



## RESEARCH PAPER

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## *In vitro* inhibition potential of *Forsskaolea tenacissima* L. against four pathogenic bacterial strains

Abd Ullah<sup>\*</sup>1, Rohul Amin<sup>2</sup>, Adnan Khan<sup>1</sup>

<sup>1</sup>School of life sciences, Northeast Normal University, Changchun, Jilin, P. R. China

<sup>2</sup>School of Forestry, Department Forest Manager, Beijing Forestry University, Beijing, P. R. China

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### Abstract

In recent past the use of plant or plant parts as potential antimicrobial agent became an attractive approach due to alarmingly increase in antibiotic resistance. The present study was aimed to evaluate the antibacterial potential of three different crude extracts (chloroform, aqueous and ethyl acetate) of *Forsskaolea tenacissima* L. against four pathogenic bacterial strains at four different concentrations (0.5, 1.0, 1.5, the 2.0 mg/ml) using agar well diffusion assay. Ethyl acetate root extracts exhibited wondrous inhibition against *Staphylococcus aureus* and *Klebsiella pneumonia* with inhibition zone recorded as 26.67 and 25.44mm at 2.0mg/ml respectively. Chloroform root extract at 2.0mg/ml concentration also showed tremendous effect with an inhibition zone of 25.34 mm. Bacterial strains were most sensitive to root crude extracts followed by stem and leaves crude extracts. *Bacillus subtilis* and *Pseudomonas aeruginosa* were least sensitive to leaves aqueous crude extracts with an inhibition zone of 5.44mm and 6.64mm at 2.00mg/ml respectively. The inhibition potential was concentration dependent. The inhibition was maximum at high concentration and showing a decrease with the decrease in concentration from 2mg/ml to 0.5mg/ml. The present findings support the use of *F. tenacissima* L. as a tool in the management of pathogenic bacterial strains.

**\*Corresponding Author:** Abd Ullah ✉ [Abdullahbotany0987@gmail.com](mailto:Abdullahbotany0987@gmail.com)

## Introduction

Pakistan has a rich floral diversity with more than 6,000 species of higher plants. Out of these estimated plants species, 12% are medicinally important (Shinwari *et al.*, 2000). Pakistan has a huge crude drug market system consisting of about 50,000 registered herbalists (William and Ahmad, 1999). They use herbal drugs for a number of humans as well as livestock illnesses. Therefore, Pakistan is amongst the leading countries exporting medicinal plants (Shinwari and Qaiser, 2011). In the developed and under developed countries more than 75% of people depend on folk medicines and this ratio is still high in the rural and remote areas (Sarker *et al.*, 2005). It has been noted that the major cause of human death is infectious disease throughout the world (Westh *et al.*, 2004). Bacteria and fungi cause different human infectious diseases that are treated with antibiotics (Abebe *et al.*, 2003). But it is also clear that such synthetic antibiotics have side effects and thus unsafe to be used by humans. Secondly, the emergence of antibiotic-resistant pathogens is a severe threat to the cure of infectious diseases (Prabuseenivasan *et al.*, 2006). In the recent years, human interest has been increased to explore the phytochemistry of medicinally important plants for pharmacological and nutritional purposes (Oktay *et al.*, 2003; Wangenstein *et al.*, 2004). The main aim is to replace synthetic antibiotics with plant-derived compounds with healing potentials. The plant chemical compounds are divided into primary and secondary metabolites (Agosta, 1996). Important plant secondary metabolites are alkaloids, flavonoids, terpenes, tannins and phenolic compounds (Edeoga *et al.*, 2005). The secondary compounds are having excellent therapeutic value due to their tremendous antimicrobial properties (Dionisi *et al.*, 2012). For exploring traditional medicines extensive studies have been conducted to evaluate the healing effect of medicinally important plants against different infectious disorders (Dias *et al.*, 2012; Qin and Xu, 1998). Such studies indicated that the inhibition potential of plant crude extracts against pathogenic bacteria is due to the presence of phenolic compounds (Baydar *et al.*, 2004; Vaquero *et al.*, 2007).

