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RESEARCH PAPER

Antimicrobial activity of Latex of *Calotropis gigantea* against some bacteria

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Key Message: In this study, it was demonstrated that *Calotropis gigantea* latex extract has an important antiperspirant activity for a variety of bacterial pathogens such as *Staphylococcus aureus, Lactobacillus spp. and Escherichia coli*. Our findings highlight the promising therapeutic potential of *Calotropis gigantea* in combating microbial infections.

Abstract

Medicinal plants contain a plethora of biologically active compounds synthesized through primary and secondary metabolism, offering diverse therapeutic potentials. *Calotropis gigantea* L., commonly known as milkweed emerges as a promising candidate due to its extensive traditional use and scientifically proven medicinal properties. The aim of the study was to evaluate the antibacterial activity of *Calotropis gigantea* latex extract against different types of harmful bacteria. The present study consisted of assembling *Calotropis gigantea* latex from plants in Rawalpindi, Islamabad, followed by extraction and preparation of an aqueous extract. The agar well diffusion method with appropriate controls was used as antimicrobial assays. In order to determine relative percentage inhibition and minimum inhibitory concentration (MIC), crude latex extract was used. The findings exhibited significant inhibition of *Staphylococcus aureus*, Lactobacillus, and *Escherichia coli* using latex extract compared to controls. The zone of inhibition for *Staphylococcus aureus* was measured as 30 ± 1 mm, for Lactobacillus was measured as 16 ± 1 mm, and for *Escherichia coli* zone of inhibition was measured as 24.6 ± 0.5 mm. With a confidence level of $p<0.05$, these results have been confirmed by data analysis. The relative percentage inhibition was measured as 290.5% for *Staphylococcus aureus*, 200.4% was measured for Lactobacillus, and a remarkable 381.1% was measured for *Escherichia coli*. In the case of *Staphylococcus aureus* and *Escherichia coli*, a minimum inhibitory concentration of 62.5 g ml was measured with *Lactobacillus* showing slightly higher MICs at 125 g ml. These findings indicate the significant antimicrobial potential of *Calotropis gigantea* latex extract against a diverse range of microorganisms, emphasizing its promising therapeutic applications in combating microbial infections. Further research is needed to explore its mechanisms and optimize its medicinal properties.

Keywords: Aqueous extract, Biologically active compounds, *Calotropis gigantean*, Minimum inhibitory concentration (MIC), Pathogenic bacteria, Therapeutic potentials

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Introduction

Medicinal plants, especially in traditional healthcare systems, are of great importance for the protection of human and community health. These systems not only contribute significantly to human health but also increase natural defenses of the body against diseases (Peterson & Dalhoff, 2004). Plants contain a plethora of potent biologically active compounds, each with distinct medicinal properties, making them precious resources for healing (Cowan, 1999; Bhavnani & Ballow, 2000). Over time, with herbal medicines meeting the health needs of approximately 80% of the global population, there has been an increase in global demand and recognition of traditional remedies. The therapeutic properties of these plants are based on special chemical constituents that produce accurate physiological responses to the human body, which form the basis for conventional treatments. (Farnsworth, 1994). In addition, the ancient practice of using plant derived medicinal products for the treatment of fungal infections in humans and animals is considered to be a valuable source of knowledge, which holds promise for the discovery of novel medicinal products (Nwosu & Okafor, 1995).

The bioactive substances encompass a variety of compounds such as tannins, alkaloids, carbohydrates, terpenoids, steroids, and flavonoids (Edeoga et al., 2005). Within living organisms, these compounds are synthesized by either primary or secondary metabolism. Secondary metabolites, characterized by their chemical diversity and often incomprehensible functions, have been historically utilized for human therapeutic purposes (Vasu et al., 2009). Traditionally, the pharmacological analysis of compounds, whether of natural or synthetic origin, has generated numerous therapeutic agents. Communicable infections, particularly those affecting the skin and mucosal surfaces are common among the general population. Fungi, including dermatophytes and Candida spp., along with certain pathogenic bacteria, constitute a significant proportion of these skin pathogens (Caceres et

al., 1993; Schmourlo et al., 2005). Moreover, in recent years, the prevalence of immunosuppressed and immunocompromised patients has increased, leading to increased concerns over multidrug resistance.

