INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 13, No. 6, p. 95-101, 2018

RESEARCH PAPER OPEN ACCESS

Plant and microbes interaction: the potential of *Calotropis gigantea* extract and latex towards bacterial strains

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Key words: *Calotropis gigantea,* Bacterial Strains, Extract, Latex.

http://dx.doi.org/10.12692/ijb/13.6.95-101 Article published on December 23, 2018

Abstract

The microbial resistance has prominently diminished the efficacy of antibiotics and also the major cause of failure to treat bacterial infections. In the backdrop of this phenomenon, phytochemical or herbal medicines are gaining attention as the alternative source of natural antibiotics. In the present study, leaves extract and milk (latex) of *Calotropis gigantea* were tested for antibacterial activity. For this purpose, well diffusion method was evaluated fortwo-Gram positive (*Staphylococcus aureus*, *Streptococcus pyogenes*) and two-Gram negative (*Escherichia coli*, *Klebsiella pneumonia*) bacterial strains. Results revealed significant antimicrobial activities of the tested samples. The inhibition zones ofbacterial strains were observed in the range of 9 to 32 mm. At a concentration of 5 mg/ml leaves,activity was found good against *S. pyogenes* and *K. pneumoniae* while 16 days old milk (latex) against *S. aureus*. Excellent zones of inhibition were showed by latex against *E. coli at a* concentration of 10 mg/ml. *E. coli* and *K. pneumoniae* showed resistant to leaves extract and fresh milk (latex) at 15 mg/ml concentration. Leaves extract at a concentration of 20 mg/ml showed excellent inhibitory zones against *S. aureus*. Moreover, extract showed excellent inhibitory zones to *S. pyogenes* as compare to 5 days old milk (latex) at a concentration of 50 mg/ml. Furthermore, it was concluded that leaves extract and milk (latex) of *C. gigantea* could be used as an alternative to antibiotics against different diseases because of their substantial antibacterial activities.

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Introduction

In ancient time, the plants were exploited to cure various diseases. Despite the improvement in the pharmacological industry, developed countries people rely on plants as a source of medicines(Newman *et al.*, 2000). A very small portion from reported plant species (250,000 – 500,000) has been screened phytochemically. In past, natural and synthetic compounds have served as a significant source of therapeutic agents during pharmacological evaluation.

The evaluation of these random experiments will helpful in the finding of new therapeutic compounds and will be mile stone in the field of antibiotics(Saket & Singh, 2017).

In the present time, different pharmaceutical industries consuming their time on developing of natural products from plants extract (Mahmood *et al.*, 2011). This type of practices will make easy availability of cost-effective drugs to common people. Besides the cost issue, it is also observed that some bacteria strains show resistance to synthetic drugs as widely reported in the literature(Davies, 1994).

Recently, pathogenic microbes demonstrate strong antibiotics resistance which is associated with side effects. To overcome these problems, researchers have turned their attention towards plant extract and biologically active compounds extracted from plants (Essawi & Srour, 2000).

The present research work was conducted to investigate the biological potential of *Calotropis gigantea* extract and latex against bacterial strains. The *C. gigantea* is a xerophyte native to Southeast Asian countries, tropical Africa, South Asia includes Pakistan, India, Srilanka,and Nepal(Wang *et al.*, 2008). These shrubs are growing about 4 meters in height. It is also considered as milky habit plant with oblong leaves (Nobel, 1980).

The purpose of the current study was to explore the alternative methods of healthcare in view of the problem of antibiotic resistance witnessed in both the developed and developing countries, particularly Pakistan.

Material and methods

Sample preparation and extraction

Leaves and milk (latex) were collected from *Calotropis gigantea* plant from Spin Khak, District Nowshera, KP province of Pakistan. Both samples of the plant were carried in the icebox to the microbiology research laboratory, Abasyn University Peshawar for further analysis.

For the preparation of extract, the leaves of C. *gigantea*were first washed with tap water and thrice times with distilled water. The leaves were then kept for shade drying at room temperature for 16 days. After that, the leaves were grinded into a fine powder using an electric grinder.

