Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Antimicrobial And Antibiofilm Activities Of Ethnomedicinal Plants Of Southern KP

Saman Shafique
Lecturer, Higher Education Department, Punjab
Email: samanshafique1@gmail.com

Nayab Iqbal
MPhil Scholar, Superior University, Sargodha
Email: nayabiqbal1304@gmail.com

ABSTRACT

The research study was based on analysis of an antibiotic effect (Antibacterial and antibiofilm activities) of traditional medicinal plants against available strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The selected plant species (*Calotropis procera*, *Fogonia indica* and *Moringa oleifera*) are native to Pahar Pur, a small tehsil of Dera Ismail Khan, Southern KP. With increasing global warming and subsequent climatic conditions, the microbial resistance against antibiotics had increased manifolds. The utilization of medicinal plants to treat illness is a communal practice in local communities. The leaves were treated with methanol as organic solvent to prepare leaf extracts. The antibacterial activity of the leaf extracts was assessed by the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) against Staphylococcus strain while antibiofilm activity was analyzed against Pseudomonas strain. The results of three medicinal plants developed a comparative evaluation of ethnomedicinal plants against resistant bacterial strains. The maximum value of the Zone of Inhibition was recorded for *Fagonia indica* i.e. 3 mm followed by *Moringa oleifera* (2 mm) and *Calotropis procera* (1 mm). In contrary, the leaf extracts exhibited parallel values for MIC (1.25 mg/ml) and MBC (>1.25 mg/ml) against *Staphylococcus aureus* strain. In case of antibiofilm activity, the S. aureus strains exhibited strong tolerance against the methanolic-leaf extracts, therefore, the *Pseudomonas aeruginosa* strain was tested for antibiofilm activity. *Fagonia indica* had shown maximum antibiofilm potential i.e. 43.3 % followed by *Moringa oleifera* (40.6 %) and *Calotropis procera* (29.4 %). This study indicated that the leaf extracts of the selected ethnomedicial plants possess antimicrobial activity along with antibiofilm potential against the selected bacteria. Thus, the leaf extracts of *F. indica*, *M. oleifera* and *C. procera* are proved efficient in treating the ailments caused by these bacteria.

Keywords: Ethnomedicinal Plants, Antimicrobial Activity, Antibiofilm Activity, *Fagonia Indica*, *Moringa Oleifera*, *Calotropis Procera*, *Staphylococcus Aureus*, *Pseudomonas Aeruginosa*, Antibiotic Resistance, Southern Khyber Pakhtunkhwa

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Introduction

Humans have always depended on nature to provide basic needs such as food, shelter and medicine. Plant resources are the most important natural resources in healthcare since it has a wide array of bioactive compounds that prevent and treat various disease in humans and animals (Ballabh & Chaursaia, 2007). Plants like poppy, henbane, and garlic have been utilized since ancient times and they are agents that have been of immense importance in the traditional medicine before modern medicine. Research indicates an increased interdependence of plants and human beings compared to that of animals, indicating the focal position of plants in the world of pharmacology (Ali and Qaiser, 2009; Babekov et al., 1999).

Plants have an enormous diversity of secondary metabolites such as alkaloids, flavonoids, saponins and terpenoids which cushion them against pathogens and environmental stress as well as offering certain therapy to humans. Such metabolites are less toxic when compared to synthetic drugs and have antimicrobial, antioxidative and anti-inflammatory effects (Berdy, 2005; Lei et al., 2016). Modern drugs take their origins in plant secondary metabolites (25-33), which is why ethnomedicinal plants are considered vital natural sources of therapeutic agents (Samuelsson, 2004).

The problem of multidrug-resistant bacteria (MDRB) and biofilm-associated infections appears to be a severe global health problem. Biofilms are organized micro-communities of microbes that are surrounded by extracellular polymeric materials (EPS) that make them more resistant to conventional antibiotics, pathogenic, and increase their survival rate (Donlan, 2002; Liu, 2005). Pathogens like Staphylococcus aureus create biofilms, which are much harder to treat with antibiotics compared to planktons, making clinical treatment difficult and causing chronic infections (Tacconelli et al., 2017; Pinheiro et al., 2014).

There are more than 6,000 plant species in Pakistan, and one out of every two-and-three hundred is of medicinal importance (Ameya et al., 2019). Southern Khyber Pakhtunkhwa is especially endowed with medicinal plants that were used in the treatment of infections and other health complications.

Fagonia indica (Figure 1) is a small, pack-shaped plant that belongs to the family of Zygophyllaceae and is up to 60 cm in height. Historically, any liver conditions, diarrhea, urinary issues, abscesses, have been treated with it (Ali and Khan, 2021; Azam et al., 2018). Its pharmacological action consists of antibacterial, anti-inflammatory, and anti oxidant action.

Figure 1. *Fagonia indica* showing leaves and flowers collected from Southern Khyber Pakhtunkhwa.

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Calotropis procera (Figure 2) of Apocynaceae family exists with diverse potent phytochemicals; flavonoids, triterpenes, glycosides, steroids, saponins, protein, and enzymes. It is traditionally applied in anti-inflammatory therapy and has

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

shown a wide range of antibacterial, antiviral, and antifungal effect (Batool et al., 2020; Singh et al., 2021; Wadhwani et al., 2021).

Figure 2. *Calotropis procera* plant with leaves and flowers used for ethnomedicinal purposes.

Moringa oleifera (Figure 3) is commonly referred to as the drumstick tree with the family of Moringaceae. It grows in tropical and subtropical zones of the world and has been extensively utilized in traditional medicine in the treatment of different diseases with the help of its leaves, seeds, roots, flowers, and bark (Anwar et al., 2007; Bhattacharya et al., 2018). It has been known to have antimicrobial, antioxidant and nutritional properties with its extracts.

Figure 3. *Moringa oleifera* plant showing leaves and flowers used in traditional medicine.

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

The growing resistance of bacteria to the traditional antibiotics especially those that develop biofilms is a factor that explains why alternative antimicrobial agents have to be investigated. The ethnomedical plants present a high potential of bioactive compound absorbed in inhibiting the growth and biofilm formation of microbes. The antimicrobial and antibiofilm properties of most of the medicinal plants in Southern Khyber Pakhtunkhwa are underutilized despite its potential.