Calotropis gigantea L. is a shrub having moderate size, commonly reaching elevations of 2 to 3 meters, with white haired young parts and it has light-colored bark. Approximately 10 to 20 cm long and 3 to 8 cm wide, the leaves are either obovate or oblong. Its corolla diameter ranges from 1.5 to 2.5 cm and is mostly white, although sometimes it has dull purple or purplish orlilac colour; its lobes are ovatelanceolate and spread outward. (Kumar et al., 2010a). *C. giganteawort*, commonly referred to as milkweed or swallowwort, is a common weed that grows in landfills. It's originally from a number of Asian countries such as Bangladesh, Burma, China, Indonesia, Malaysia, Pakistan, the Philippines, Thailand and Sri Lanka., However, in India, where people have been using *C. gigantea* in traditional medicine for a long time, a practice supported by scientific research, it is quite common. (Witit et al., 2002; Bharathi et al., 2011).

Today, the *Calotripis gigantea* plant is an important asset worldwide for its wide range of therapeutic uses and related health benefits. Its ingredients like seeds, leaves, latex or roots have been treated for diseases in old systems such as Ayurveda and Unani because of the development of their basic components into modern medicines, cosmetic products, toiletries and herbal remedies Apart from its herbal properties and ecological compatibility, *Calotripis gigantea* has a complex therapeutic profile. A good tonic, expectorant, depurative and antihelminthic agent is provided by the dried whole plant. In addition, its dried root bark contains febrifuge, antihelminthic, depurative, expectorant and laxative properties as an alternative to ipecacuana. The bark can be used to treat skin diseases, stomach infections, bacterial infections, coughs and ascites. Moreover, root powder has also been shown to be effective in treating asthma, bronchitis, indigestion, lupus, tuberculosis, leprosy, syphilis ulcers and other diseases, as well as treating liquid exhaust gas. The leaves exhibit therapeutic potential in treating paralysis, arthralgia, swellings, and intermittent fevers, while the bitter flowers provides digestive, astringent, stomachic, bechic, antiasthmatic, antihelminthic, tonic, and analgesic properties. There is also a significant place for Calotropis in homeopathy. In addition, its latex has potent fungicidal activity and shows a wide range of medicinal potential; both leaves and latex also exhibit antimicrobial properties (Almagboul et al., 1985; Pathak & Argal, 2007; Kumar et al., 2010a; Kumar et al., 2010b; Sharma et al., 2015; Dubey et al., 2020).

The plant has a reputation for its large production of latex. Latex is an excellent source of a wide range of bioactive compounds, including glycosides, tannins and many proteins. In the treatment of external swelling and diarrhoea, leaves and aerial parts of the plant shall be used (Das et al., 2011). Furthermore, latex is known to have a purgative effect and wound healing activity that makes it an important medicinal product (Kareem et al., 2008). In this study, the antibacterial activity of *C. gigantea* against three different species of *Staphylococcus aureus, Lactobacillus and Escherichia coli* was studied under laboratory conditions.

Materials and Methods

Plant material

Giant plants have been picked up in the wastelands of Rawalpindi and Islamabad in December 2012.

Collection of the plant's latex

Apical segments of *C. gigantea* were collected and fresh latex was extracted from them. the samples of latex were collected and sent to Department of Biology, AIOU, Islamabad., where they were dried in a hot oven at the temperature of 42 °C. Afterward, the dried latex underwent pulverization using a mortar and pestle to achieve a powdered form.

Chemicals

Various chemicals including supplement broth (NB), Mueller-Hinton agar (MHA), Sabouraud dextrose agar (SDA), penicillin G plate, polymyxin-B plate, amoxicillin plate, amphotericin-B circle, and dimethyl sulfoxide (DMSO) were obtained from Rawalpindi. Additionally, potassium bromide (KBr-FTTR review) was also obtained from Rawalpindi.

Preparation of aqueous extract

The powdered latex (10 g) underwent extraction using 100 ml of distilled water for a two-day period at room temperature. Four layers of muslin cloth were used to filter the resulting extract, followed by concentration using a rotavapor, and then drying. The resulting extract powder was dissolved in DMSO, yielding a 100 mg/ml (10% w/v) solution.

Determination of antimicrobial activity

Test microorganisms

Pathogenic bacteria, including *S. aureus*, *Lactobacillus*, and *E. coli*. aureus, has been tested against the liquid extract of *C. gigantea latex*.

