Antimicrobial Activity of *Calotropis procera* against Pathogenic Microorganisms

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Abstract

Plants and their active compounds are safely used in treatment of several diseases. The leaf extract and latex of Calotropis procera (C. procera) are prepared in chloroform, ethanol and aqueous extracts against clinically important pathogenic bacteria and fungi. Antibacterial activity of C. procera extract and latex is done using agar well diffusion method. The leaves extract of C. procera has antibacterial activity on pathogenic microorganisms. The widest zone of inhibition of 19mm for M. morganii, E. coli and S. Typhi is demonstrated while minimum of 13mm for P. alcalifaciens by the ethanolic leaf extract of C. procera latex in ethanol shows good activity. M. morganii, Y. enterocolitic, S. Typhi,S. marcescens with a zone inhibition of 13mm, followed by B. megaterium, S. aureus, and P. fluorescens of 12 mm. C. procera latex extracts in Chloroform possess good activity against S. Typhi with an inhibition zone of 12mm. The extract shows low activity against C. albicans (yeast) (5, 9, & 3mm) and F. oxysporum (6, 9 & 5mm). While the extract was active H. maydis and F. oxysporum 11mm zone of ethanol extract. We recommend C. procera latex in comparison to leaf extract for medicinal purposes in pharmaceutical industries. It is concluded that chloroform, ethanol and aqueous of C. procera leaf extract and latex have antibacterial as well as the antifungal potentials against clinical strains and thus derive antimicrobial agents especially M. morganii, E. coli, S. Typhi and P. alcalifaciens.

Keywords**:** *Calotropis procera*; Antimicrobial Activity; Minimum Inhibitory Concentration; Plant Extract.

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Introduction

Herbal Plants from the very beginning in human life are used as a medicine in the case of various health issues and harmful diseases. Usage of plants for therapeutic purposes and in the improvement of human health has been under consideration since the ancient times. Basically, the plants secondary metabolites known as phytochemicals derived from a Greek word, has the potential against various types of infection caused by multiple microorganisms (Shakya, 2016). The practices of traditional medicine are widespread in various countries including Pakistan, India, Japan, Sri Lanka, Thailand, and China, almost 46% of the total medicinal consumption is attributed to traditional tribal medicines (Batool et al., 2020).

Calotropis procera (*C. procera*) is a flowering plant species which belongs to the family of Asclepiadaceae. Furthermore, this species is distributed globally with different socially accepted names such as milkweed, dead sea apple, swallowwort, and Sodom apple, whereas in India it is known as madar in Hindi. This plant is known as alarka in Sanskrit and akanda in Bengali. According to Ayurveda formulations, it is commonly known as *C. procera*. It is widely known due to its medicinal use (Kundu, 2021). *C. gigantean* and *C. procera* are the two species distributed globally for a long time. The most common type of *C. procera* is a purple flowered plant which is seen more commonly as compared to other species (Verma, 2014).

Distribution of *C. procera* is found in Africa, America, and Asia. Its distribution is also found in different regions of Pakistan in pasture, plains, and roadways (Azhar et al., 2014). *C. procera* leaves have secondary metabolites including flavonoids, terpenoids, alkaloids, glycoside, saponins, calotropin phenols, cardenolides, sugars, tannins, and steroids. It includes different bitter contents such as calotoxin, uscharin, calactin, and other volatile organic compounds (Shrivastava et al., 2013).

Different parts of *C. procera* are used in folk medicine and a therapeutic agent for treating leprosy, fever, diarrhea, jaundice, dysentery, and eczema (Meena et al., 2010). Moreover, the latex and oil of the plant is used to treat joint pain and paralyze parts of the body respectively (Abhishek et al., 2010). It is also used in antifungal drugs to treat *Tinea capitis* in children (Aliyu et al., 2015). The laticifer proteins obtained fron the latex of *C. procera* also shows antibacterial activity (Saher et al., 2023). The plant also contains antioxidant and hepatoprotective agents besides antiviral and antidiarrheal compounds (Muhammad et al., 2015). The proposed study evaluates the leaf extract and latex of *C. Procera* prepared in chloroform, ethanol, and aqueous extracts against clinically important pathogenic bacteria and fungi.

Materials and Methods

The study is carried out in Microbiology Research Laboratory Abasyn University Peshawar during February to July 2019.