As such, the given research paper is going to investigate the antimicrobial properties and the antibiofilm capabilities of the leaf extracts of Fagonia indica, Calotropis procera, and Moringa oleifera against Staphylococcus aureus to gain a perspective on potential uses of leaf extracts as natural therapeutic agents.

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Objectives

To assess the antimicrobial activity of leaf extracts of *Moringa oleifera*, *Calotropis procera*, and *Fagonia indica* against *Staphylococcus aureus* strains.

To investigate the antibiofilm potential of leaf extracts against *Staphylococcus aureus*.

Table 1.1 Some indigeous plants along with their uses

COMMON NAMES	BOTANICAL NAMES	USES
Saijna	*Moringa oleifera*	Diabetes, anti-inflammatory
Arka	*Calotropis procera*	Anti-inflammatory, constipation
Sachi booti	*Fagonia indica*	Analgesic, antitumor

Literature Review

Biofilms consist of complicated microbial mixtures comprising of surface fixed colonies rooted in a self generating extra cellular polymere substance (EPS). The structures safeguard the microbes against environmental stresses such as the increased temperatures, reduced oxygen levels, chemical stress, and above all against antibiotic working (Aslam et al., 2021). The fact that biofilm-forming bacteria are able to survive under extreme conditions is impressive and plays a significant role in the continuance and reemergence of infectious diseases. Biofilms raise the resistance of microbes to antibiotics making not only more patients develop infection but also raising the mortality rate linked with such infections.

Research shows that biofilm-associated infections can only be counteracted by using agents that would interfere with the formation of biofilms and prevent microbial adhesion and quorum sensing systems (Guzzo et al., 2020; Tilberg et al., 2017). Biofilms can often be resistant to the traditional antibiotics, and other measures such as utilizing natural plant-based antimicrobials can be used.

Antimicrobial and antibiofilm activity effects have extensively been tested on methanolic leaf extracts. As an illustration, the findings of Sanchez et al. (2016) have shown that Prosopis laevigata, Opuntia ficus-indica, and Gutierrezia microcephala primarily exhibit good antibacterial activity with MIC values between 0.7 and 15 mg/mL and MBC values within the same range. Biofilm formation was also inhibited according to the Specific Biofilm Formation Index (SBF) and its toxicity was detected according to Brine Shrimp Mortality Assay which proved the comparatively low cytotoxicity. The analysis of phytochemicals showed that the major active compounds were flavonoids, coumarins and tannins.

The Leaves, seeds, fruits, flowers, and immature pods all contain many bioactive compounds in Moringa oleifera, also known as the plant of life or miracle plant in the world. These ingredients cure malnutrition and improve health conditions of all age groups by covering infants and lactating women (Anwar et al., 2007; Estrella et al., 2000). Its seeds have been used in the filtration of water and treatment of wastes (Fuglie, 1999).

The moringa leaves are rich in nutrients in terms of vitamins, carotenoids, flavonoids, phenols, calcium, and potassium, vitamin A, C, and E, b-carotene, proteins, and iron

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

(Hekmat et al., 2015; Wasif et al., 2016; Dillard and German, 2000; Saini et al., 2014). The moringa extracts have antioxidant, antibacterial, antidiabetic, hepatoprotective, and anticancer properties (Coz-Bolanosa et al., 2018; Arora & Onsare, 2014; Ruckmani et al., 1998; Makonnen et al., 1997). Research has proven to be effective against E. coli, S. aureus, and M. kristinae (Moyo et al., 2012) and chloroform and methanolic extracts can have greater antibacterial activity than aqueous extracts in most instances (Dalukdeniya et al., 2016; Abdulkadir et al., 2016). The existence of alkaloids, glycosides, flavonoids, saponins, and tannins in Moringa has been discovered by conducting phytochemical screening, which makes it a medicinal compound with its many attributes. Evaporation techniques including drying under regulated temperatures in the oven have proved to retain antioxidant activity and phenolic content (Bennour et al., 2020). These researches establish Moringa oleifera as an important source of antimicrobial and antioxidant, which can be used in food preservation, pharmaceutical industry and natural medication.

Calotropis procera is a herb of the Asclepiadaceae family, which creates its natural forest in arid and semi-arid areas, and is extensively employed in traditional medicine in North Africa, South-East Asia, the Middle East, and some parts of Asia and Africa (Dirir et al., 2017; Al Sulaibi et al., 2020). It has bioactive chemicals like alkaloids, cardiac glycosides as well as steroid, especially in its latex as well as in the leaves, the causes of which are the pharmacological effects (Parihar and Balekar, 2016; Mohamed et al., 2015).

C. procera leaf methanolic leaf extracts have been shown to have an antibacterial effect against methicillin-resistant Staphylococcus aureus (MRSA) and other pathogenically relevant organisms (Shafat et al., 2023; Joshua et al., 2022). The plant also has anticancer effects as shown in research done on MCF7 cellules of breast cancer. Nevertheless, it is dangerous and needs some care because of the effect of toxicity when ingested by latex or accumulated into plant tissues as heavy metals associated with it: Cr, Cd, Ni, and Pb (Naz et al., 2020; Tossou et al., 2018). The antibacterial activity of C. procera differs based on the extraction technique, the type of solvent, the part of the plant, and the dosage, and in general, methanolic and ethanolic extracts tend to be more active (Balial et al., 2020; Salem et al., 2014).

Zygophyllaceae (Fagonia indica) is a medical plant that is found in Iran, Pakistan, and India in arid and semi-arid areas (Pullaiah, 2006). It is also applied in the traditional blood purification, treatment of anemia, diabetes, skin diseases, and even in the treatment of cancer (Shinwari and Shah, 2003; Ansari, 1983; Graham et al., 2000). Bioactive compounds found in the plant include triterpenes, saponins, flavonoid, alkaloids, and this characteristic has earned the plant antimicrobial, antioxidant, analgesic, anticancer, and anti-inflammatory properties (Sharma et al., 2009; Saleem et al., 2020).