*Forsskaolea tenacissima* L. belongs to family Urticaceae, consisting of herbs, shrubs, and small trees. It is a small shrub consisting of six species which are present mainly in the tropical region from the Canary Islands and south Spain to India and Pakistan (Qaisar *et al.*, 2008; Loutfy, 1999). It is commonly known as nettle desert. It has two synonyms including *Caidbeja adhaerens* Forssk and *Forsskaolea cossoniana* Webb. (Tackholm *et al.*, 1976; Lyman, 1957). It is a non-cultivated species found in low rain area in sandy and stony soil. It is also found in arid and semi-arid waste lands, shrub lands to extreme deserts, rock crevices (Earl, 1962; Wickens, 2013). It is highly resistance to drought and salinity (Özcan, 2005). *Forsskaolea tenacissima* L. is generally used in folk medicine as anti-inflammatory, antispasmodic, antidiabetic and antipyretic (Shah *et al.*, 2010). It also showed antioxidant (Alali *et al.*, 2007), hepatoprotective (Assaf *et al.*, 2017), antiviral and antibacterial activities (Assaf *et al.*, 2015). The present study was aimed to evaluate the antibacterial potential of *Forsskaolea tenacissima* L. against four pathogenic bacterial strains.

## Materials and methods

### Experimental site

The present work was carried out at the laboratory of plant sciences, Department of Botany, Bacha University Charsadda, Khyber Pakhtunkhwa Pakistan.

### Plant materials collection

Healthy plants of *F. tenacissima* L. were collected from Tehsil Tangi, District Charsadda, Pakistan (34-03' and 34-38' north latitudes and 71-28' and 71-53' east longitude). The plants were separated into leaves, stem, and roots.

### Plant materials processing

The plant parts were washed properly with distilled water and shade dried at room temperature. The plant parts were then grounded into powder form using homogenizer. The powder was stored at room temperature for experimental purpose.

*Preparation of crude extract*

The plant parts were then soaked in 200 ml of chloroform, ethyl acetate and water using clean and sterilized beakers, incubated for two weeks at room temperature (25°C). After 14 days of extraction, the mixture was filtered twice; using Whatman-41 filter paper and the extract was reduced to dryness by removing the respective solvents. Agar well diffusion method was used for antibacterial activity (Carron *et al.*, 1987).

*Bacteria strains used*

Four strains of bacteria were used in the study. Two were gram-positive, *S. aureus* and *B. subtilis* and two were gram negatives which was *K. pneumonia* and *P. aeruginosa*. The organisms were maintained on nutrient agar medium at 4°C.

*Positive control*

Chloramphenicol (1.0 mg/ml) was used as a positive control for the test bacterial strains.

*Assay for antibacterial activity*

Agar well diffusion method was used for antibacterial activity (Carron *et al.*, 1987). Nutrient agar medium was prepared by adding nutrient agar (MERCK) 2.3g in 100ml of distilled water; pH was adjusted at 7.0 and was autoclaved at 121°C. It was allowed to cool up to 45°C. Petri plates were prepared by pouring 75ml of seeded nutrient agar and allowed to solidify. Four wells per plate were made with a sterile cork borer

(5mm). Using micropipette; 100µl of test solutions was poured into respective wells. These plates were incubated at 37°C. After 24 hours of incubation, the diameter of the clear zones of inhibitions was measured by a ruler. Antibacterial activity of 4 dilutions of each plant part extract was determined against four bacterial strains.

*Statistical analysis*

The clear zones of inhibition were measured in millimeter. All the data values are the means of three replicates. The data were presented in tables as a Mean ± standard deviation.

**Results**

*Antibacterial activity against S. aureus*

Our results (Table 1) showed that maximum inhibition against *S. aureus* has been exhibited by *F. tenacissima* L. ethyl acetate and chloroform root extract (2mg/ml) with 25-26mm zone of inhibition. Furthermore, ethyl acetate stem extract, chloroform leaves and stem extracts (2mg/ml) had considerable inhibition against *S. aureus* with 23.34, 22.34 and 21.00mm zone of inhibition respectively. Least zone of inhibition (10.67mm) against *S. aureus* has been reported for aqueous leaves extract at 2mg/ml.