The 25g powder was added into 500 ml flask and boiled by adding 100ml of distilled water for 20 mins at 150°C.The mixture was then kept overnight in an incubator at 37°C for maximum extraction.

The extract was then filtered by using Whatman filter paper. In addition, different concentrations i.e. 1 mg/ml, 5 mg/ml, 10 mg/ml, 15 mg/ml, 20 mg/ml and 50 mg/ml were prepared from the stock by using distilled water. The sample extracts were kept in the refrigerator (4°C) for further analysis (Wilson, 1995).

The milk (latex) of *C. gigantea* were collected from the aerial parts of the said plant in airtight glass tubes and kept in the refrigerator (4°C) for further analysis (Wilson, 1995).

Bacterial strains

The potency of leaves extract and milk (latex) was investigated against four pathogenic bacterial strains, two-Gram positive (*Staphylococcus aureus*and *streptococcuspyogenes*) and two-Gram negative (*Escherichia coli* and*Klebsiella pneumonia*). All bacterial strains were kindly donated by Department of Microbiology, University of Peshawar, KP,

Pakistan. Different biochemical tests were performed for the strains identification and then kept in agar slant at 4°C(Cowan, 2003).

Antibacterial assay

The antibacterial activity of leaves extract and milk (latex) of *C. gigantea* plant was carried out by well diffusion method. First of all, nutrient agar plates were equipped, which is then labeled and inoculated with test organisms under aseptic conditions. Afterward, cork borer was used to make 5mm diameter wells in the agar plate. The equal volumes (50 µl) of leaves extract, milk (latex) were poured into the wells using micropipette. Four wells at the side of Petri dishes and one well at the center were made. Gentamycin (1 mg/ml) was used as a positive control in the center well. The incubation period for 24 h at 37°C was carried out for all the Petri dishes. The zone of inhibition was measured in mm after the incubation period.

Results

In this study, *C. gigantea* leaves extract and milk (latex) were tested against various identified bacterial strains. Furthermore, the antibacterial activity of leaves extract was measured by the presence or absence of zone inhibition at different concentrations i-e. 1, 5, 10, 15, 20 and 50 mg/ml.