Fagonia indica leaves have demonstrated a great mode of antibacterial effect on E. coli, S. aureus, P. aeruginosa and B. cereus with highest inhibition occurring against B. cereus (Sharma et al., 2009). Its chloroform extract had also shown good biofilm inhibition of S. aureus (71.84) and K. pneumoniae (70.83) and low bacterial adhesion and increased protein leakage, demonstrating potential as a low-cost biofilm inhibitor (Saleem et al., 2020). Also, its wound-healing and anti-inflammatory effects have been confirmed in animals (Alqasoumi et al., 2011).

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

With the help of molecular biology and DNA barcoding, phytochemical inquiries confirmed that Fagonia indica is the strongest species in its family regarding antioxidant, antimicrobial, and antifungal actions (Saleem et al., 2019).

A number of researches have been carried out on plant derived compounds as biofilm inhibitors. Essential oils, flavonoids, terpenes, and phenolic acid have demonstrated both the ability to disrupt biofilms without necessarily killing bacteria, but instead focusing on phenotypic and quorum sensing pathways (Artini et al., 2018; Guzzo et al., 2020). Synergistic biofilm inhibition with copper ions (Cu2 +) with quaternary ammonium compounds (QACs) have also been reported to be effective in P. aeruginosa and other pathogens (Harrison et al., 2018).

The ability to inhibit biofilms by P. aeruginosa is important in a clinical setting because biofilms of this bacteria are implicated in chronic lung infections in cystic fibrosis patients and in skin infections, septic arthritis, and infectative endocarditis with S. aureus. Natural plant compounds disrupting biofilm matrix production, adhesion of microbes or quorum sensing may serve as alternative or supplementary therapy to biofilm-related infections (Tilberg et al., 2017; Guzzo et al., 2020).

The literature illustrates therapeutic value of the Moringa oleifera, Calotropis procera and Fagonia indica sources of antimicrobial and antibiofilm compounds. They possess bioactive components such as flavonoids, alkaloids, saponins, phenolics and terpenes that can be attributed to their antibacterial, antifungal, antioxidant and anti inflammatory properties. These medicinal plants are good natural substitutes of drugs used to control infection and prevent biofilm due to the growing risk posed by multidrug-resistance pathogens and biofilm-mediated infections.

Research Methodology

Leaves of *Calotropis procera*, *Fagonia indica*, and *Moringa oleifera* were collected from Paharpur, Dera Ismail Khan, and the plant species were identified and authenticated using standard botanical references. The collected leaves were thoroughly washed with running and fresh water, air-dried, and ground into a fine powder. For extraction, 200 g of powdered leaves from each species were soaked in 2 L of methanol for three days at room temperature with hourly agitation to ensure maximum extraction of bioactive compounds. The mixtures were subsequently filtered through Whatman Grade 1 filter paper, and the filtrates were concentrated using a rotary evaporator at 50°C to obtain crude methanolic extracts. Pure isolates of *Staphylococcus aureus* were obtained from the Department of Gomal Centre of Biochemistry and Biotechnology (GCBB), Dera Ismail Khan. Bacterial cultures were streaked on Mueller-Hinton Agar (MHA) to confirm purity, and 3–5 pure colonies were suspended in saline with turbidity adjusted to 1.5×10^8 CFU/mL according to the McFarland standard. Mueller-Hinton Agar was sterilized by autoclaving at 121°C for 20 minutes and poured into Petri dishes. Stock solutions of plant extracts were prepared at 10,000 µg/mL, and working concentrations of 50, 100, 200, and 400 µg/mL were incorporated into the agar before solidification. Plates without extracts were used as controls. Inoculation of the plates was performed using a Steer's replicator equipped with sixteen 4 mm pins, allowing simultaneous inoculation of sixteen samples, and the plates were incubated at 37°C for 24 hours. The antimicrobial activity of the plant extracts was evaluated using the agar well diffusion

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

method, in which 100 µL of bacterial culture (~10^6 CFU/mL) was swabbed on MHA plates, wells were bored into the agar, and 100 µL of each extract was added, while methanol served as a control. Plates were incubated at 37°C for 18 hours, and inhibition zones were measured in millimeters. Minimum inhibitory concentration (MIC) was determined using the broth microdilution method with extract concentrations ranging from 1 to 25 mg/mL, with 100 µL of Mueller-Hinton Broth and 10 µL of bacterial culture added per well, and incubated at 37°C for 24 hours; MIC was recorded as the lowest concentration showing no visible bacterial growth. Minimum bactericidal concentration (MBC) was assessed by sub-culturing wells showing no growth in the MIC assay onto fresh Mueller-Hinton Broth, incubated at 37°C for 24 hours, using methanol as a blank control. Sub-lethal effects were further evaluated at 75%, 50%, and 25% concentrations of the extracts, and bacterial counts were performed using the plate count technique. The antibiofilm potential of the plant extracts was assessed using a crystal violet staining assay in 96-well microplates. Wells were washed multiple times with distilled water, air-dried, stained with 1% crystal violet for 15 minutes, rinsed to remove unbound dye, and destained with methanol. Absorbance of the destaining solution was measured at 590 nm using a microplate reader, and biofilm inhibition was calculated as Specific Biofilm Formation (SBF) using the formula SBF = (AB − CW)/G, where AB represents the optical density of the bacterial strain, CW the optical density of the control medium, and G the optical density of bacterial growth. All experiments were performed in triplicate, and the results were expressed as mean ± standard error, with statistical analysis conducted to determine significant differences between treated and control groups.