Positive and negative controls

For *S. aureus* and Lactobacillus, a penicillin G plate (10 µg/disc) was utilized as the positive control (PC), while for *E. coli*, a polymyxin-B plate (10 µg/disc) served as the positive control. The negative control was performed using DMSO (NC).

Antimicrobial assay

The agar well diffusion method has been used to assess the antimicrobial activity of the crude latex extract. Prior to the test, microbiological suspensions have been adjusted to a 0.5 McFarland standard. Bacterial suspensions were inoculated onto Mueller-Hinton Agar (MHA) plates and fungal suspensions onto Sabouraud Dextrose Agar (SDA) plates in triplicates.

On each plate, the sterilized cork borer was used to create two wells aseptically. 100 l of oil extract and adverse control were added to separate wells by the use of micropipette. For comparison, a positive antimicrobial control plate has also been included on the same plate. After inoculation bacterial plates were placed in a refrigerator at 37 °C for 24 hours of incubation and fungal plates are left to incubate up to 72 hours under room temperatures. For the purpose of assessment of antibacterial activity, a zone of inhibition has been established in each borehole.

Determination of relative percentage inhibition

For the determination of the relative inhibition percentage of the crude latex extract compared to the positive control, the following formula has been used:

$$\text{Relative percentage inhibition} = \frac{100 \times (a-b)}{(c-b)}$$

Where

a denotes the total area of inhibition of the test extract.
b denotes the total area of inhibition for a solvent.
c denotes the total area of inhibition of the standard drug.

Using the formula, the total area of inhibition was calculated. Area = πr^2; where, r denotes radius of zone of inhibition.

Determination of minimum inhibitory concentration (MIC)

a modified agar well diffusion method was used to find the minimum inhibitory concentration(MIC) of the crude extract. The extract was dissolved in dimethyl sulfoxide (DMSO) to achieve a concentration range spanning 62.5, 125, 250, 500, 1000, 5000, and 10000 µg/ml. Bacterial suspensions were inoculated onto Mueller-Hinton Agar (MHA) plates and fungal suspensions onto Sabouraud Dextrose Agar (SDA) plates in triplicates. Four wells were aseptically created on each plate using a sterile plug borer. Following this, 100 µl of each dilution of the extract was dispensed into the wells using a micropipette. For 24 hours bacterial plates were incubated at 37 C, while for 48 to 72 hours the fungus plate was housed at room temperatures. The minimum concentration of the extract, which has created a zone of inhibition in the vicinity of the well, was considered to be minimum inhibitory concentration (MIC). This method allows for the determination of the lowest concentration of extract to prevent microorganism growth and provides essential information on its potency as an anticonvulsive agent.

Statistical analysis

The mean ± standard deviation from three independent measurements per experiment is represented by the antimicrobial activity results of *C. gigantea* latex extract. Using the Student's twinning test at significance level of $p < 0.05$, statistical significance was established. Microsoft Excel 2007 data analysis was used to determine statistical significance (Roselle, IL, USA). In order to ensure the accuracy and reliability of the results, this statistical approach allows for meaningful comparisons and conclusions to be drawn from the experimental data.

Results

Antimicrobial activity of *Calotropis gigantean*

Calotropis gigantea was selected for study because of its pharmacological properties and the history of therapeutic uses. *Calotropis gigantea* is known for its antimicrobial, anti-diarrheal, antipyretic, wound healing, and central nervous system (CNS) modulating effects. In this research study, a crude aqueous extract of *Calotropis gigantea* latex was tested against pathogenic organisms.

The experiment was conducted on *Staphylococcus aureus*, Lactobacillus and *Escherichia coli* by means of a Positive or Negative Control to assess the antibacterial activity of latex extract from *Calotropis gigantea*. Compared to controls, the area of inhibition that served as an indication of microbial growth inhibition was significantly larger for latex extract and has shown a powerful antimicrobial effect (Table 1). Statistical analysis confirmed these findings with a confidence level of $p < 0.05$. In addition, against the test bacteria, latex extract showed more potent inhibitory effects than traditional antibacterial agents (Table 1). *Calotropis gigantea* was selected on the basis of its traditional medicinal uses, including antimicrobial properties. These results demonstrate the potential of *Calotropis gigantea* latex extract for further studies in pharmaceutical and medical applications due to its significant antimicrobial activity against the tested organisms.

