{CHCl}_3$	22.0	Oily	Pinkish-Purple
MeOH	23.0	Gummy	Red

EtOAC = Ethyl acetate;  $\text{CHCl}_3$  = Chloroform; MeOH = Methanol

The minimum inhibitory concentration (MIC) of the roots, stem and leaves extracts of *F.* was determined at five concentrations against the seven mentioned strains (Table 4). Overall the MIC value of all the tested plant extracts was found in the range of 3.12 to 50.0  $\text{mg ml}^{-1}$  (Table 4). The most active plant extract was the *n*-hexane root extracts with MIC value of 3.12  $\text{mg ml}^{-1}$  for the Gram (-) *E. coli*, *K. pneumonia*, and *S. typhi*. The MIC values of all the stem extracts were reasonably good whereas the leaves extracts of the plant displayed activity with high MIC values (Table 4).

Our results of viable cell count (VCC) are in line with those obtained by zone of inhibition and MIC values recorded for the three compounds. The three compounds *viz.*,

nonacosane-10-ol, 23a-homostigmast-5-en-3 $\beta$ -ol, and *cis*- and *trans*-prtopinium at a conc. of 100  $\mu\text{g ml}^{-1}$  completely killed bacteria at 8 h or 10 h of time span incubation (Figure 1a-c). The nonacosane-10-ol completely killed the *E. coli* at a time span of 10 h followed by *S. typhi* (Figure 1a). The second compound (23a-homostigmast-5-en-3 $\beta$ -ol) effectively killed *S. epidermis* after 10 h whereas *Staphylococcus aureus* was completely inhibited after 14 h of time interval (Figure 1b). The *cis*- and *trans*-

prtopinium was more effective than the former two compounds and killed the Gram (+) *S. epidermis* and Gram (-) *K. pneumonia* exactly after 8 and 10 h of time span respectively (Figure 1c). Whereas *S. typhi* was least sensitive to protopine and took longer time (22 h) for complete inhibition. However, all the three compounds were bactericidal and did not allow bacterial growth after 24 h (Figure 1a-c).

**Table 2:** Antibacterial activities (diameter of growth inhibition zone) of n-hexane, ethyl acetate (EtOAC), chloroform (CHCl<sub>3</sub>) and methanol (MeOH) extracts (10 mg/ml) from the root bark, stem bark, leaves and flowers of *F. parviflora*.