Results and Discussion

The Zone of Inhibition exhibited by the leaf extracts of *Fagonia indica*, *Moringa oleifera*, and *Calotropis procera* were obtained using the Disc Diffusion Method against *Staphylococcus aureus*. The maximum zone of inhibition was demonstrated by *Fagonia indica* i.e. 3 mm followed by *Moringa oleifera* (2 mm) and *Calotropis procera* (1 mm) as shown in Table 4.1. Similar results were reported by Sajid et al. (2011) where aqueous, methanolic and ethanolic whole plant extracts had shown inhibitory effect of *Fagonia indica* against *Staphylococcus aureus*, Bacillus subtilis, Escherichia coli and *Pseudomonas aeruginosa* by means of Disc Diffusion Method. Comparing the antibacterial effect detected by the overlay method, the aqueous and methanolic extracts performed significantly higher than the ethanolic extract. Moreover, the methanolic leaf extract of *Calotropis procera* had shown the ability to inhibit the growth of both Gram-positive and Gram-negative bacteria such as *Staphylococcus aureus*, Bacillus subtilis, Escherichia coli and *Pseudomonas aeruginosa* in natural environment (Nenaah, 2013). In case of Fagonia indica, the inhibitory property of extracts of ariel parts was found to be more effective than the extracts prepared from roots.

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Zone of Inhibition

Extract	Zone of inhibition (mm)
Fagonia indica	3
Moringa oleifera	2
Calotropis procera	1

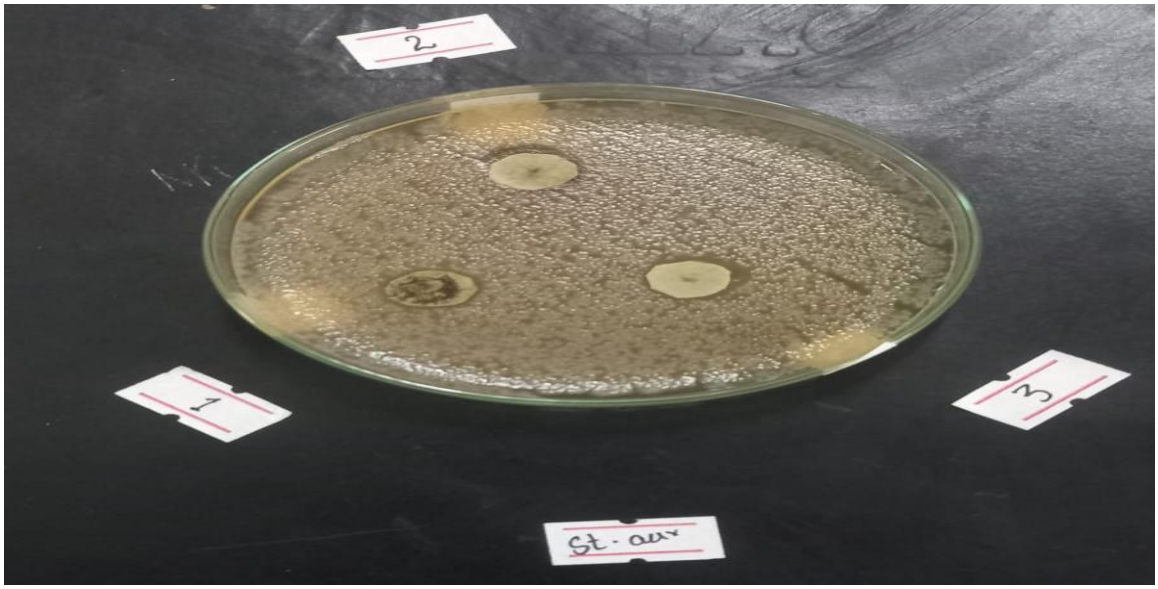


Figure 4: Zone of Inhibition of the tested plant samples against *Staphylococcus aureus*

Minimum Inhibitory Concentration

Minimum Inhibitory Concentration (MIC) of the tested samples was detected against the *Staphylococcus aureus* by Broth Dilution Method on 96 well microplate as shown in Figure 2. The difference between the mean values of MIC was found to be non-signifcant as shown in Table 4.2. The maximum mean value of 1.253 mg/ml was showed by leaf extracts of *Fagonia indica* followed by *Moringa oleifera* (1.247 mg/ml) and *Calotropis procera* (1.243 mg/ml). These results are comparable to the study conducted by Aslam et al. (2021) on *Fagonia indica* from Cholistan Desert. The chloroform extracts of Fagonia indica exhibited MIC in range of 125-500 µg/ml. The chloroform extracts both roots and aerial parts of *Fagonia indica* exhibited MIC value of 250 µg/ml.

Minimum Inhibitory Concentration (mg/ml) of the tested Medicinal Plants

Strain	MIC mg/ml		
Staphylococcus aureus	*Fagonia indica*	*Moringa oleifera*	*Calotropis procera*
	1.253±0.01	1.247±0.01	1.243±0.01

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

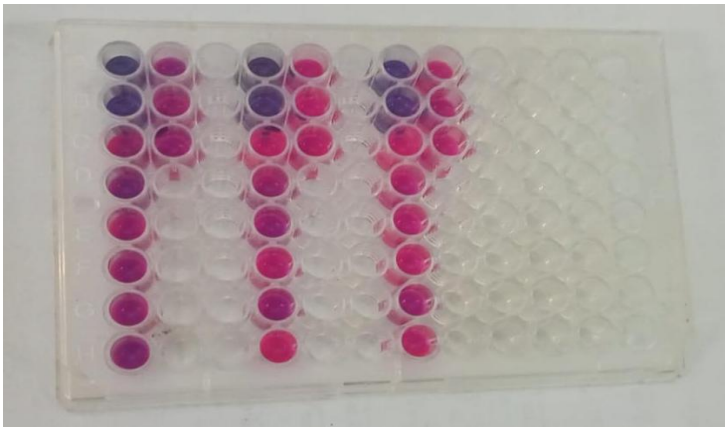
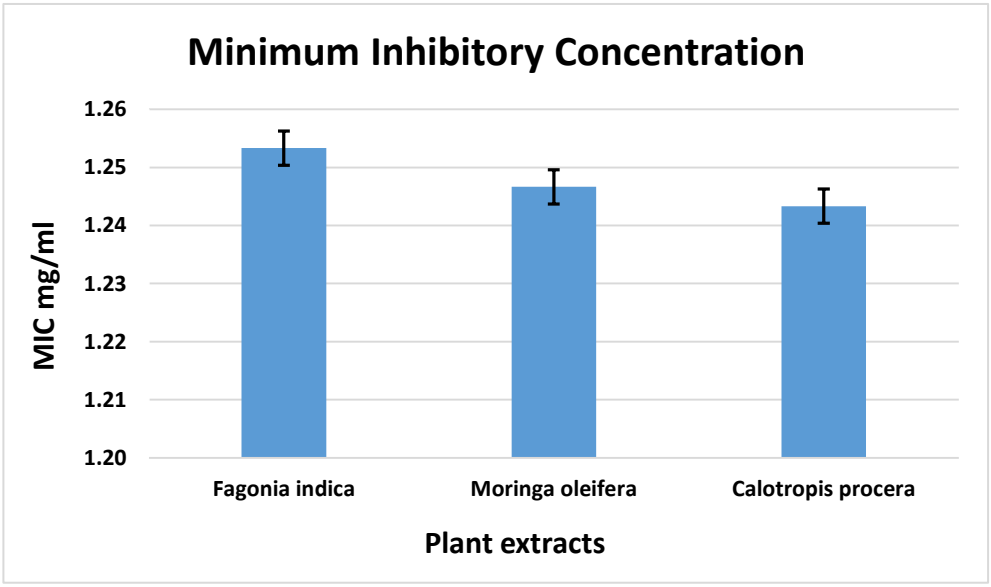


