Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/t4t94d93

EVALUATION OF ANTIMICROBIAL EFFICACY AND GROWTH DYNAMICS OF HUMAN PATHOGENS EXPOSED TO PROSOPIS JULIFLORA POD EXTRACT

Khalid Hassan[1]*, Roohi Abid[1], Urooj Haroon[2], Ambreen Aziz[3], Rabya Fatima[4], Taba Rauf[1] Farina Ahmed[5], Noor Afshan Hassan[3]

[1]Centre for Plant Conservation, University of Karachi
[2]Department of Chemistry, Federal Urdu university sciences arts and technology, Karachi
[3]Institute of Environmental Studies, University of Karachi
[4]CESAT, Islamabad
[5]Jinnah Sindh Medical University, Karachi

***Corresponding author:** Khalid Hassan
*Email: env.khalid@gmail.com

Abstract

Introduction

Prosopis juliflora is a woody shrub indigenous to Northern South America, possessing resistance to harsh climatic conditions. The ethnobotanical value of the plant has long been established, especially in folk medicine for the treatment of a range of diseases. Yet, its antimicrobial potential has not been thoroughly investigated. This research is focused on testing the antibacterial activity of P. juliflora pod extract against prevalent human pathogens and the growth kinetics of these pathogens in the presence and absence of the extract.

Materials and Methods

Fruits of *Prosopis juliflora* were harvested from the Karoonjhar Mountains, Tharparkar District, Sindh, Pakistan. The crude extract was obtained with the use of methanol as a solvent. Antibacterial activity was determined using the agar well diffusion assay against Gram-positive as well as Gram-negative bacterial pathogens. In addition, growth kinetics of the preferred microbial strains were observed through the measurement of optical density (OD) at 600 nm at different intervals.

Results

The pod extract was shown to have high antibacterial activity against all but one of the bacterial strains tested, and well-defined zones of inhibition were observed. The extract did not exhibit inhibitory activity against Streptococcus pyogenes and Pseudomonas aeruginosa. The growth patterns of the bacterial strains showed an inhibition of bacterial growth when in contact with the extract, reflected by lower readings for OD values than the control groups.

Conclusion

The findings indicate that *Prosopis juliflora* pods are antibacterial, most probably due to the presence of a range of bioactive compounds. The work demonstrates the potential of this plant as a source of new antimicrobial agents. Isolation and identification of the active compounds responsible for these activities need to be done, which would lead to the creation of new pharmaceuticals.

Keywords: *Prosopis juliflora*, antibacterial activity, Streptococcus pyogenes, Pseudomonas aeruginosa, growth kinetics, agar well diffusion assay, bioactive compounds, antimicrobial agents.

Introduction

Prosopis juliflora, also referred to as mesquite, is a perpetually green tree with widespread distribution in arid and semi-arid areas known for its dense phytochemical content and medicinal value. It is rich in bioactive compounds like alkaloids, flavonoids, tannins, phenolic, terpenes, and steroids, which are responsible for its pharmacological activities such as antimicrobial, antioxidant, and anti-inflammatory properties. (Khandelwal p *et al*., 2016) Prosopis *juliflora*, or the Keekar, has been identified as a green plant producing a broad diversity of novel and biologically active compounds (Ibrahim et al., 2003). Prosopis juliflora falls under the Fabaceae family that consists of nearly 45 identified genera. This plant is globally distributed in Pakistan (Sathiya *et al*., 2008). Different components of *Prosopis juliflora* have been used in the management of infectious diseases from time immemorial, and the plant occupies a central position in traditional medicine practices (Malik *et al*., 2018). Crude extracts obtained from the plant have shown potential therapeutic activity, including anti-cancer activity. From 2012, there has been a significant increase in the occurrence of different cancers, especially those occurring in the lip, oral cavity, lungs, and liver. In addition to the mounting cancer burden, a large percentage of cases—calculated at 25%—have been seen in Pakistan. The World Health Organization (WHO) states that the economic burden of cancer in developing countries is rising. In the year 2010 alone, the combined global economic burden due to cancer was estimated at US$1.16 trillion (Selvamohan *et al*., 2012).