Plant Samples Collection and Processing

The latex and leaves are collected from the plain area of Bara Kajoori, Khyber District and brought to Microbiology laboratory at Abasyn University Peshawar. Latex is directly collected from the stem and shoot of *C. procera* and stored in sterilized plastic bottles. The leaves are dried for a duration of 16-20 days at room temperature and then blended into powder for further necessary processes.

Extraction of Plant Extracts

Leaf and latex extracts of *C. procera* are prepared with 60% ethanol, water, and chloroform. The 10 gram latex and leaf powder each are used to prepare labeled suspension of 100 ml solvent and left for a duration of 5 days. Finally the plant extracts are filtered and stored at 4^{o}C.

Nutrient agar Medium Preparation

The Nutrient agar medium is prepared as per manufacturing instruction by autoclaving sterilization at 121°C, cooling to 50°C, and pouring into Petri dishes. Finally, the solidified agar stored at 4°C is used for inoculation purposes.

Bacterial Species Isolates

Ten identified bacterial spp. are used in the study including *M. morganii, P. alcalifaciens, Y. intermedia, Y. enterocolitica, E. coli, S. aureus, S. Typhi, B. megaterium, S. marcescens,* and *P. fluorescens.* All the fully identified bacterial spp. are obtained in the lab and sub-cultured on Nutrients agar medium to fresh the bacterial culture.

Anti-bacterial Activity

The Muller Hinton Agar (MHA) medium are prepared, and wells are made in the petri plates with 24mm distance from the center one for control. An inoculum of 2-6 hours are spread on the surface of MHA plates using sterile swabs. Test sample with concentration of 0.1g/ml are prepared in Dimethyl sulfoxide (DMSO) and then added to the corresponding wells. These plates are incubated at 37°C for 24 hours. The zone of inhibition are determined by measuring the zone in millimeter (mm). The experiments are repeated three times using positive control (ciprofloxacin) and negative control (DMSO).

Antibacterial Activity of C. procera Extract and Latex

Agar well diffusion method (Janovska et al., 2003) is used to check the antimicrobial activity of plant extract based on liquid dilution method. A 0.1gram of plant extract is suspended in 1ml of DMSO (0.1g/1ml). The McFarland standard (0.5M) is used as a turbidity standard. A 7mm Sterile cork borer is used to make wells in the agar medium. Different concentrations are used to observe the antibacterial activity in *C. procera* of water, ethanol, and chloroform extract and latex of plant by using micropipette in the marked wells in plates with concentration of 100 µl. All the plates are incubated for 24 hours at 37°C.

Anti-fungal Activity

Jonvska et al. (2013) demonstrate agar well diffusion method for the antifungal activity of plant extract. A 0.1g of plant extract and latex is used in 1 ml DMSO (0.1g/1ml) suspension. The potato dextrose broth is used for the antifungal activity. A 2µl of broth is taken through a micropipette and prepared a lawn on potato dextrose agar (PDA) using a sterilized swab to perform a uniform lawn. A 7mm Cork borer is used for well formation in the agar plates. A 100µl aliquot plant extract and latex is placed in the well aseptically. All culture plates are incubated at 27°C for 48 hrs.

Experimental Results

The leaves extract of *C. procera* has antibacterial activity on pathogenic microorganisms. Ethanol is proved to be the best solvent for plant extracting compared to chloroform and water as shown in the Table 1. The maximum zone of inhibition of 19mm for *M. morganii, E. coli* and *S. Typhi* is demonstrated with a minimum of 13mm for *P. alcalifaciens* by the ethanolic leaf extract of *C. procera* (Table 1).

C. procera latex in ethanol shows good activity against *M. morganii, Y. enterocolitic, S. Typhi, S. marcescens* with a zone inhibition of 13mm, followed by *B. megaterium, S. aureus,* and *P. fluorescens* of 12mm. *C. procera* latex extracts in Chloroform possess good activity against *S. Typhi* with an inhibition zone of 12mm followed by *S. marcescens* and *M. morganii* of 11mm. Rest of the details are shown in Table 2.

Antifungal Activity

Chloroform, ethanol, and aqueous of *C. procera* leaf extract are also screened antifungal activity against selected fungal spp. The extract shows low activity against *C. albicans* (yeast) (5mm, 9mm and 3mm) and *F. oxysporum* (6mm, 9mm, and 5mm). While the extract is active for *H. maydis* and *F. oxysporum* in 11mm zone of ethanol extract (Figure 1).