Figure 5. Minimum Inhibitory Concentration exhibited by Broth Dilution Method in the tested plant samples against *Staphylococcus aureus*



Graph 1: Minimum Inhibitor Concentration of the tested plant samples against *Staphylococcus aureus* strain

Minimum Bactericidal Concentration

Minimum Bactericidal Concentration (MBC) is described as the concentration of the drug which is sufficient to kill the bacterial cell (Parvekar et al., 2020). The leaf extracts of all tested ethnomedicinal plants (*Fagonia indica, Moringa oleifera* and *Calotropis procera)* had shown MBC more than 1.25 mg/ml against the *Staphylococcus aureus* (Table 4.3). The results are comparable to those reported by Aslam et al. (2021) for both extracts of aerial parts (0.5 mg/ml) and roots (0.5 mg/ml) of *F. indica*.

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

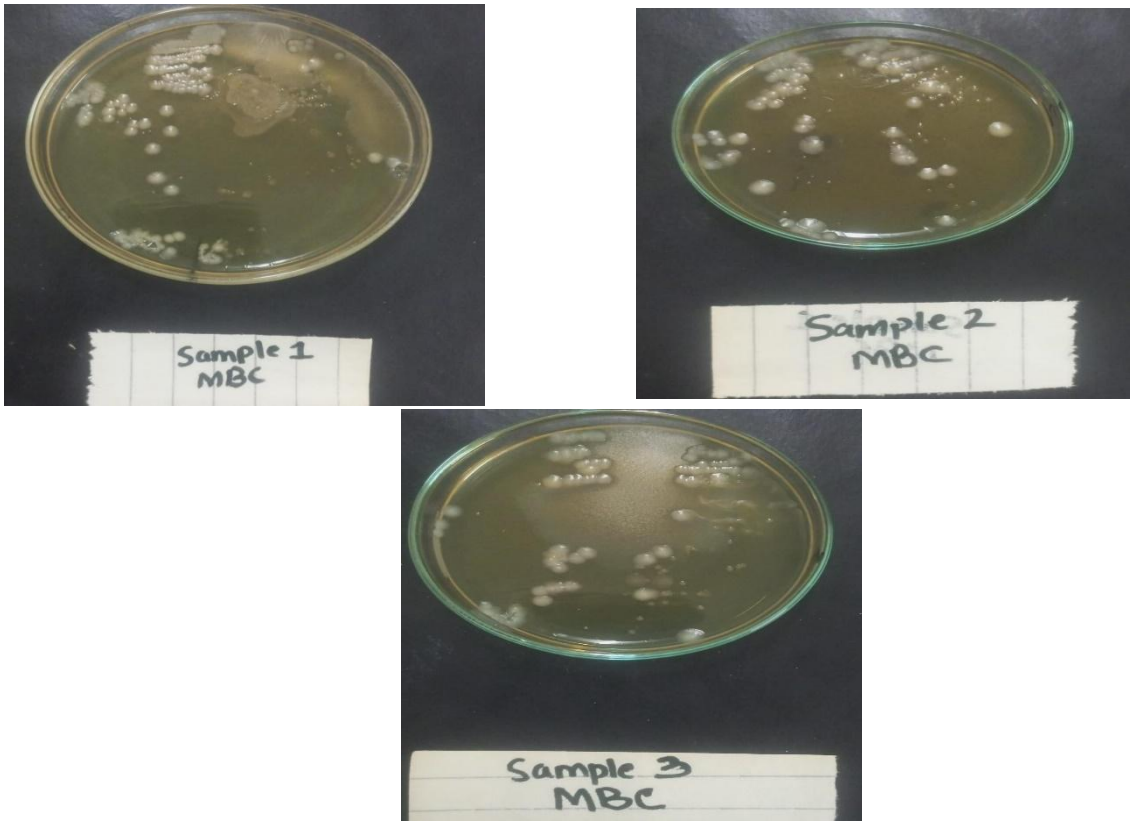


Figure 6. MBC analysis of Fugania indica (Sample1), *Calatropis proceira* (Sample 2) *and Moringa oliefera* (Sample 3)

Minimum Bactericidal Concentration

Leaf Extracts	MBC (mg/ml)
Fagonia indicaindica	
Moringa oleifera	MBC >1.25
Calotropis procera	

Antibiofilm analysis

Staphylococcus aureus strain was highly-tolerant to the methanolic-leaf extracts in case of antibiofilm. The potential cause of this failure of the examined leaf extract in antibiofilm was perhaps facilitated by the selection of the organic solvent i.e. methanol. In one of the studies, Aslam et al. (2021) have tested chloroform extracts of F.indica in favor of antibiofilm activity in comparison with other organic extracts (i.e. ethanol, methanol and ethyl acetate). The antibiofilm activity of the chloroform extracts was 71.84 percent of Staphylococcus aureus. Thus, the selected plants of methanolic extracts were used against the strain of Pseudomonas aeruginosa in the present study. The findings are recorded in Table 4.4 wherein, Fagonia indica had indicated the highest antibiofilm potential i.e. 43.3 % then the Moringa indica (40.6%) and Calotropis procera (29.4%). Such data show better quantitative results than in Suleiman et al. (2022) whereby methanolic extracts of F.indica had exhibited inhibition of P. aeruginosa with the rates of 33.69 to 17.78%. It is mentioned that F.