The overall inhibition was concentration-dependent showing a progressive decrease in inhibition with a decrease in concentration of crude extract from 2mg/ml to 0.5mg/ml.

**Table 1.** Antibacterial potential of *F. tenacissima* L. against *S. aureus*.

Solvent extracts	2 mg/ml	1 mg/ml	1.5 mg/ml	0.5 mg/ml
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
PC-1.0mg/ml	29.34 ±0.43	27.39±0.33	26.01±0.33	24.45±0.43
Chloroform leaves extract	22.34±0.33	19.39±0.33	15.01±0.58	14.12±0.33
Ethyl acetate leaves extract	17.00±0.58	11.72±0.58	10.34±0.33	10.45±0.67
Aqueous leaves extract	10.67±0.63	9.39 ±0.33	8.34 ±0.73	8.45 ±0.58
Chloroform stem extract	21.00±0.58	15.39±0.33	12.34±0.33	11.12±0.53
Ethyl acetate stem extract	23.34±0.38	17.72±0.53	14.34±0.53	12.79±0.33
Aqueous stem extract	20.67±0.33	18.39±0.33	14.34±0.33	12.79±0.43
Chloroform root extract	25.34±0.58	23.06±0.63	20.01±0.58	18.12±0.53
Ethyl acetate root extract	26.67±0.33	24.39±0.33	21.01±0.58	17.12±0.33
Aqueous root extract	19.67±0.58	15.72±0.73	11.34±0.33	9.79±0.0.58

PC= Positive control (Standard drug, Chloramphenicol) Mean ± SD= Mean Zone of inhibition in mm and standard deviation.

*Antibacterial activity against P. aeruginosa*

Table 2 revealed that the maximum inhibition against *P. aeruginosa* has been exhibited by *F. tenacissima* L. ethyl acetate and chloroform root extract (2mg/ml) with inhibition zone recorded as 22.64 and 21.31mm at 2mg/ml crude extracts. considerable inhibition showed by ethyl acetate stem extract (19.31mm) and chloroform leaves extract (18.31mm) at 2mg/ml

concentration. The minimum inhibition zone recorded as 6.64mm for aqueous leaves extract indicating that *P. aeruginosa* was least susceptible to aqueous leaves extracts even at high concentration (2mg/ml). The susceptibility of *P. aeruginosa* decreases with a decrease in concentration of all the crude extract.

**Table 2.** Antibacterial potential of *F. tenacissima* L. against *P. auregonosa*.

Solvent extracts	2 mg/ml	1.5 mg/ml	1 mg/ml	0.5 mg/ml
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
PC-1.0mg/ml	25.31 ± 0.04	24.70 ± 0.19	22.45 ± 0.08	24.45 ± 0.06
Chloroform leaves extract	18.31 ± 0.09	16.70 ± 0.17	11.45 ± 0.16	14.12 ± 0.12
Ethyl acetate leaves extract	12.98 ± 0.06	9.04 ± 0.67	6.79 ± 0.03	10.45 ± 0.29
Aqueous leaves extract	6.64 ± 0.06	6.70 ± 0.35	4.79 ± 0.33	8.45 ± 0.33
Chloroform stem extract	16.98 ± 0.06	12.70 ± 0.10	8.79 ± 0.08	11.12 ± 0.04
Ethyl acetate stem extract	19.31 ± 0.17	15.04 ± 0.08	10.79 ± 0.10	12.79 ± 0.04
Aqueous stem extract	16.64 ± 0.15	15.70 ± 0.07	10.79 ± 0.06	12.79 ± 0.13
Chloroform root extract	21.31 ± 0.20	20.37 ± 0.01	16.45 ± 0.02	18.12 ± 0.07
Ethyl acetate root extract	22.64 ± 0.20	21.70 ± 0.06	17.45 ± 0.14	17.12 ± 0.10
Aqueous root extract	15.64 ± 0.33	13.04 ± 0.02	7.79 ± 0.19	9.79 ± 0.08

PC= Positive control (Standard drug, Chloramphenicol) Mean ± SD= Mean Zone of inhibition in mm and standard deviation.