Table 1: Antibacterial activity of C. procera leaf extracts using well diffusion method.

S. No	Bacterial species	Zone of inhibition (mm) concentration of 100µl						
		Chloroform Extract		Ethanol Extract		Aqueous Extract		Positive control Ciprofloxacin
		mm	%	mm	%	mm	%	
1.	*M. morganii*	15	60	19	76	13	52	25
2.	*P. alcalifaciens*	8	32	13	52	7	28	24
3.	*Y. intermedia*	0	0	15	60	6	24	19
4.	*Y. enterocolitica*	11	44	17	68	8	32	25
5.	*E. coli*	9	36	19	76	8	32	23
6.	*S. aureus*	13	52	18	72	9	36	22
7.	*S. Typhi*	15	60	19	76	10	40	24
8	*B. megaterium*	14	56	18	72	12	48	21
9.	*S. marcescens*	11	44	17	68	8	32	25
10.	*P. fluorescens*	11	44	15	60	5	20	25

Table 2: Antibacterial activity of C. procera latex using well diffusion method.

S. No	Bacterial species	Zone of inhibition (mm) concentration of 100µl						
		Chloroform Extract		Ethanol Extract		Aqueous Extract		Positive control Ciprofloxacin
		mm	%	mm	%	mm	%	
1.	*M. morganii*	11	39	13	46	9	32	28
2.	*P. alcalifaciens*	5	23	9	43	3	14	21
3.	*Y. intermedia*	0	0	7	35	0	0	20
4.	*Y. enterocolitica*	9	50	13	72	6	33	18
5.	*E. coli*	5	31	10	62	4	25	16
6.	*S. aureus*	9	64	11	78	7	50	14
7.	*S. Typhi*	12	70	13	76	8	47	17
8.	*B. megaterium*	9	56	12	75	6	37	16
9.	*S. marcescens*	11	42	13	50	9	34	26
10.	*P. fluorescens*	8	47	11	65	5	29	17

Antifungal Activity of C. procera Latex

Various fungal strains are analyzed for the antifungal activity against *C. procera* latex. Ethanol extracts show the good antifungal activity *H. maydis,* and *R. nigricans* of 9mm zone on inhibition, followed by *C. albicans* (*yeast*) of 7mm as shown in Figure 2.

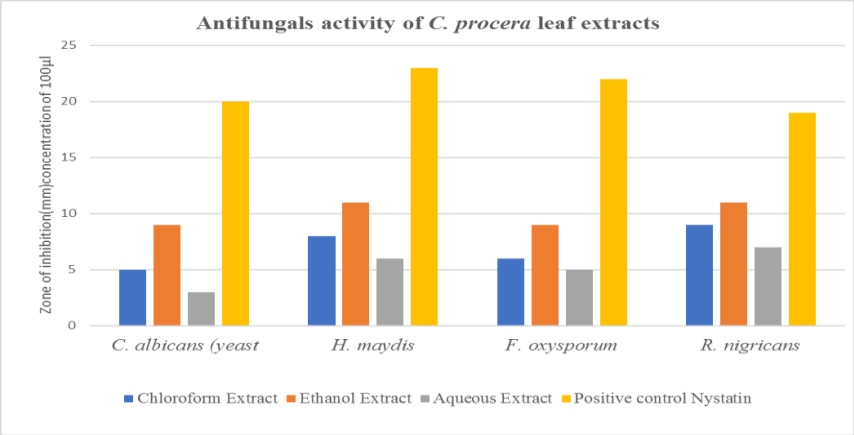

Figure 1: Antifungal activity of C. procera leaf extracts using well diffusion method.