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

indica is a formidable contender which needs further studies in order to employ it to deal with multidrug resistant diseases.

Biofilm Inhibition Activity of Plant extracts against *Pseudomonas aeruginosa*

Strain	**% Biofilm inhibition**		
Psudomonas aeruginosa	*Fagonia indica*	*Moringa oleifera*	*Calotropis procera*
	40.6±0.15	43.3±0.15	29.4±0.35

Biofilms are also organized and firmly fixed or fixed on a surface community of microorganisms, which are installed in an extracellular polymeric substance Biofilm (Karygianni et al., 2020). These groups of microbes enable the colonization of bacteria in adverse conditions such as desiccation and starvation (Krishnan et al., 2023) and a primary cause of infected individuals with compromised immune systems (Di Domenico et al., 2022). Normal tissue implants, such as contact lenses, catheters, heart valves, and pacemakers, dentures, and joint prostheses, are the ideal biofilm surface (Nagaiah et al., 2023).

Pseudomonas aeruginosa is an opportunistic and common Gram-negative pathogen that causes nosocomial infections and fatal pathologies especially those in immune-compromised individuals such as cancer, surgical and HIV infected patients (Thi et al., 2020). P aeruginosa is a multidrug-resistant pathogen identified by the WHO as one of the priority outcomes where it is recommended to develop new antibiotics to address the high mortality rates (Mancuso et al., 2021). It is also strengthened by the fact that it is able to become biofilms to protect the bacteria against environmental pressures, reduces their vulnerability to phagocytosis and, hence, improves their capacity to colonize and survive in the host (Fernández-Billón et al., 2023). Regarding the measuring of biofilm-forming activity of plant extracts, the crystal violet technique was used. They noted that, the presence of glucose in broth media was promoting the growth of biofilm and it was essential to have flat bottomed 96-well plates where bacteria could adhere to during the experiment.

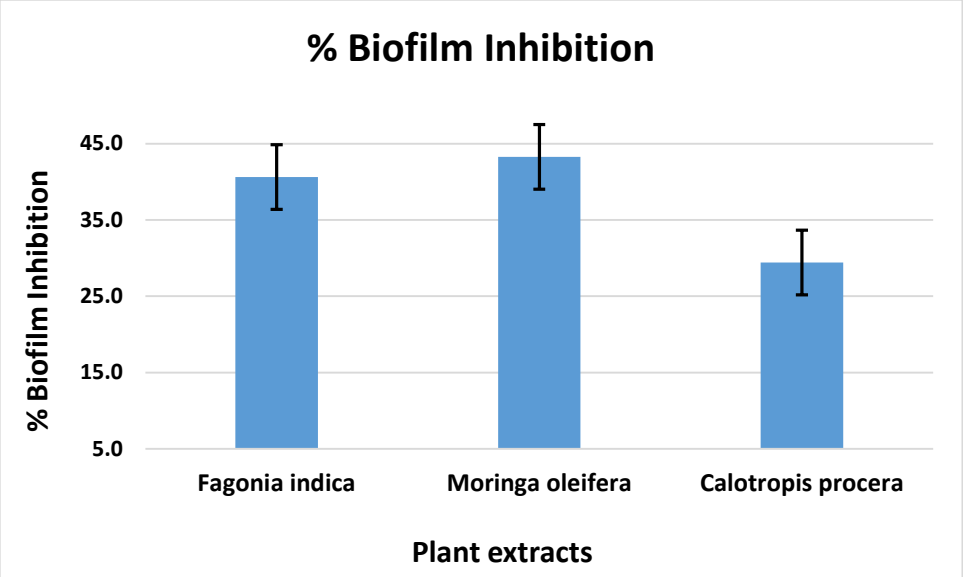
The modification of bacterial cell adherence and dissolving bacterial cell membrane has been revealed in the bioclimat effect of Fagonia indicaceae family Aslam et al., 2021. F. indica has a healthcare use in the Indo-Pak region, in which it is utilized to treat disorders/ diseases including fever, urinary diseases, asthma, liver diseases, tumor, and peptic ulcer disease among others (Aslam et al., 2021). It was observed that the anitbiofilm activity of F. indica was 40.1920.60% with B. subtilis, and 33.6917.78% with P aeruginosa (Ali and Khan, 2021). These studies are a good indication of the possibility of using F. indica against multi drug resistant bacteria. Moringa oleifera is a medicinal plant, which is fortified with several polysaccharides and phytochemicals with good therapeutic effects that in the end lead to its antibiofilm effect against pathogens (Kaur et al., 2020). Calotropis procera is also referred to as Sodom apple and it has been widely sold all over the globe particularly those areas that are arid to semi arid. Plant components are highly employed in the curing of the cold, fever, respiratory diseases such as asthma and skin illnessers on conventional foundations (Ahmed et al., 2021). This plant has a great concentration of

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

the metabolites that comprise flavonoids, tannins, terpenoids, saponins, alkaloids, steroids, and cardenolides and these qualities are due to the various pharmacological applications of this plant (Chiocchio et al., 2021). According to Tanimu et al (2022), C. procera showed anti biofilm effects on Staphylococcus aureus and Pseudomonas aeruginosa.