Table 1 Assessment of the antimicrobial activity of *Calotropis gigantea* latex extract on bacteria

	Zone of inhibition (mm)		
Test organisms	Latex	PC	NC
Staphylococcus aureus	30 ± 1	17.6 ± 0.5	-
Lactobacillus	16 ± 1	11.3 ± 0.5	-
Escherichia coli	24.6 ± 0.5	12.6 ± 0.5	-

All reported values are mean ± standard deviation (n=3); PC denotes positive control; NC denotes negative control; Zone of inhibition measurements exclude the well diameter.

Relative percentage inhibition of *Calotropis gigantean*

The results presented in Table 2 shows the relative percentage inhibition and minimum inhibitory concentration (MIC) of latex extract from *Calotropis gigantea* on various tested organisms. In the test organisms

from latex extracts, significant antimicrobial activity was observed. The highest relative percentage inhibition against *Escherichia coli* was achieved with a remarkable value of 381.1%. The latex extract showed a potent inhibitory effect against *Staphylococcus aureus* and *Escherichia coli* with MIC values of 62.5 g mL or more. The MIC of 125 g mL has been shown to be a little higher in Lactobacillus, indicating that the extract is somewhat less susceptible. These results demonstrate that the latex extract of *Calotropis gigantea* has a strong antibacterial activity against many different types of organisms. Its potent inhibition effects, especially with *Escherichia coli*, demonstrate the positive therapeutic use of this extract in the fight against bacterial infections. Further research and examination of the mechanisms that underlie its antibacterial activity is needed in order to make full use of its therapeutic potential.

Table 2 The relative percentage inhibition of *Calotropis gigantea* latex extract on the test organisms

Test organisms	Relative percentage inhibition (%)	MIC (µg/ml)
Staphylococcus aureus	290.5	62.5
Lactobacillus	200.4	125
Escherichia coli	381.1	62.5

MIC = Minimum inhibitory concentration

Discussion

The world has a rich array of vegetation. In many rural areas across different nations, plant-based remedies are crucial due to their affordability and minimal side effects (Numan et al., 2018). Plants serve as a primary source of potentially valuable compounds for the development of novel chemotherapeutic agents. It is well known that traditional use of plant extracts is used to fight a variety of infectious diseases caused by bacteria, fungi, protozoa and viruses (Nejad & Deokule, 2009). However, the emergence of multidrug resistance poses a major challenge in the healthcare industry, as various pathogenic microbes exhibit resistance to a wide range of drugs (Sathish Kumar et al., 2011).

These multidrug-resistant organisms pose significant challenges, often proving lethal and difficult to treat (Maragakis et al., 2008; Raghunath, 2008). In immunocompromised patients, infection rates and the severity of multidrug resistant organisms are very high (Sharma et al., 2011). Addressing these problems necessitates the discovery and development of new drug molecules. Secondary metabolites in plants include phytochemical constituents such as aromatic compounds, flavonoids, alkaloids, metabolites and other bioactive substances. These metabolites function as a defense mechanism against the predation of various organisms, insects and herbivores (Cowan, 1999). *C. gigantea* has been shown to have antibacterial activity against both gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacteria in the current study.

Various bioactive substances, including alkaloids, flavonoids, glycosides, saponins, tannins, steroids, triterpenoids, and phenols, have been detected in phytochemical screening of ethanolic extracts of latex from a specific plant species. (Sharma et al., 2015). In previous studies, the presence of Cardenolides, flavonoids, terpenes, pregnanes, nonprotein amino acids and cardiac glycosides was noted as significant constituents of *C. gigantea* suggesting that they may have therapeutic properties (Saratha & Subramanian, 2010). A bioactive compound was separated from the crude chloroform extract (Rf value: 0.95) via a thin layer chromatography (TLC) technique and prestandardized solvent system (chloroform: methanol, 8: 2). Bioautographs against *S. mutans* have been performed on the resulting chromatogram. In addition, against the plant pathogenic fungus *Fusarium mangiferae*, the antifungal potential of *C. gigantea* has been reported (Usha et al., 2000).