*Antibacterial activity against B. subtilis*

Against *B. subtilis* (Table 3) the highest zone of inhibition were recorded for ethyl acetate and chloroform root extracts at the concentration of 2mg/ml, which showed 21.44 and 20.10mm mm

inhibition zones respectively. The ethyl acetate and chloroform leaves extract extracted samples (2mg/ml) showed considerable inhibition zone recorded as 18.10 and 17.10mm respectively as shown in table 3.

**Table 3.** Antibacterial potential of *F. tenacissima* L. against *B. subtilis*.

Solvent extracts	2.00 mg/ml	1.5 mg/ml	1.00 mg/ml	0.5 mg/ml
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
PC-1.0mg/ml	24.10 ± 0.07	23.45 ± 0.12	23.46 ± 0.04	21.11 ± 0.07
Chloroform leaves extract	17.10 ± 0.17	15.45 ± 0.03	12.46 ± 0.15	10.78 ± 0.04
Ethyl acetate leaves extract	11.77 ± 0.05	7.79 ± 0.12	7.80 ± 0.06	7.11 ± 0.08
Aqueous leaves extract	5.44 ± 0.08	5.45 ± 0.17	5.80 ± 0.06	5.11 ± 0.21
Chloroform stem extract	15.77 ± 0.09	11.45 ± 0.09	9.80 ± 0.13	7.78 ± 0.14
Ethyl acetate stem extract	18.10 ± 0.01	13.79 ± 0.21	11.80 ± 0.07	9.45 ± 0.16
Aqueous stem extract	15.44 ± 0.05	14.45 ± 0.09	11.80 ± 0.01	9.45 ± 0.06
Chloroform root extract	20.10 ± 0.05	19.12 ± 0.10	17.46 ± 0.08	14.78 ± 0.03
Ethyl acetate root extract	21.44 ± 0.05	20.45 ± 0.06	18.46 ± 0.08	13.78 ± 0.05
Aqueous root extract	14.44 ± 0.07	11.79 ± 0.21	8.80 ± 0.23	6.45 ± 0.56

PC= Positive control (Standard drug, Chloramphenicol) Mean ± SD= Mean Zone of inhibition in mm and standard deviation.

The minimum inhibition (5.44mm) against *B. subtilis* has been showed by aqueous leaves extract 2mg/ml concentration.

Table 3 also reveals that the endurance of *B. subtilis* increases with a decrease in the concentration of the crude extract.

*Antibacterial activity against K. pneumoniae*

The ethyl acetate and chloroform root extract at

2mg/ml concentration showed tremendous inhibition against *K. pneumoniae* recorded as 25.44 and 24.11mm respectively. Considerable inhibition has been showed by ethyl acetate stem extract (22.11mm) and chloroform leaves extract (21.11mm) at 2mg/ml concentrations of the extracted samples. *K. pneumonia* was least susceptible to aqueous leaves extract with an inhibition zone of 9.44mm at 2mg/ml concentration as shown in Table 4.

**Table 4.** Antibacterial potential of *F. tenacissima* L. against *K. pneumonia*.

Solvent extracts	2 mg/ml	1.5 mg/ml	1 mg/ml	0.5 mg/ml
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
PC-1.0mg/ml	28.11 ± 0.56	25.22 ± 0.50	23.00 ± 0.27	20.00 ± 0.35
Chloroform leaves extract	21.11 ± 0.58	17.22 ± 0.68	12.00 ± 0.67	9.67 ± 0.52
Ethyl acetate leaves extract	15.78 ± 0.50	9.56 ± 0.35	7.34 ± 0.44	6.00 ± 0.43
Aqueous leaves extract	9.44 ± 0.43	7.22 ± 0.42	5.34 ± 0.32	4.00 ± 0.47
Chloroform stem extract	19.78 ± 0.78	13.22 ± 0.64	9.34 ± 0.38	6.67 ± 0.49
Ethyl acetate stem extract	22.11 ± 0.35	15.56 ± 0.61	11.34 ± 0.40	8.34 ± 0.41
Aqueous stem extract	19.44 ± 0.47	16.22 ± 0.38	11.34 ± 0.35	8.34 ± 0.55
Chloroform root extract	24.11 ± 0.52	20.89 ± 0.63	17.00 ± 0.59	13.67 ± 0.58
Ethyl acetate root extract	25.44 ± 0.85	22.22 ± 0.55	18.00 ± 0.44	12.67 ± 0.55
Aqueous root extract	18.44±0.62	13.56±0.35	8.34±0.52	5.34±0.55

PC= Positive control (Standard drug, Chloramphenicol) Mean ± SD= Mean Zone of inhibition in mm and standard deviation.