Graph 2: Biofilm Inhibition activity of the tested plant samples against *Psudomonas aeruginosa* strain

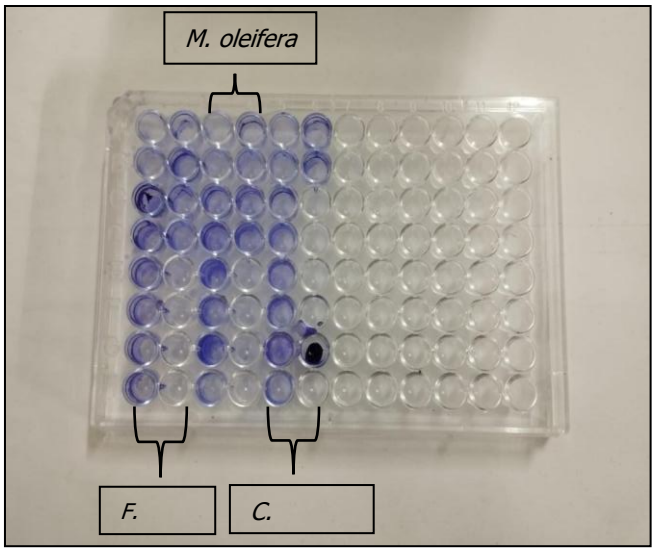


Figure 7: Comparison of the Antibiofilm potential of the plant extracts against *Psudomonas aeruginosa*

Annual Methodological Archive Research Review
http://amresearchreview.com/index.php/Journal/about
Volume 4, Issue 3 (2026)

Online ISSN 3007-3197
Print ISSN 3007-3189

http://amresearchreview.com/index.php/Journal/about

Conclusion

This paper examined three of the most popular ethnomedicinal foods in terms of the potential of antimicrobial and antibiofilm against common strains of Staphylococcus aureus and Pseudomonas aeruginosa. Fagonia indica methanolic extracts showed superior properties over Moringa oleifera and Calotropic procera by showing maximum zone of inhibition (3 mm) and antibiofilm development (43.3%). The said ethnomedicinal plants have the prospect of curing illnesses introduced by antimicrobial resistance (AMR) compounded by the prevailing climate change. In future research, it is possible to prepare the leaf extracts with other organic solvents like chloroform and ethyl acetate to get a bigger picture of the identification of antioxidant, neuroprotective and anticancer antifungal properties.

Work Cited

Abdulkadir, S., et al. (2016). Antibacterial activity of Moringa oleifera leaf extracts against pathogenic bacteria. *Journal of Medicinal Plants Research*, 10(5), 100–108.

Ahmed, A., et al. (2021). Phytochemical composition and medicinal potential of Calotropis procera. *Journal of Ethnopharmacology*, 278, 113–122.

Ali, A., & Khan, S. (2021). Ethnomedicinal applications and pharmacological properties of Fagonia indica in South Asia. *Phytotherapy Research*, 35(4), 2000–2015.

Ali, S. I., & Qaiser, M. (2009). Medicinal flora of Pakistan. *Pakistan Journal of Botany*, 41(6), 233–245.

Alqasoumi, S., et al. (2011). Anti-inflammatory and wound healing activities of Fagonia indica extracts. *Journal of Ethnopharmacology*, 133(2), 420–426.

Ameya, G., et al. (2019). Medicinal plant diversity in Pakistan: An overview. *Herbal Medicine Journal*, 4(2), 23–38.

Anwar, F., et al. (2007). Moringa oleifera: A food plant with multiple medicinal uses. *Phytotherapy Research*, 21(1), 17–25.

Ansari, M. A. (1983). Traditional uses of Fagonia indica. *Indian Journal of Traditional Knowledge*, 2(3), 12–18.

Artini, M., et al. (2018). Plant-derived compounds as biofilm inhibitors. *Frontiers in Microbiology*, 9, 1002.

Aslam, B., et al. (2021). Antimicrobial and antibiofilm potential of Fagonia indica from Cholistan Desert. *Journal of Applied Microbiology*, 130(5), 1560–1573.

Azam, F., et al. (2018). Pharmacological evaluation of Fagonia indica: An updated review. *Journal of Pharmacognosy and Phytochemistry*, 7(2), 101–108.

Babekov, Y., et al. (1999). Role of medicinal plants in healthcare systems. *Ethnobotany Research & Applications*, 1(1), 45–50.

Balial, A., et al. (2020). Comparative antimicrobial activity of Calotropis procera extracts. *Journal of Natural Remedies*, 20(3), 150–162.

Batool, R., et al. (2020). Phytochemical and pharmacological properties of Calotropis procera: A review. *Saudi Journal of Biological Sciences*, 27(2), 871–880.

Berdy, J. (2005). Bioactive microbial metabolites. *Journal of Antibiotics*, 58(1), 1–26.

Bhattacharya, S., et al. (2018). Nutritional and medicinal uses of Moringa oleifera. *Plant Foods for Human Nutrition*, 73(1), 1–12.

Online ISSN 3007-3197
Print ISSN 3007-3189
http://amresearchreview.com/index.php/Journal/about

Bennour, H., et al. (2020). Preservation of antioxidant activity in plant extracts: Effect of drying methods. *Food Chemistry*, 309, 125–136.

Chiocchio, I., et al. (2021). Bioactive metabolites from Calotropis procera: Pharmacological significance. *Frontiers in Pharmacology*, 12, 643–656.

Coz-Bolañosa, M., et al. (2018). Antioxidant and antibacterial activity of Moringa oleifera leaves. *Food Science and Technology*, 92, 65–72.

Dalukdeniya, D., et al. (2016). Comparative antimicrobial potential of Moringa oleifera extracts. *Asian Pacific Journal of Tropical Biomedicine*, 6(4), 303–308.

Dirir, A., et al. (2017). Traditional uses of Calotropis procera in arid regions. *Journal of Ethnopharmacology*, 199, 20–31.

Dillard, C. J., & German, J. B. (2000). Phytochemicals: Nutritional and functional properties. *Journal of Nutrition*, 130(12), 3297–3302.

Di Domenico, E. G., et al. (2022). Clinical implications of bacterial biofilms. *Frontiers in Cellular and Infection Microbiology*, 12, 789–804.

Donlan, R. M. (2002). Biofilms: Microbial life on surfaces. *Emerging Infectious Diseases*, 8(9), 881–890.

Estrella, M., et al. (2000). Nutritional and therapeutic properties of Moringa oleifera. *Food Science & Nutrition*, 40(6), 510–517.

Fernández-Billón, P., et al. (2023). Pseudomonas aeruginosa biofilms and multidrug resistance. *Microorganisms*, 11(2), 456.

Fuglie, L. (1999). The miracle tree: Moringa oleifera. Church World Service, Dakar.