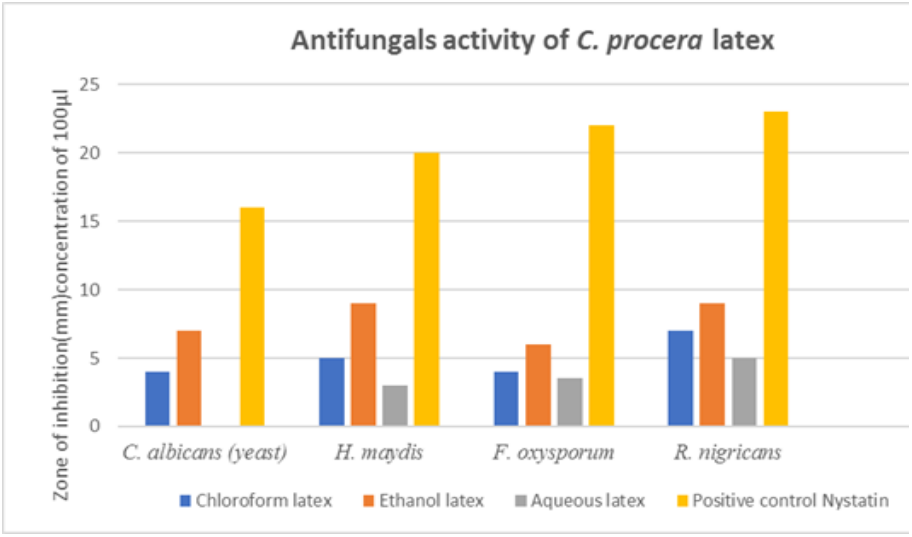

Figure 2: Antifungal activity of C. procera latex using well diffusion method.

Discussion

This research study analyzes the antibacterial and antifungal activities of the leaf extract and latex of the medicinal plant known as *C. procera.* Ethanol has shown the most active antibacterial activity. Saddiq et al. (2022) reported that the antibacterial efficacy of *C. procera* extract is significantly active against *K. pneumoniae*, *S. aureus*, and *E. coli* with maximum zone of inhibition of 21.26mm, 18.66mm, and 21.93mm respectively, and noted that the antifungal activity against *C. albican* with

21mm zone of inhibition. The proposed study supports these findings as an 18 mm zone of inhibition is found against *S. aureus* and 19mm against *E. coli*.

Mehmood et al. (2020) reported the antibacterial activity of bark and leaves of *C. procera* against *Bacillus* strains maximum 20 mm zone of inhibition in 50% methanol extract, a 21mm zone against *K. pneumoniae*, and 17mm against *E.* coli which support the current study results. Amini et al. (2021) reported the antibacterial activity of *C. procera* against routine pathogens and showed good activity against Gram-positive and Gram-negative bacteria, which also support the current findings.

Salem et al. (2014) described the antibacterial potential of leaves and latex of *C. procera* against common routine pathogens such as *E. coli*, *S. aureus*, *S. flexeneri* recorded good activity, which support the current study findings as *E. coli* shows maximum zone of inhibition of 18mm and 19mm against *S. typhi*.

Amit et al., 2013 used agar well diffusion method antibacterial activity of *C. procera* leaves examined against *S. aureus* and *S. typhi* with no zone of inhibition. This study is not similar to the current study, as it observes the maximum zones of 18mm and 19mm against *S. aureus* and *S. typhi*, respectively. The reason for showing no activity of *C. procera* leaves against bacterial pathogens may be the strain dependent ability of the bacterial pathogen.

Rani et al. (2017) showed the potential impact of *C. procera* leaves against fungal pathogens, as *Fusarium genus* and *Aspergillus* with 17.3mm of maximum zone of inhibition and observes 9mm zone of inhibition against *Fusarium oxysporium*, which is not similar to the current study. The same study also reported the antibacterial activity of *C. procera* leaves which is not consistent with the current study.

Researchers from Brazil also reported its significant effectiveness against bacterial and multiple fungal species (Nascimento et al., 2015). Fawzi et al. (2021) demonstrated that extracts of *C. procera* leaves showed strong antibacterial effects against *E. coli, S. aureus, K. Pneumoniae, S. typhi* which exhibited good antibacterial activity against these pathogen. Their work is well aligned with the findings of the current study. Similar report from Pakistan evaluated that *C. procera* is a latex producing plant and its laticifer proteins shows antibacterial activity (Saher et al., 2023). The current study results also support the results collected in previous studies including Farooq et al. (2017) and Abegunde et al. (2020), which show that ethanol extracts exhibited good antibacterial activity against Gram-positive and Gram-negative pathogens. *C. procera* activity against a variety of pathogens can be used as an alternative medicine in the case of drug resistant pathogens.

Conclusions

From this study, it was concluded that the chloroform, ethanol, and aqueous extracts of *C. procera* leaf extract and latex have antibacterial and antifungal potentials against clinical strains and thus could serve as antimicrobial agents, especially *M. morganii, E. coli*, *S. Typhi* and *P. alcalifaciens,* as well as *H. maydis* and *F. oxysporum.*

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