Plants have been a resource of bioactive compounds for centuries, which are synthesized in different parts of the plant and widely used in the pharmaceutical sector. The discovery of antibiotic-resistant pathogens is now a cause of concern worldwide in recent years (Tajbakhsh *et al*., 2000). The increasing rate of mortality caused by bacterial infections has led researchers to seek new natural and synthetic compounds that are therapeutically effective with fewer side effects on humans (Thakur et al., 2014). P. juliflora has been traditionally used in folk medicine to cure a number of diseases, and recent scientific research has confirmed its potential as a natural source of antimicrobial agents against human pathogens like Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa. (Tajbakhsh S *et al*., 2015). The World Health Organization (WHO) states that about 80% of the world's population depends on herbal medicines as part of their traditional healthcare system (Qureshi et al., 2014).

Prosopis juliflora is a species under the family *Fabaceae* and subfamily *Mimosoideae* and has around 44 identified species (William *et al.,* 2015). It originates from northern South America and is a robust, drought-tolerant shrub that can thrive in extreme environments. It is a versatile plant with several ecological and economic advantages such as soil conservation, nitrogen fixation, roadside plantation, charcoal, building material, fuel, fodder, and medicinal application (Gayathri *et al*., 2014). In the latter part of the 20th century, P. juliflora was introduced to many parts of the world as a measure to prevent desertification, deforestation, and climate change. It has now extended to millions of hectares of rangeland in South Africa, East Africa, and coastal Asia. It was introduced in Sindh in Pakistan and has, since then, been reported in Punjab and along the coast of Baluchistan. It is known locally as "Kabuli Kikar" or "Valayati Jand" (Dos *et al*., 2013).

A survey of the literature demonstrates the high ethnobotanical significance of *Prosopis juliflora*, especially its extensive use in traditional systems of medicine for curing multiple diseases. The crude extracts from the shoot and root of *Prosopis juliflora* have been found to be rich in nutrients. Pods, though bioactive, contain anti-nutrients like saponins and cyanogenic compounds. However, P. juliflora is packed with bioactive phytochemicals including tannins, flavonoids, alkaloids, and polyphenolic compounds, which are all renowned for their extraordinary therapeutic activities. All

these bioactive compounds are heavily used by the pharmaceutical industry for their high potential against pathogenic bacterial isolates (Gayathri *et al*., 2014).

These compounds have antibacterial actions not just through the inhibition of bacterial growth but also through the disruption of microbial activity, particularly through the interference with enzyme active sites (Dos *et al*., 2013). Microbial kinetic analysis is one of the effective methods to assess the antimicrobial action of plant-derived compounds, through which a thorough understanding of inhibitory mechanisms can be achieved. Considering the antibacterial activity of P. juliflora pod extracts, the current research was planned to evaluate them in vitro pharmacological activity with a view to establishing their therapeutic utility and human health benefits. With the rise in antibiotic resistance among pathogens and the limitation of traditional antimicrobial agents, identification of plant-derived compounds such as P. juliflora presents a rich source for the design of new drugs. This study intent to perform a thorough phytochemical characterization of P. juliflora and assess its potential as an antimicrobial agent against various human pathogens, along with analysing the growth kinetics of these pathogens treated with the plant extracts to determine their mechanisms and effectiveness of inhibition.

Taxonomic position:

Kingdom: Plantae
Phylum: Tracheophyta
Class: Magnoliopsida
Order: Fabales
Family: *Fabaceae*
Genus: Prosopis
Species: *Prosopis juliflora*

Material and Methods

Study site:

Prosopis juliflora pods were harvested from the Karoonjhar Mountain area, District Tharparkar, Sindh, Pakistan, in November 2019. The plant specimen was taxonomically recognized by the experts of the Centre for Plant Conservation, University of Karachi. A voucher specimen was conserved and deposited for future reference.

Fig 01: sample collection from district Tharparkar, Karoonjhar

Source: http://earth. Google/web/search/karoonjhar+mountain +sardharo

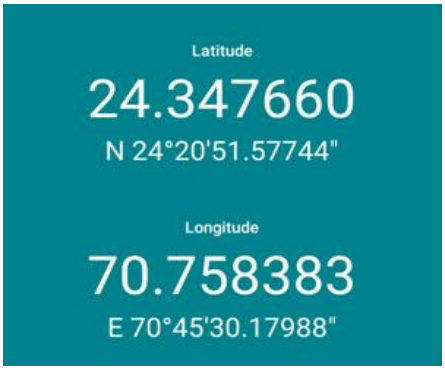

Fig 02: GPS coordinates of sample collection point.

Collection of plant material, drying and storage:
Prosopis juliflora pods were harvested at the fruiting stage. The harvested pods were washed with running water to eliminate surface contaminants and then air-dried at room temperature for 15 days. After complete drying, the pods were powdered using a mortar and pestle. The powdered material was placed in airtight glass containers and stored in a dark, dry place at room temperature to avoid moisture uptake and maintain phytochemical integrity.