Extracts of <i>F. parviflora</i>	Bacteria <sup>a</sup> tested zone of inhibition (IZ) (mm)						
	Sa	Se	Ec	Sty	Kp	Bs	St
<b>Roots</b>							
n-Hexane	16 ± 0.3 (S) <sup>d</sup> 0.53 ± 0.1	18 ± 0.2 (S) 0.6 ± 0.2	28 ± 0.9 (SS) <sup>c</sup> 0.93 ± 0.3	15 ± 0.4 (S) 0.5 ± 0.6	22 ± 0.4 (SS) 0.73 ± 0.4	16 ± 0.9 (S) 0.53 ± 0.3	22 ± 0.4 (SS) 0.73 ± 0.9
EtOAC	12 ± 0.4 (S) 0.4 ± 0.6	9 ± 0.4 (MS) <sup>e</sup> 0.3 ± 0.2	5 ± 0.3 (MS) 0.16 ± 0.4	11 ± 0.5 (S) 0.36 ± 0.2	14 ± 0.3 (S) 0.46 ± 0.4	14 ± 0.6 (S) 0.46 ± 0.5	0 ± 0 (NS) 0 ± 0
CHCl <sub>3</sub>	9 ± 0.2 (MS) 0.3 ± 0.6	8 ± 0.8 (MS) 0.26 ± 0.2	8 ± 0.1 (MS) 0.26 ± 0.5	15 ± 0.9 (S) 0.5 ± 0.3	12 ± 0.9 (S) 0.4 ± 0.1	12 ± 0.3 (S) 0.4 ± 0.3	9 ± 0.3 (MS) 0.3 ± 0.2
MeOH	11 ± 0.6 (S) 0.36 ± 0.5	11 ± 0.9 (S) 0.36 ± 0.4	17 ± 0 (S) 0.5 ± 0.5	15 ± 0.4 (S) 0.5 ± 0.3	16 ± 0.2 (S) 0.53 ± 0.2	11 ± 0.3 (S) 0.36 ± 0.6	8 ± 0.9 (MS) 0.26 ± 0.5
<b>Stem</b>							
n-Hexane	12 ± 0.4 (S) 0.4 ± 0.2	12 ± 0.4 (S) 0.4 ± 0.6	9 ± 0.9 (MS) 0.3 ± 0.5	9 ± 0.5 (MS) 0.3 ± 0.3	13 ± 0.4 (S) 0.43 ± 0.2	14 ± 0.9 (S) 0.46 ± 0.3	8 ± 0.6 (MS) 0.26 ± 0.3
EtOAC	9 ± 0.5 (MS) 0.3 ± 0.2	11 ± 0.4 (S) 0.36 ± 0.3	8 ± 0.2 (MS) 0.26 ± 0.2	8 ± 0.7 (MS) 0.26 ± 0.2	15 ± 0.6 (S) 0.5 ± 0.2	9 ± 0.4 (MS) 0.3 ± 0.3	5 ± 0.9 (MS) 0.16 ± 0.8
CHCl <sub>3</sub>	11 ± 0.3 (S) 0.36 ± 0.2	9 ± 0 (MS) 0.3 ± 0.2	7 ± 0.9 (MS) 0.23 ± 0.3	12 ± 0.2 (S) 0.4 ± 0.3	9 ± 0.2 (MS) 0.3 ± 0.2	8 ± 0.2 (MS) 0.26 ± 0.1	6 ± 0.3 (MS) 0.2 ± 0.3
MeOH	13 ± 0.7 (S) 0.43 ± 0.3	6 ± 0.2 (MS) 0.2 ± 0.2	6 ± 0.2 (MS) 0.2 ± 0.2	6 ± 0.9 (MS) 0.2 ± 0.1	12 ± 0.6 (S) 0.4 ± 0.3	5 ± 0.3 (MS) 0.16 ± 0.6	5 ± 0.7 (MS) 0.16 ± 0.3
<b>Leaves</b>							