The overall results showed that all the four bacterial strains were most susceptible to ethyl acetate and chloroform root extracted samples with maximum zone on inhibitions. On the other hand, all the aqueous extracted samples were least effective against the respective bacterial pathogens.

Results also revealed that the inhibition of the pathogenic bacterial strains was maximum at high concentration and progressively showing a decrease with a decrease in concentration from 2mg/ml to 0.5mg/ml, indicating that the inhibition is concentration dependent.

*Standard drug vs crude extracts*

Comparison of the inhibition potential of the crude extracts of Ethyl acetate and chloroform with the standard drug has been shown in Fig. 1.

The overall results showed that although all the crude extracts were effective but the selected pathogenic bacterial strains were most susceptible to ethyl acetate and chloroform root extract as compared to standard drug (Chloramphenicol). Standard drug has tremendous effect against all the four pathogenic bacterial strains, followed by ethyl acetate root extract with inhibition zone of 26.67 and 25.44mm against *S. aureus* and *K. pneumonia* respectively. tremendous inhibition i.e 25.34 and 24.11mm had also been

reported for chloroform root extract against *S. aureus* and *K. pneumonia*. It is obvious from figure 1 that the root extracts of ethyl acetate and chloroform were highly effective as compared to stem and leaved extracts.

**Discussion**

For the past two decades, there has been an increasing interest in the investigation of various extracts obtained from traditional medicinal plants as potential sources of new antimicrobial agents (Bonjar and Farrokhi, 2004). Results showed that all extracts showed considerable inhibition, which was

concentration dependent. Ethyl acetate and chloroform root extract of *F. tenacissima* L. had the highest antibacterial activity against both Gram-positive and Gram-negative bacteria i.e. *S. aureus*, *B. subtilis*, *P. aeruginosa* and *K. pneumonia* at higher concentrations which are in agreements with the previous findings of the use of this shrub as a potential antibacterial agent (Assaf *et al.*, 2015). Ethyl acetate root extracts exhibited tremendous inhibition against *S. aureus* and *K. pneumoniae* with inhibition zone recorded as 26.67 and 25.44mm at 2.0mg/ml respectively which reflect similar findings from previous studies (Negi and Dave, 2010).