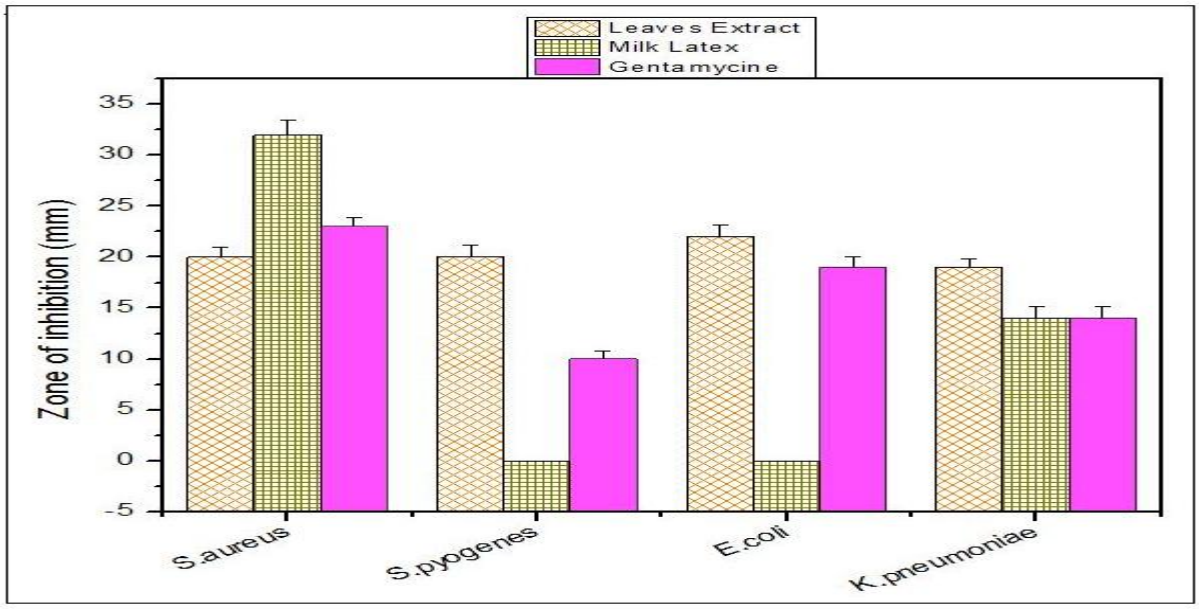

Fig. 1. Antibacterial activity of leaves extract at conc. of 5 mg/ml and 16 days old milk (latex).

The activity of leaves extract at conc. of 1 mg/ml and fresh milk (latex)

No potential activity was observed while using 1mg/ml concentration of the extract as well as fresh milk (latex).

It is clear from these findings that at a concentration of 1 mg/ml leaves extract showed resistance to *S. aureus, S. pyogenes, E. coli, K. pneumoniae* as well as to fresh milk (latex) respectively.

Activity of leaves extract at conc. of 5 mg/ml and 16 days old milk (latex)

The 16 days old *C. gigantea* milk (latex) showed excellent inhibitory zone as compared to leaves extract against *S. aureus*, while *S. pyogenes* showed resistance to 16 days old milk (latex) and the leaves extract showed best inhibitory zone against *S. pyogenes*. On other hand, the old milk (latex) showed no activity against *E. coli*, and the leaves extract showed excellent inhibitory zone against *E. coli*. Leaves extract showed better activity against *K. pneumoniae* while latex showed minimum inhibitory zone as compare to leaves extract against *K. pneumoniae* as shown in Fig. 1.

Activity of leaves extract at conc. of 10 mg/ml and 7 days old milk (latex)

Leaves extract and milk (latex) of *C. gigantea* showed best inhibitory zones at 10 mg/ml. Leaves extract showed best activity as compared to latex against *S. pyogenes* while milk (latex) showed excellent inhibitory zone as compared to leaves extract against *E. coli* and *K. pneumoniae*. As shown in Fig. 2.

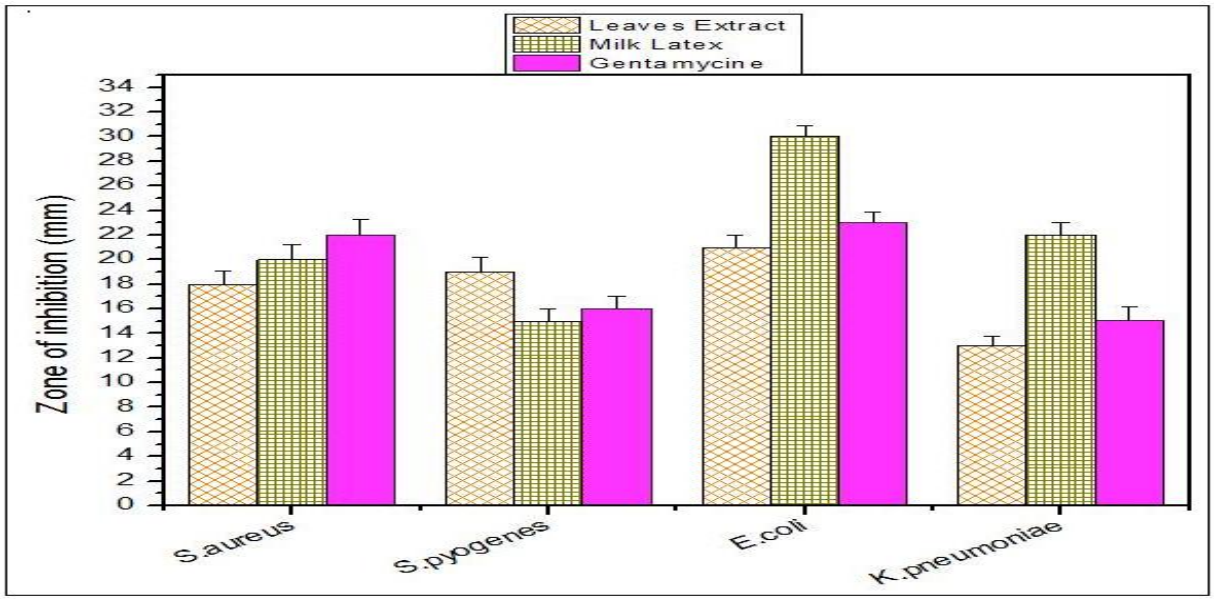

Fig. 2. Antibacterial activity of leaves extract at conc. of 10 mg/ml and 7 days old milk (latex).