n-Hexane	6 ± 0.7 (MS) 0.2 ± 0.2	4 ± 0.6 (NS) 0.13 ± 0.4	7 ± 0.5 (MS) 0.23 ± 0.1	5 ± 0.2 (MS) 0.16 ± 0.3	4 ± 0.1 (NS) 0.13 ± 0.2	8 ± 0.9 (MS) 0.26 ± 0.3	8 ± 0.8 (MS) 0.26 ± 0.1
EtOAC	8 ± 0.7 (MS) 0.26 ± 0.2	5 ± 0.8 (MS) 0.16 ± 0.5	5 ± 0.9 (MS) 0.16 ± 0.5	6 ± 0.7 (MS) 0.2 ± 0.3	4 ± 0.2 (NS) 0.13 ± 0.3	8 ± 0.5 (MS) 0.26 ± 0.4	7 ± 0.7 (MS) 0.23 ± 0.4
CHCl <sub>3</sub>	6 ± 0.2 (MS) 0.2 ± 0.2	6 ± 0.7 (MS) 0.2 ± 0.4	7 ± 0.8 (MS) 0.23 ± 0.2	8 ± 0.3 (MS) 0.26 ± 0.3	3 ± 0.9 (NS) 0.13 ± 0.3	9 ± 0.6 (MS) 0.3 ± 0.5	5 ± 0.8 (MS) 0.16 ± 0.4
MeOH	9 ± 0.8 (MS) 0.3 ± 0.2	7 ± 0.3 (MS) 0.23 ± 0.1	8 ± 0.9 (MS) 0.26 ± 0.1	7 ± 0.2 (MS) 0.23 ± 0.4	8 ± 0.9 (MS) 0.26 ± 0.2	10 ± 0.4 (MS) 0.33 ± 0.1	9 ± 0.8 (MS) 0.3 ± 0.1
<b>Flowers</b>							
n-Hexane	2 ± 0.6 (NS) <sup>f</sup> 0.06 ± 0.6	2 ± 0.1 (NS) 0.06 ± 0.3	3 ± 0.9 (NS) 0.1 ± 0.9	3 ± 0.8 (NS) 0.1 ± 0.9	5 ± 0.4 (MS) 0.16 ± 0.4	5 ± 0.9 (MS) 0.16 ± 0.4	6 ± 0.7 (MS) 0.2 ± 0.2
EtOAC	1 ± 0.5 (NS) 0.03 ± 0.5	3 ± 0.2 (NS) 0.1 ± 0.9	0 ± 0 (NS) 0 ± 0	2 ± 0.4 (NS) 0.06 ± 0.3	0 ± 0 (NS) 0 ± 0	3 ± 0.6 (NS) 0.1 ± 0.9	10 ± 0.2 (MS) 0.33 ± 0.1
CHCl <sub>3</sub>	5 ± 0.2 (MS) 0.16 ± 0.3	4 ± 0.7 (NS) 0.13 ± 0.3	5 ± 0.7 (MS) 0.16 ± 0.2	3 ± 0.9 (NS) 0.1 ± 0.6	0 ± 0 (NS) 0 ± 0	2 ± 0.7 (NS) 0.06 ± 0.2	0 ± 0 (NS) 0 ± 0
MeOH	6 ± 0.7 (MS) 0.2 ± 0.1	5 ± 0.4 (MS) 0.16 ± 0.2	6 ± 0.5 (MS) 0.2 ± 0.2	5 ± 0.5 (MS) 0.16 ± 0.3	8 ± 0.4 (MS) 0.26 ± 0.3	5 ± 0.8 (MS) 0.16 ± 0.3	6 ± 0.8 (MS) 0.2 ± 0.2
Streptomycin (+ control)	30 ± 0.4 (VSS)	30 ± 0.3 (VSS)	30 ± 0.4 (VSS)	30 ± 0.4 (VSS)	30 ± 0.4 (VSS)	30 ± 0.4 (VSS)	30 ± 0.4 (VSS)
DMSO (- control)	0 ± 0 (NS)	0 ± 0 (NS)	0 ± 0 (NS)	0 ± 0 (NS)	0 ± 0 (NS)	0 ± 0 (NS)	0 ± 0 (NS)