Preparation of extracts:
100 g of Prosopis juliflora pod powder was macerated in 500 mL of 98% methanol (1:5 w/v ratio) for seven days at room temperature. After the first maceration, the mixture was then homogenized and sonicated employing a sonicator in order to break cell membranes and allow intracellular contents to be released by disrupting intermolecular forces. The resulting solution was then filtered through Whatman filter paper, and the filtrate was concentrated by evaporation of methanol using a rotary evaporator (Bio-base RE 2010D) under reduced pressure. The crude metabolic extract obtained was transferred to sterile bottles and kept at 4°C for subsequent analysis.

Selection of microorganisms:
Twelve strains of bacteria, selected from the organisms whose known pathogenicity for humans provided the basis of selection, included six Gram-positive and six Gram-negative bacterial strains. These Gram-positive strains included *Bacillus subtilis, Corynebacterium diphtheria, Staphylococcus aureus, Staphylococcus epidermidis, Streptococcus pyogenes, and Streptococcus faecalis*, and these Gram-negative strains were *Enterobacter spp., Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa,* and *Salmonella typhi.* Each of these microbes' pellets was reconstituted in a lyophilization buffer, followed by standard microbiological methods to form a homogeneous mixture of lyophilized form.

***In vitro* antibacterial Activity Determination of (ZOI) zone of inhibition**
Prosopis juliflora pod extract in vitro antibacterial activity was determined by well diffusion method. In brief, 50 µL of every bacterial culture was inoculated in sterilized Petri plates containing about 15 mL of molten nutrient agar. The medium was left at room temperature for solidification. After solidification, wells of 5 mm diameter were punched in the agar using a sterile metallic cork borer. Each well was subsequently filled with 50 µL of crude pod extract.

For effective diffusion of the extract, inoculated plates were pre-incubated for one hour at 4°C. Then the plates were incubated at 37°C for 24 hours. Sterile controls were performed to authenticate the experimental process. On incubation, the antibacterial activity was evaluated by measuring the zone of inhibition around the wells, expressed in millimetres (mm).

Growth kinetics of microorganisms

The growth kinetics of some bacterial strains were determined to determine the antibacterial activity of Prosopis juliflora pod extract. Nutrient broth was made in a 500 mL flask, and 50 mL of the broth was poured into two different flasks for each of the bacterial strains. Flask A had 50 mL of nutrient broth inoculated with the particular microbial strain, which was the control. Flask B held 50 mL of nutrient broth with the same microbial strain added to it along with the crude P. juliflora pod extract, which was the treatment group. This process was repeated for all the bacterial species being investigated.

All the flasks were kept at 37°C. The readings of optical density (OD) were recorded at 600 nm at two-hourly intervals for 24 hours using a UV-Vis spectrophotometer (Shimadzu 1700, Model YDL-7, Biobase, Japan) to check for bacterial growth and the inhibitory activity of the extract.

Table.1: Yield of *Prosopis juliflora* extract obtained with solvent by infusion and

Extraction Method	Solvent	Solvent Volume (ml)	Weight of Powder (g)	Weight of Extract (g)	Extract Yield (%)
Infusion	Methanol	500ml	100g	11.706	11.706
Rotary Evaporator	Methanol	500ml	100g	27.361	27.361

Formula for getting Yield:

The extract yield was calculated using the following formula:

$$\text{Yield (\%)} = \frac{\text{weight of extracted plant residues}}{\text{weight of plant raw sample}} * 100$$

Phytochemical analysis

Carbohydrates test:

Few drops of concentrated sulphuric acid in 2ml extract. 1 ml molish regent added. Purple colour shown presences of Carbohydrates.

Terpenoids test:

1 ml extract add in 1 ml chloroform and mixed 1 ml concentrated sulpuric acid. Reddish brown colur shown presence of terpenoids**.**

Steroids Test:

1 ml chloroform mixed with 1ml extract. Then add 10 drops of acetic anhydride and 5 drops of concentrated sulphuric acid. Then shake well. Dark red or dark pink color shown presence of steroids.

Saponins Test:

in graduate cylinder, 2 ml of extract and 2 ml of distilled water shaken 15 minutes' formation of 1 cm layer of foam indicator presence of saponins.

Anthraquinone Test:

1 ml of extract and 1 ml of 10% ammonia solution pink precipitate shown the presence of Anthraquinone.