The activity of leaves extract at conc. of 15 mg/ml and fresh milk (latex)

Leaves extract showed the best inhibitory zone against *S. aureus* as compare to Fresh milk (latex) while*E. coli* and *K. pneumoniae* showed resistant to leaves extract and fresh milk (latex). Moreover, it was also observed that *S. pyogenes* also showed resistant to fresh milk (latex) as shown in Fig. 3.

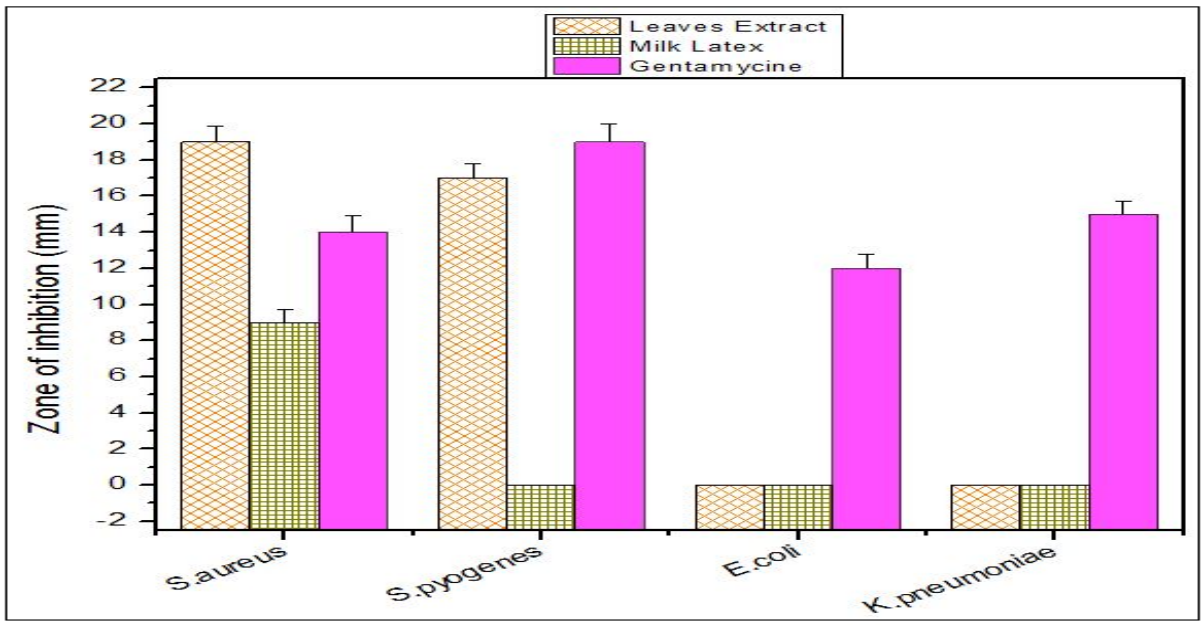

Fig. 3. Antibacterial activity of leaves extract at conc. of 15 mg/ml and fresh milk (latex).

The activity of leaves extract at conc. of 20 mg/ml and fresh milk (latex)

Fresh milk (latex) was used against *E. coli, K. pneumoniae, S. aureus* and *S. pyogenes* which showed significant results while *E. coli* showed resistance to leaves extract at conc. of 20 mg/ml.

Furthermore, it was observed that *S. pyogenes* showed resistance to extract as well as to fresh milk (latex) while leaves extract at conc. of 20 mg/ml showed excellent inhibitory zones against *S. aureus* as compared to fresh milk (latex) as shown in Fig. 4.