Bacteria<sup>a</sup> Sa = *Staphylococcus aureus*; Se = *Staphylococcus epidermis*; Ec = *Escherichia coli*; Sty = *Salmonella typhimurium*; Kp = *Klebsiella pneumonia*; Bs = *Bacillus subtilis*; St = *Salmonella typhi*. VSS<sup>b</sup> = Very strongly sensitive ( $\geq 30$  mm or above); HS<sup>c</sup> = Strongly sensitive ( $\geq 20$  mm); S<sup>d</sup> = Sensitive ( $\geq 10$  mm); MS<sup>e</sup> = Moderately sensitive ( $\geq 5$  mm); NS<sup>f</sup> = Non-sensitive ( $< 5$  mm); Upper values in column 1 represents means of zone of inhibition (IZ) (mm)  $\pm$  S.D of five replicates. Lower values in column 1 represents Activity Index (AI) = ZI for each extract/IZ for standard

**Table 3:** Zone of inhibition in mm of 100, 200 and 300 µg ml<sup>-1</sup> concentrations of different compounds of *Fumaria parviflora*

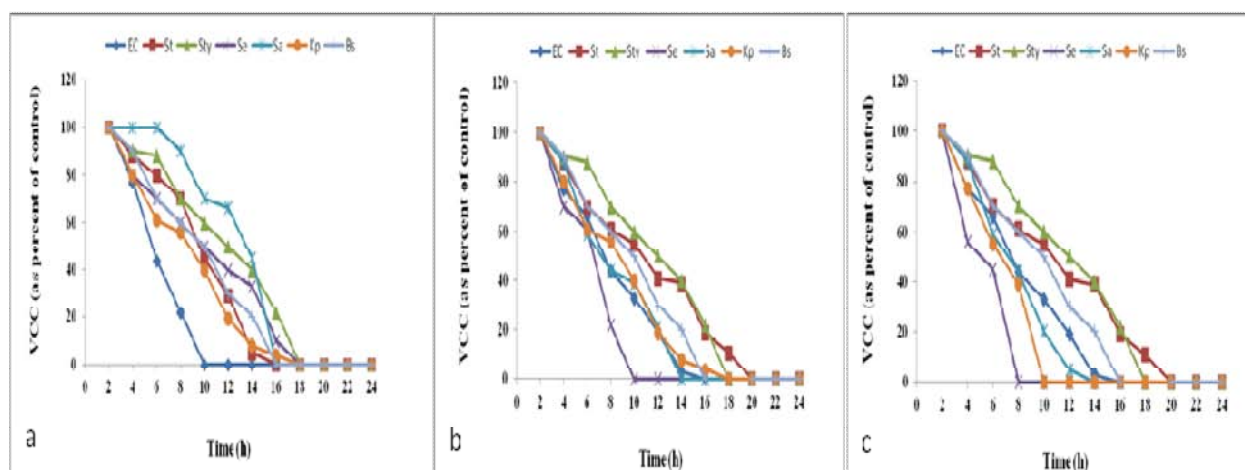
Concentrations (µg ml <sup>-1</sup> )	Bacteria <sup>a</sup> tested zone of inhibition (IZ) (mm)						
	Sa	Se	Ec	Sty	Kp	Bs	St
<b>Nonacosane-10-ol</b>							
100	11 ± 0.8 (S) 0.36 ± 0.8	12 ± 0.4 (S) 0.4 ± 0.3	13 ± 0.1 (S) 0.43 ± 0.2	9 ± 0.9 (MS) 0.3 ± 0.5	14 ± 0.5 (S) 0.46 ± 0.4	11 ± 0.6 (S) 0.36 ± 0.5	11 ± 0.2(S) 0.36 ± 0.4
200	21 ± 0.1 (SS) 0.7 ± 0.2	19 ± 0.1 (S) 0.63 ± 0.2	22 ± 0.9 (SS) 0.73 ± 0.3	11 ± 0.3 (S) 0.36 ± 0.3	19 ± 0.3 (S) 0.63 ± 0.2	14 ± 0.5 (S) 0.46 ± 0.3	29 ± 0.5 (SS) 0.9 ± 0.5
300	33 ± 0.3 (VSS) 1.1 ± 0.3	38 ± 0.9 (SS) 1.2 ± 0.2	37 ± 0.6 (VSS) 1.23 ± 0.3	31 ± 0.1 (VSS) 1.03 ± 0.2	29 ± 0.9 (SS) 0.9 ± 0.2	19 ± 0.1 (S) 0.63 ± 0.5	37 ± 0.8 (VSS) 1.23 ± 0.2
<b>23a-homostigmast-5-en-3β-ol</b>							
100	29 ± 0.4 (SS) 0.9 ± 0.1	30 ± 0.5 (SS) 1.0 ± 0.32	20 ± 0.2 (SS) 0.66 ± 0.2	22 ± 0.9 (SS) 0.73 ± 0.3	19 ± 0.2 (S) 0.63 ± 0.3	26 ± 0.3 (SS) 0.86 ± 0.3	19 ± 0.3 (S) 0.63 ± 0.4
200	39 ± 0.6 (SS) 1.3 ± 0.1	29 ± 0.8 (SS) 0.9 ± 0.1	22 ± 0.4 (SS) 0.73 ± 0.3	28 ± 0.3 (SS) 0.93 ± 0.1	33 ± 0.1 (VSS) 1.1 ± 0.3	29 ± 0.6 (SS) 0.9 ± 0.1	37 ± 0.5 (VSS) 1.2 ± 0.1
300	48 ± 0.8 (VSS) 1.6 ± 0.1	40 ± 0.9 (VSS) 1.33 ± 0.3	26 ± 0.6 (SS) 0.86 ± 0.5	40 ± 0.7 (VSS) 1.3 ± 0.4	34 ± 0.6 (VSS) 1.1 ± 0.1	32 ± 0.8 (VSS) 1.1 ± 0.1	41 ± 0.8 (VSS) 1.3 ± 0.1
<b>Cis- and trans-protopinium</b>							
100	25 ± 0.4 (SS) 0.8 ± 0.3	30 ± 0.3 (VSS) 1.0 ± 0.1	23 ± 0.9 (SS) 0.76 ± 0.1	22 ± 0.2 (SS) 0.73 ± 0.3	29 ± 0.3 (SS) 0.9 ± 0.1	12 ± 0.4 (S) 0.4 ± 0.3	19 ± 0.2 (S) 0.63 ± 0.4
200	33 ± 0.6 (VSS) 1.1 ± 0.3	38 ± 0.4 (VSS) 1.26 ± 0.3	27 ± 0.9 (SS) 0.9 ± 0.3	30 ± 0.8 (VSS) 1.0 ± 0.3	37 ± 0.4 (VSS) 1.2 ± 0.1	29 ± 0.8 (SS) 0.9 ± 0.1	27 ± 0.4 (SS) 0.9 ± 0.1
300	39 ± 0.9 (VSS) 1.3 ± 0.1	39 ± 0.4 (VSS) 1.3 ± 0.1	46 ± 0.9 (VSS) 1.53 ± 0.3	39 ± 0.9 (VSS) 1.3 ± 0.1	40 ± 0.9 (VSS) 1.33 ± 0.3	38 ± 0.3 (VSS) 1.2 ± 0.3	43 ± 0.1 (VSS) 1.4 ± 0.3
Streptomycin (+)	30 ± 0.2 (VSS)	30 ± 0.1 (VSS)	30 ± 0.7 (VSS)	30 ± 0.4 (VSS)	30 ± 0.4 (VSS)	30 ± 0.4 (VSS)	30 ± 0.6 (VSS)
DMSO (-)	0 ± 0 (NS)	0 ± 0 (NS)	0 ± 0 (NS)	0 ± 0 (NS)	0 ± 0 (NS)	0 ± 0 (NS)	0 ± 0 (NS)