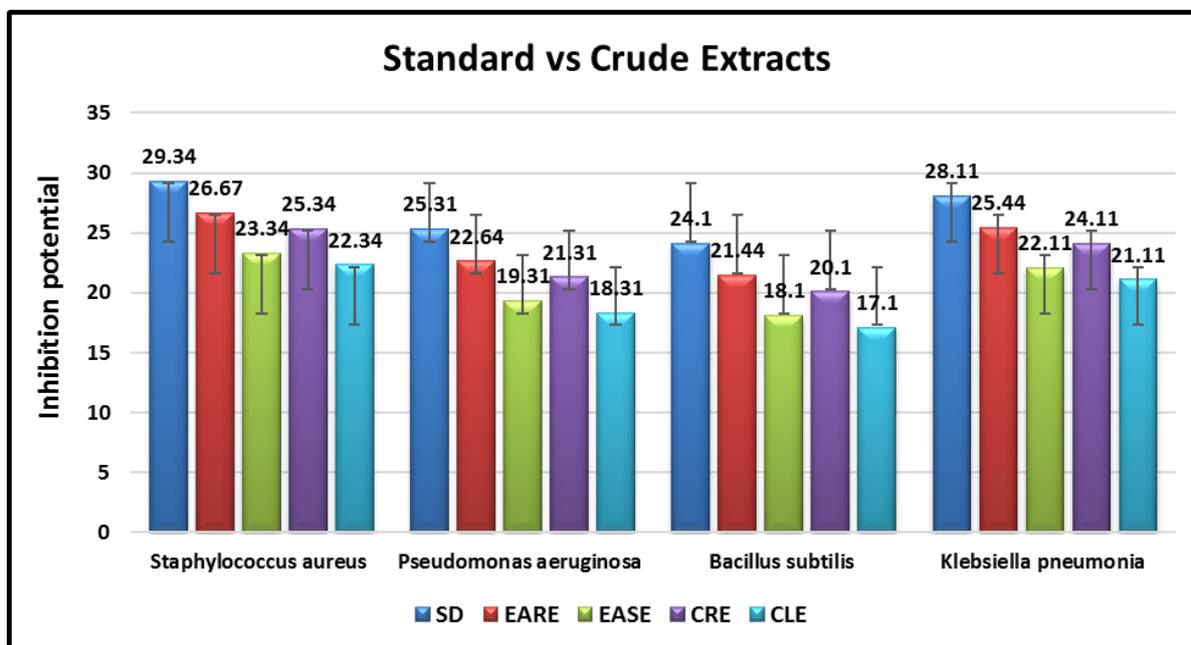


Fig. 1. Comparison of inhibition zone of crude extracts with standard drug.

The roots are used traditionally as chewing stick are known to have an antibacterial effect (Owoseni and Ogunnusi, 2006). The nettle desert is used widely in traditional medicines in Pakistan as antispasmodic, anti-diabetic and antipyretic and anti-inflammatory (Shah *et al.*, 2010), hepatoprotective (Assaf *et al.*, 2017), antioxidant (Alali *et al.*, 2007), antiviral and antibacterial activities (Assaf *et al.*, 2015). In the present work ethyl acetate root extract had the highest antibacterial activity against both Gram-positive and Gram-negative bacteria i.e. *S. aureus*, *B. subtilis*, *P. aeruginosa* and *K. pneumonia* at higher concentrations (2mg/ml) which is supported by a previous study in which *n*-hexane, dichloromethane,

ethyl acetate, methanol extracts of aerial parts of nettle desert (*F. tenacissima* L.) was used for the possible antimicrobial activities via agar cup diffusion method and observed that ethyl acetate fraction showed remarkable antibacterial activity against both Gram-negative bacteria (*E. coli* and *P. aeruginosa*) and Gram-positive bacteria (*S. aureus* and *Bacillus subtilis*). In other studies (Rani *et al.*, 2010; Gulfranz *et al.*, 2011; Ali *et al.*, 2014; Malik, 2015) *E. sativa* L. aqueous, chloroform and ethyl acetate crude extract were used against *S. aureus*, *B. subtilis*, *P. aeruginosa*, *K. pneumonia* and observed that maximum inhibition has been shown by *Eruca sativa* ethyl acetate stem extract against *S. aureus* which

also supports our results in which *S. aureus* was found to be most susceptible. All the bacterial strains were least affected by aqueous crude extracts even at high concentration (2mg/ml). The lowest inhibition zone has been recorded against *B. subtilis* (5.44mm) followed by *P. aeruginosa* (6.64mm), *K. pneumonia* (9.44mm) *S. aureus* (10.67mm) and *K. pneumonia* (9.44mm) at 2mg/ml concentration of aqueous crude extracts. Another study (Aslam *et al.*, 2018) also suggests the anti-microbial activity of three different crude extracts (ethanol, aqueous and n-hexane) of *F. tenacissima* L. leaves against gram-negative and gram-positive bacteria and fungi using well diffusion method. This study showed N-hexane extract exhibit maximum inhibition against *S. aureus* and *B. subtilis* at 1000 µg/ml concentration which also supports the present work.

### Conclusion

Ethyl acetate root extracts exhibited wondrous inhibition against *S. aureus* and *K. pneumoniae* with inhibition zone recorded as 26.67 and 25.44mm at 2.00mg/ml respectively. Phytochemical screening of the plant is required for further justification.

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