Antho-Cyanosides

5 ml of dilute hydrolic acid mixed with 1 ml of extract. Pale pink colour shown the activity of antho -cyanosides**.**

Alkaloids Test:

1 ml extract mixed with 1 ml picric acid saturated solution. Yellow precipitate from indicate alkaloids is present.

Tannin Test:

1 ml extract add with 2 ml of 5% ferric chloride solution dark blue colour indicate tannin is present.

Flovoinds Test:
1 ml extract with ferric chloride solution brown colour appeared indicate flavonoids is present.
Phenol Test:
Few drops of ferric chloride solution were treated with 1 ml of extract. Bluish color indicate phenol is present. (Gayathri *et al.* 2014)

Result and Discussion
Two methods of extraction were utilized, and a yield of 27.361% using the rotary evaporator was far greater than by the infusion technique, as summarized in Table 1. *Prosopis juliflora* pod extract showed impressive antibacterial action against all of the tested Gram-positive and Gram-negative bacterial strains. The zone of inhibition tended to be largely uniform in the majority of strains; however, Streptococcus pyogenes and Pseudomonas aeruginosa were relatively less susceptible, as summarized in Tables 2 and 3.

In addition to evaluating antibacterial activity, growth and inhibitory kinetics of each bacterial strain were investigated with and without the pod extract. Optical density (OD) was measured at 600 nm in two-hour intervals to observe growth patterns of the bacteria.

Table 2 showed the microbial growth and inhibitory kinetics with extracts of pods against Gram positive. *S. aureus* and S. *epidermiditis* showed a well-defined zone of inhibition while *B. subtilis, C. diphtheria* and *S. faecalis* showed approximately same activity with extract *S. pyogenes* showed no zone of inhibition with extract.
Table 3 showed similar size of zone in case of Gram –ve bacteria except *P. aeruginosa* which showed no activity and *K. pneumonia* and *Proteus* were highly active in all selected –ve organisms. *P. aeruginosa, Proteus* and *Enterobacter* showed high optical density which showed less inhibitory activity with extract. However, *E. coli, S. typhi* and *K. pneumoniae* were actively inhibit the growth in presence of extract in nutrient broth. Approximately after 18 hours' organisms started to decline due to extract as showed in table 5. It is clearly seen in figure 1 and 3 that organisms were following a normal bacterial growth cycle without extract a long stationary phase followed by decline phase after exponential growth in log phase but this cycle was completely disturbed in case of extract in both cases of Gram +ve and -ve bacteria as mentioned in figure 2 and 4 a very slow growth due to compounds which slow down the enzymatic activity of bacterial cells. Plant extract not only inhibit the bacterial growth it can also slow down the bacterial activity as well.
Table 4 showed the optical density of gram +ve organisms with and without extract. Optical density slowly increasing with extract in initial readings of log phase. However, after 14 hours' decline phase started and optical density going down except in case of *S. epidermiditis.* It was observed that S. pyogenes showed strong activity with extract in nutrient broth but no zone of inhibition observed in solid nutrient ager. In case of without extract the organism's growth was higher as compare to with extract and showed longer log phase and high optical density. Stationary phase started after approximately 12 hours due to limited availability of nutrients. Figure 1showed dose response curve obtained at 600 nm.
Table 5 showed the activity of gram negative bacteria against the activity against the with extract and without extract. Initial 2 hours' organisms trying to adjust in surrounding according to the table and Fig No. 2
Presence of phenol and its derivative cause the deactivation of bacterial growth by destruction of *p*-hydroxyphenyl acrylate (M2)>allyl *p*-hydroxyphenyl acetate (M1)≈*p*-2-propenoxyphenol (M3) and also effects on the synthesis of protein (Dos *et al*. 2013). Flavonoids are having strong antibacterial activity against gram positive and gram negative bacteria. In which include *E.coli P.aeroginosa* and *Saureus.* (Agarwal et al. 2016). it acts as anti MRSA agent and become a part of bioactive region (Ahmad et al. 2019). Tanin and its compounds are very for the field of medical sciences now synthetic compounds of tannin use as antibacterial agents. These are giving high activity against *S. aureus, S.*

pyrogens, *Salmonilla typhi*, and *E.coli* (Chandra *et al.* 2017). Terpenoids has ability to shown the strongest DDPH free radical scavenging and the highest reducing power. The steroids and it compound inhibit the growth of bacterial strains (Chew *et al.* 2011).