Bacteria<sup>a</sup> Sa = *Staphylococcus aureus*; SE = *Staphylococcus epidermis*; Ec = *Escherichia coli*; Sty = *Salmonella typhimurium*; Kp = *Klebsiella pneumonia*; Bs = *Bacillus subtilis*; St = *Salmonella typhi*; VSS<sup>b</sup> = Very strongly sensitive (≥ 30 mm or above); HS<sup>c</sup> = Strongly sensitive (≥ 20 mm); S<sup>d</sup> = Sensitive (≥ 10 mm); MS<sup>e</sup> = Moderately sensitive (≥ 5 mm); NS<sup>f</sup> = Non-sensitive (< 5 and 0 mm); Upper values in column 1 represents means of zone of inhibition (IZ) (mm) ± S.D of five replicates. Lower values in column 1 represents Activity Index (AI) = ZI for each extract/IZ for standard

**Table 4:** Minimum Inhibitory Concentration (MIC) of the active plant extracts against Gram (-) and Gram (+) bacteria in an agar-dilution assays.

Extracts of <i>F. parviflora</i>	Minimum Inhibitory Concentration (MIC) <sup>*</sup>						
	Sa	Se	Ec	Sty	Kp	Bs	St
<b>Roots</b>							
n-Hexane	3.12	6.24	3.12	6.24	3.12	6.24	3.12
EtOAC	3.12	12.5	6.24	6.24	12.5	12.5	12.5
CHCl <sub>3</sub>	6.24	6.24	12.5	12.5	12.5	12.5	12.5
MeOH	12.5	12.5	3.12	6.24	12.5	25.0	12.5
<b>Stem</b>							
n-Hexane	6.24	6.24	3.12	12.5	12.5	25.0	12.5
EtOAC	12.5	12.5	3.12	12.5	12.5	12.5	25.0
CHCl <sub>3</sub>	6.24	12.5	6.34	12.5	25.0	25.0	25.0
MeOH	12.5	12.5	3.12	12.5	25.0	12.5	12.5
<b>Leaves</b>							
n-Hexane	12.5	12.5	12.5	25.0	25.0	25.0	12.0
EtOAC	25.0	25.0	25.0	12.5	20.0	50.0	50.0
CHCl <sub>3</sub>	12.5	12.5	12.5	12.5	25.0	12.5	25.0
MeOH	6.24	12.5	12.5	25.0	25.0	25.0	25.0
<b>Streptomycin**</b> (standard/control)	6.24	12.5	12.5	12.6	6.24	12.5	12.5