Our findings differ from Sukanya et al. (2009), who stated that *C. gigantea* is not effective or shows poor inhibition in testing for human pathogens like *Escherichia coli* and *Staphylococcus aureus*. In contrast, Kumar et al. (2010) reported a significant high effect on *S. aureus*, *B. cereus*, and *E. coli*, with a moderate effect on *C. krusei*, while no effect was noticed on *M. luteus*, *K. pneumonia*, *P. aeruginosa* and *A. niger*. Different levels of efficacy on all tested microorganisms have been verified by the antimicrobial activity of different solvent extracts from *C. gigantea* (Saratha & Subramanian, 2010). Moreover, the latex (milk) of the plant exhibited maximum antibacterial activity against all strains. Our findings align strongly with the findings of Nenaah and Ahmed (2011), who reported strong antibacterial activity using latex from *Calotropis procera*. Similarly, Jayakumar et al. (2010) found that the methanolic extract of *C. gigantea* inhibited the growth of *E. coli*, *S. aureus*, and *K. pneumonia*. Our current work indicates even higher activity. The antibacterial activity of the ethanolic extract of *C. gigantea* against all pathogenic strains was demonstrated in another study conducted by Saratha and Subramanian (2010) which supports our finding. According to our research, *C. gigantea*'s extract has great potential against harmful bacterial species. However, in order to ensure the safety and effectiveness of these preparations for pathogen control it is advisable to consider them as medicines with caution. Traditional use of medicinal plants is scientifically validated by the results of our study.

The researchers used an ethanolic extract of *C. gigantea* in an early research study, which proved to be more effective than other extracts (Sharma et al., 2015). The polar character of ethanol, which allows more active ingredients to be leached from calotropis instead of other solvents for the extraction of antimicrobial substances, may have contributed to this superiority (Ahmad et al., 1998; Kareem et al., 2008). The antimicrobial potential of *C. gigantea* extract for various bacteria, including Sarcina lutea, *Bacillus megaterium*, *B. subtilis*, *Shigella sonnei*, *Escherichia coli* and *Pseudomonas aeruginosa* has been shown in prior studies. Therefore, in this study we sought

to assess the antibacterial activity of *Calotropis gigentica* against *Staphylococcus aureus*, *Lactobacillus* and *Escherichia coli*. In order to assess the anticarbogenic potential of *Calotropis gigentica*, the presence or absence of inhibition zones and their respective diameter have been used. Our studies showed that the ethanol in *Calotropis gigentica* has been shown to have a maximum inhibitory effect on *Staphylococcus* aureus and *E.coli* at doses which are unpredictable. Medicinal products from plants and phytochemicals are recognized for their antibacterial properties, which have a substantial therapeutic potential. A number of studies have shown the efficacy of these substances during the last decade. Due to the presence of different compounds synthesized in their secondary metabolism, several plant species have been examined for antimicrobial properties. Therefore, against a wide range of microorganisms, plant extracts are considered promising sources for antimicrobial agents (Sharma et al., 2015).

Previous studies have demonstrated the broad-spectrum antibacterial potential of acetone, ethanol, and aqueous extracts derived from both shade-dried leaves and fruits of *C. gigantea*, indicating their suitability for integration into ethnomedical practices (Ishnava, 2012; Kori & Alawa, 2014). We have established the minimum inhibitory concentration (MIC) in our study through modified agar well diffusion method. The aqueous extract expressed an MIC of 62.5 µg/ml against *Staphylococcus aureus* and *Escherichia coli*, and 125 µg/ml against *Lactobacillus*. The use of multitarget screening techniques for medicinal plants extracts and herbal medicines presents a promising opportunity to discover novel therapeutic activities (Wang et al., 2008). We've found that the latex extract of *C. gigantea* has a great deal of antibacterial activity in relation to many different microorganism types. The results show the effectiveness of organic cultivation to combat infectious diseases.

Conclusion

In comparison to control, the latex extract of *Calotropis gigantea* has been shown to significantly inhibit bacterial growth and indicates that it can have antibacterial properties. An inhibitory effect of the latex extract on the tested bacteria, which is superior to traditional antibacterial agents, has been demonstrated. The relative percentage inhibition against *Escherichia coli* is of particular importance. The potential effects of latex extract on *Staphylococcus aureus* and *Escherichia coli* were further demonstrated by the minimum inhibitory concentrations (MIC). Lactobacillus showed a slight reduction in susceptibility. Further research on the mechanisms underlying its antimicrobial activity is needed to enhance the medicinal properties of this product for potential pharmaceutical and medical applications.

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