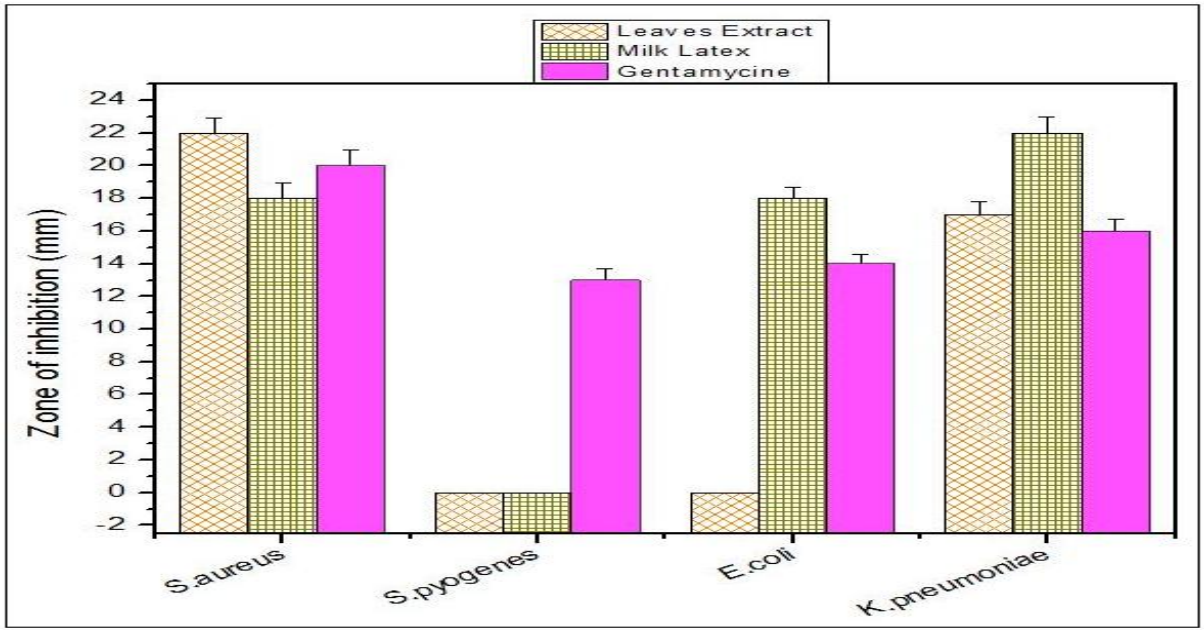

Fig. 4. Antibacterial activity of leaves extract at conc. of 20 mg/ml and fresh milk (latex).

The activity of leaves extract at conc. of 50 mg/ml and fresh milk (latex)

Milk (latex) of *C. gigantea* showed good results against *E.coli* as compare to leaves extract. Moreover, extract showed excellent inhibitory zone to *S. pyogenes* as compare to 5 days old milk (latex). Furthermore, milk (latex) showed less zone of inhibition against *K. pneumoniae* as compare to leaves extract while*S. aureus* showed less resistance to 5 days old *C. gigantea* milk (latex) as compare to leaves extract as shown in Fig. 5.