Graham, H., et al. (2000). Pharmacognostic studies of Fagonia indica. *Fitoterapia*, 71(6), 699–705.

Guzzo, C., et al. (2020). Biofilm inhibition by natural compounds. *Antibiotics*, 9(12), 867.

Harrison, J. J., et al. (2018). Synergistic biofilm suppression with copper ions and QACs. *Applied Microbiology and Biotechnology*, 102(15), 6479–6490.

Hekmat, S., et al. (2015). Nutritional composition of Moringa oleifera leaves. *International Journal of Food Science*, 50(5), 1001–1010.

Joshua, R., et al. (2022). Antibacterial activity of Calotropis procera methanolic extracts. *Phytochemistry Letters*, 46, 77–84.

Karygianni, L., et al. (2020). Biofilm formation mechanisms and clinical relevance. *Frontiers in Microbiology*, 11, 580–595.

Kaur, M., et al. (2020). Therapeutic potential and antibiofilm activity of Moringa oleifera. *Biomedicine & Pharmacotherapy*, 128, 110–121.

Krishnan, S., et al. (2023). Biofilms: Adaptive strategies of microbial communities. *Microbial Pathogenesis*, 173, 105–112.

Lei, Y., et al. (2016). Antimicrobial activities of plant secondary metabolites. *Journal of Ethnopharmacology*, 192, 123–132.

Liu, Y. (2005). Biofilm-associated infections: Mechanisms and challenges. *Clinical Microbiology Reviews*, 18(2), 289–304.

Makonnen, E., et al. (1997). Pharmacological activities of Moringa oleifera leaves. *Phytotherapy Research*, 11(8), 555–558.

Mancuso, G., et al. (2021). Clinical relevance of Pseudomonas aeruginosa infections. *Infection and Drug Resistance*, 14, 1457–1470.

Online ISSN 3007-3197
Print ISSN 3007-3189

Mohamed, R., et al. (2015). Phytochemistry of Calotropis procera: A review. *Journal of Natural Products*, 8(2), 121–133.

Moyo, B., et al. (2012). Antimicrobial activity of Moringa oleifera extracts. *African Journal of Biotechnology*, 11(19), 4523–4531.

Nagaiah, C., et al. (2023). Biofilms on medical devices: Risks and prevention. *Journal of Hospital Infection*, 133, 34–45.

Naz, S., et al. (2020). Toxicity studies of Calotropis procera: Heavy metals accumulation. *Environmental Toxicology and Pharmacology*, 77, 103–112.

Nenaah, G. (2013). Antimicrobial activity of Calotropis procera extracts. *Saudi Journal of Biological Sciences*, 20(3), 265–272.

Parihar, P., & Balekar, N. (2016). Pharmacological properties of Calotropis procera. *International Journal of Pharmacology*, 12(6), 550–561.

Parvekar, P., et al. (2020). Determination of minimum bactericidal concentration in antimicrobial studies. *Journal of Microbiological Methods*, 174, 105–112.

Pinheiro, M. G., et al. (2014). Biofilm-associated antibiotic resistance. *Current Opinion in Microbiology*, 21, 21–26.

Pullaiah, T. (2006). Encyclopedia of world medicinal plants. Regency Publications.

Ruckmani, K., et al. (1998). Therapeutic applications of Moringa oleifera. *Journal of Medicinal Plant Research*, 2(2), 23–30.

Saini, R., et al. (2014). Phytochemical content of Moringa oleifera leaves. *Journal of Food Science*, 79(6), 104–112.

Salem, S., et al. (2014). Extraction and antimicrobial evaluation of Calotropis procera leaves. *Asian Pacific Journal of Tropical Biomedicine*, 4(1), 40–45.

Samuelsson, G. (2004). Drugs of natural origin: A textbook of pharmacognosy. 5th Edition. Stockholm: Apotekarsocieteten.

Saleem, A., et al. (2019). Molecular identification and bioactivity of Fagonia indica. *Phytochemistry Letters*, 29, 101–110.

Saleem, M., et al. (2020). Antibiofilm and antibacterial activity of Fagonia indica chloroform extracts. *Journal of Ethnopharmacology*, 250, 112–120.

Sánchez, G., et al. (2016). Antibacterial and antibiofilm activity of Prosopis laevigata and related plants. *Journal of Applied Microbiology*, 121(2), 514–526.

Shafat, A., et al. (2023). Antibacterial potential of Calotropis procera against MRSA. *Microbial Pathogenesis*, 172, 105–115.

Sharma, P., et al. (2009). Phytochemical screening and antibacterial activity of Fagonia indica. *Pharmacognosy Magazine*, 5(20), 248–254.

Shinwari, Z. K., & Shah, S. S. (2003). Medicinal plants research in Pakistan. *Pakistani Journal of Botany*, 35(1), 1–9.

Singh, R., et al. (2021). Pharmacological properties of Calotropis procera: A review. *Journal of Herbal Medicine*, 26, 100–110.

Suleiman, M., et al. (2022). Antibiofilm activity of Fagonia indica against Pseudomonas aeruginosa. *Journal of Ethnopharmacology*, 287, 114–122.

Tacconelli, E., et al. (2017). WHO priority list of antibiotic-resistant bacteria. *Lancet Infectious Diseases*, 17(3), 318–327.

Tanimu, S., et al. (2022). Antibiofilm properties of Calotropis procera. *BMC Complementary Medicine and Therapies*, 22(1), 223.

Thi, T., et al. (2020). Clinical challenges of Pseudomonas aeruginosa infections.

Online ISSN 3007-3197
Print ISSN 3007-3189

Pathogens, 9(6), 456.

Tossou, M. C., et al. (2018). Heavy metal toxicity and Calotropis procera. *Environmental Science and Pollution Research*, 25, 344–355.

Wadhwani, R., et al. (2021). Phytochemical and pharmacological profiling of Calotropis procera. *Journal of Ethnopharmacology*, 268, 113–123.

Wasif, S., et al. (2016). Nutritional evaluation and bioactivity of Moringa oleifera leaves. *International Journal of Food Science*, 2016, 345–355.