<sup>\*</sup>Bacteria, Sa = *Staphylococcus aureus*; SE = *Staphylococcus epidermis*; Ec = *Escherichia coli*; Sty = *Salmonella typhimurium*; Kp = *Klebsiella pneumonia*; Bs = *Bacillus subtilis*; St = *Salmonella typhi*; <sup>\*\*</sup>Minimum inhibitory concentration of Streptomycin (µg ml<sup>-1</sup>)



**Figure 1a-c:** Viable Cell Count (VCC) of all bacteria tested with three different compounds. a) Viable Cell Count (VCC) of different bacteria with nonacosane-10-ol of *Fumaria parviflora*; b) Viable Cell Count (VCC) of different bacteria with 23a-homostigmast-5-en-3 $\beta$ -ol of *Fumaria parviflora*; c) Viable Cell Count (VCC) of different bacteria with *cis*- and *trans*-prtopinium of *Fumaria parviflora* (Sa = *Staphylococcus aureus*; Se = *Staphylococcus epidermis*; Ec = *Escherichia coli*; Sty = *Salmonella typhimurium*; Kp = *Klebsiella pneumonia*; Bs = *Bacillus subtilis*; St = *Salmonella typhi*).

## DISCUSSION

Traditional medicines play important role in the world, treating millions of people. Nearly 25 % of all modern medicines have been derived from medicinal plants.<sup>14</sup> The medicinal or antibiotic activity of herbs is due to the presence of many complex substances termed as secondary metabolites or phytochemicals, which are accumulated at varying concentration in different plant parts.<sup>19</sup> Many clinical and even non-clinical bacteria have become resistant to antibiotics and drug development strategies seem insufficient to provide therapeutic covers in the coming 10-20 years.<sup>20</sup> The search for novel phytochemical based strategies for bacteria control is a global challenge.<sup>19</sup>

The family Fumariaceae contains an important species *F. parviflora* Lam. In the present study the antibacterial activity of *F. parviflora* plant part extracts (roots, stem, leaves and flowers) and compounds from the roots were *in vitro* tested against seven clinical bacteria *viz.*, *Staphylococcus aureus*, *Staphylococcus epidermis*, *Escherichia coli*, *Salmonella typhimurium*, *Klebsiella pneumonia*, *Bacillus subtilis* and *Salmonella typhi*. The *n*-hexane roots extracts of the plant showed good antibacterial activity against Gram (-) bacteria i.e *E. coli*, *S. typhimurium* and *K. pneumonia*, compared with the standard streptomycin and a negative control. The MeOH roots extracts ranked second in terms of zone of inhibition and activity index. Better activities of the *n*-hexane and MeOH root extracts could be attributed to the presence of seven bioactive classes of compounds previously detected in the plant roots and stem.<sup>10</sup> These compounds either singly or jointly inhibited the growth of Gram (+) and (-) bacteria. These findings agree with those of others who reported that ethyl acetate and acetone root bark extracts of *Moringa oleifera* (drumstick) showed maximum activity against *Staphylococcus aureus*, *Escherichia coli*, *Salmonella gallinarum* and *Pseudomonas aeruginosa* in *in vitro* study.<sup>21</sup>

Stem extracts (*n*-hexane, EtOAc, CHCl<sub>3</sub> and MeOH) inhibited bacterial growth and most of the tested strains were moderately sensitive when exposed to the four stem extracts. The antibacterial activity of *F. parviflora* aerial plant extracts against *Actinomyces viscosus*, *Streptococcus mutans*, *Streptococcus sobrinus*, *Lactobacillus fermentum*, *Lactobacillus casei* and *Eikenella corrodens* have been reported, but with low efficacy.<sup>22</sup> The aerial plant extracts exhibited minimum activity, however, showed no zone of inhibition against *Eikenella corrodens* even at the highest concentration of 100% (w/v). These results are in congruence to our results of the extracts from the aerial parts especially the foliage extracts which exhibited minimum activity against all the strains tested. Plant extracts and phytochemicals from diverse plant species for example *Achillea millefolium* (yarrow), *Caryophyllus aromaticus* (clove), *Melissa officinalis* (lemon-balm), *Ocimum basilicum* (basil), *Psidium guajava* (guava), *Punica granatum* (pomegranate), *Rosmarinus officinalis* (rosemary), *Salvia officinalis* (sage), *Syzygium joabolanum* (jambolan) and *Thymus vulgaris* (thyme) were found effective against *P. aeruginosa*, *K. pneumonia*, *K. pneumonia*, *Shigella* spp, *Proteus* spp, *S. aureus* and *E. aerogenes*.<sup>23</sup>