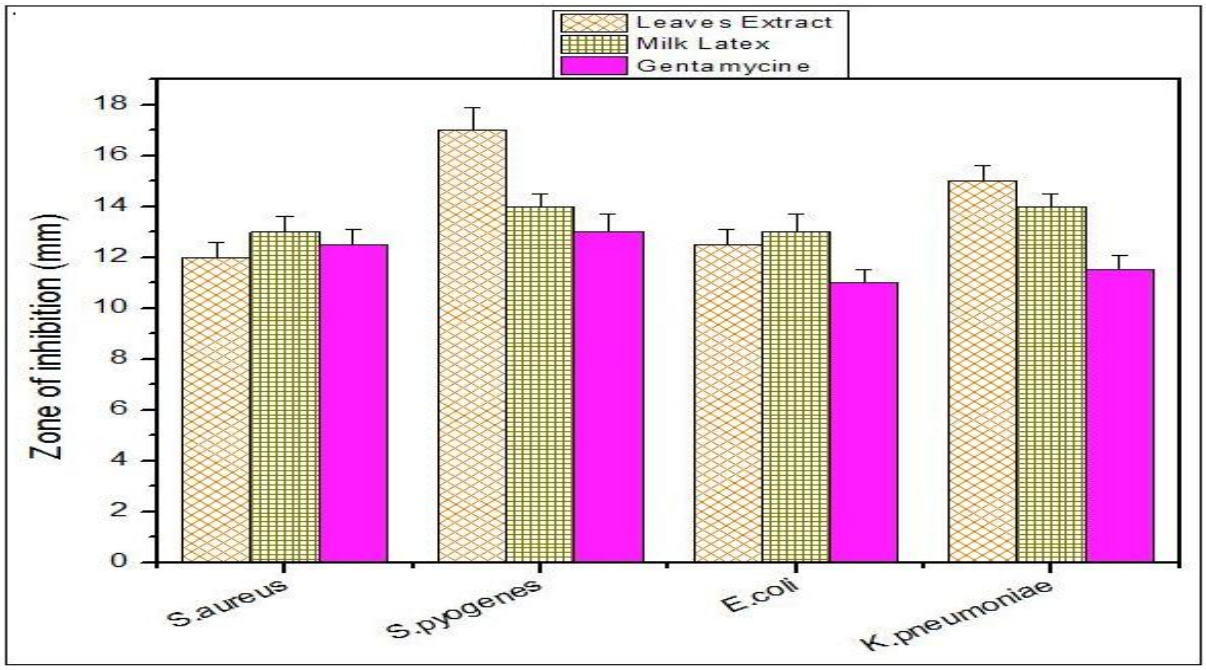

Fig. 5. Antibacterial activity of leaves extract at conc. of 50 mg/ml and fresh milk (latex).

Discussion

World has diverse vegetation. The rural area of many countries mainly relays on plant-based drugs because they are inexpensive and has no side effects.

In the present work, *C. gigantea* displayed antibacterial activity againstGram-positive (*Staphylococcus aureus*, *Streptococcus pyogenes*) and Gram-negative (*Escherichia coli*, *Klebsiella pneumonia*).

The leaves extract were found to have most susceptibility against *S. aureus* (22 mm) and *E. coli* (21 mm). However, the current activity is less as compared to the study reported by (Kumar *et al.*, 2010), where they carried out (24.6 mm) for *E. coli* and (30 mm) for *S. aureus*.

Furthermore, milk (latex) of the plant indicated maximum antibacterial activity against all the strains. Our study has a strong agreement with the previous study reported by (Nenaah & Ahmed, 2011), where they revealed strong antibacterial activity while using Latex of *Calotropis procera*.

(Jayakumar *et al.*, 2010)the study showed that methanolic extract of *C. gigantea* was found to exhibit the growth of *E. coli, S. aureus,*a nd *K. pneumonia*. *Calotropis gigantea* evidenced a higher activity as indicated in current work.

Another study reported by (Subramanian & Saratha, 2010) indicated that ethanolic extract of *C. gigantea*possesses antibacterial activity against all pathogenic strains which shows strong agreement with the current study.

Plants produce an antioxidant effect, owing to the presence of flavonoids, polyphenols, tannins, and phenolic terpenes (Rahman *et al.*, 2007). A number of neurodegenerative diseases and AIDS, cancer could be cured due to the presence of free radicals in plants. Present research work suggested that *C. gigantea* extract has a strong potential for pathogenic bacterial strains. However, it is recommended that botanical preparations as medicine could be considered safe and to be effective against pathogenic organisms. The results of the present study provide a scientific validation for the popular use of medicinal plants.

Acknowledgments

The authors are thankful to the Department of Microbiology, Abasyn University Peshawar for providing research facilities.

Declaration of interest

None of the authors of this paper had any personal or financial conflicts of interest.

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