In the present study the three compounds of *F. parviflora* roots revealed marvelous antibacterial activity at a conc. of 100, 200 and 300  $\mu$ g ml.<sup>-1</sup> To the best of our knowledge these compounds of *Fumaria parviflora* have not previously reported for their antibacterial activity against the tested clinical pathogens. Nonacosane-10-ol restricted both the Gram (-) and Gram (+) bacterial growth at the highest concentration compared with the standard Streptomycin and control. Nonacosane-10-ol is a white amorphous long chain alcohol (C-29) with a melting point of 81-82  $^{\circ}$ C.<sup>15</sup> Many long chain alcohols have proven effective antibacterial against *Staphylococcus aureus* and *Propionibacterium acnes* and maximum activity was found to depend on the chain length from the hydrophilic hydroxyl group as well as the bacteria tested.<sup>24</sup> Another

compound i.e 23a-homostigmast-5-en-3 $\beta$ -ol in the present study restricted the bacterial growth and proved effective antibiotic at all conc. compared with the standard Streptomycin. Except *E. coli*, all other bacteria were very strongly sensitive (VSS) to this compound at 300  $\mu\text{g ml}^{-1}$ . Our results are in congruence to those who reported that many phytosterols proved effective antibiotics than the *n*-alkanes against Gram (+) *Staphylococcus* spp., and Gram (-) *Escherichia coli*, *Pseudomonas pvoicyanea*, and *Klebsiella*.<sup>25</sup> Similar results were reported by many other scientists who reported that sterol from *Euphorbia hirta* restricted the growth of *Enterobacter aerogens* with inhibition zone of 21 mm.<sup>18</sup> The third compound i.e *cis*- and *trans*-protopinium in the present study showed toxic potency to the seven strains of bacteria at all the tested concentrations. The bacterial zone of inhibition was the highest and all bacteria were very strongly sensitive (VSS) at 300  $\mu\text{g ml}^{-1}$ . Reports revealed that protopine is the most active isoquinoline alkaloid of *F. parviflora*.<sup>8</sup> The effect of protopine from *Fumaria* and *Corydalis* species against antibacterial and antiviral activities is well documented.<sup>26</sup> In addition a number of antibacterial activities have been conducted on natural isoquinoline alkaloids or their synthetic derivatives.<sup>27</sup> All extracts in the present study were evaluated against the seven bacterial strains for MIC values. The *n*-hexane roots extracts displayed good activity and inhibited *E. coli*, *K. pneumonia* and *S. typhi* growth with MIC value of 3.12 mg ml<sup>-1</sup>. Similar results were reported by other researchers who reported MIC value for acetone (5-15 mg ml<sup>-1</sup>) and seed extracts (20-80 mg ml<sup>-1</sup>) of *Anethum graveolens* Linn., *Foeniculum vulgare* Mill. and *Trachyspermum ammi* L, against clinical pathogens.<sup>14</sup> The antimicrobial activities of various natural products belonging to the terpenoids, alkaloids and phenolics against many multidrug-resistant Gram-negative bacteria have been reported.<sup>28</sup> Medicinal and pharmacological activities of *F. parviflora* are well reported. For example, extracts from *F. parviflora* have reported to reduce gum inflammation.<sup>29</sup> Use of plant derived natural products or secondary metabolites with known antimicrobial properties can be of great significance in therapeutic treatments. The present studies showed that extracts and compounds of *F. parviflora* possess a broad-spectrum antimicrobial activity against Gram (+) and Gram (-) bacteria. This broad activity of the plant could be due to the identified compounds i.e alkaloid, alcohol and sterol found in the plant roots. The roots and stem extracts and compounds of *F. parviflora* exhibited growth inhibitory activity against one or the other bacterial strains selected, hence *F. parviflora* can be used in formulations of novel chemotherapeutic agents. Further studies are needed to establish the exact mechanism for antibacterial action of the plant extracts and pure compounds.

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