Table 2. Zone of inhibition (mm) of pods extract

Gram +ve Organisms	Zone of Inhibition
Bacillus subtilis	25
Corynebacterium diphtheria	25
Staphylococcus aureus	30
Staphylococcus epidermiditis	29
Streptococcus pyogenes	00
Streptococcus faecalis	26
Pseudomonas aeroginosa	00
E. coli	25
Salmonella typhi	26
Proteus	29
Klebsiella pneumonia	30
Enterobacter	25

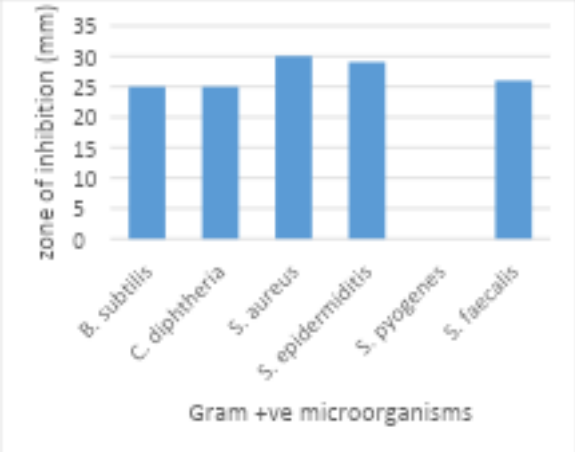

Figure. 3: Zone of inhibition of Gram +ve Bacteria

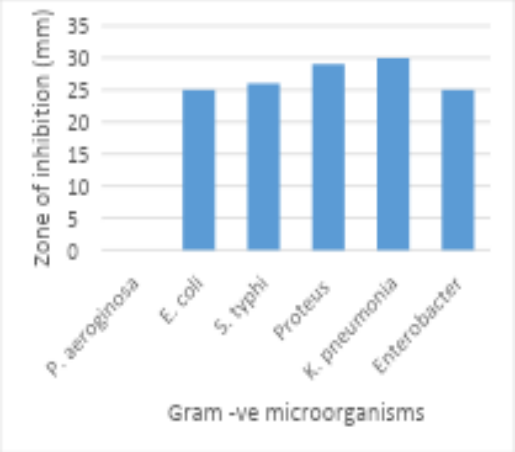

Figure. 4: Zone of inhibition of Gram -ve Bacteria

Table. 4: Gram +ve bacterial growth with and without extract of Prosopis juliflora (Optical Density at 600nm)

Time Duration (hrs)	*B. subtilis*		*C. diphtheria*		*S. aureus*		*S. epidermiditis*		*S. pyogenes*		*S. faecalis*	
	With Extract	Without Extract	With Extract	Without Extract	With Extract	Without Extract	With Extract	Without Extract	With Extract	Without Extract	With Extract	Without Extract
2	0.08	0.02	0.6	0.32	0.148	0.026	0.01	0.005	0.09	0.032	0.101	0.03
4	0.11	0.08	0.723	0.206	0.172	0.036	0.125	0.121	0.101	0.033	0.11	0.029
6	0.19	0.57	0.778	0.907	0.649	0.511	0.213	0.32	0.122	1.176	0.623	0.901
8	0.23	0.82	0.811	1.003	0.788	0.646	0.386	0.46	0.321	1.134	0.633	1.12
10	0.29	0.88	0.823	1.006	0.799	0.646	0.556	0.812	0.412	1.135	0.634	1.231
12	0.31	0.89	0.702	1.008	0.811	0.646	0.561	0.806	0.563	1.136	0.633	1.232
14	0.354	0.89	0.701	1.008	0.898	0.645	0.576	0.806	0.598	1.134	0.632	1.232
16	0.35	0.88	0.698	1.007	0.755	0.645	0.602	0.805	0.578	1.132	0.565	1.232
18	0.341	0.87	0.677	1.007	0.703	0.643	0.846	0.803	0.531	1.131	0.478	1.233
20	0.213	0.86	0.611	1.007	0.612	0.642	0.846	0.802	0.501	1.130	0.411	1.231

Table. 5: Gram -ve bacterial growth with and without extract of Prosopis juliflora (Optical Density at 600nm)

Time Duration (hrs)	*P.aeruginosa*		*E. coli*		S. typhi		Proteus		*K.pneumoniae*		*Enterobacter*	
	With Extract	Without Extract	With Extract	Without Extract	With Extract	Without Extract	With Extract	Without Extract	With Extract	Without Extract	With Extract	Without Extract
2	0.123	0.028	0.08	0.027	0.101	0.034	0.6	0.32	0.023	0.025	0.148	0.021
4	0.118	0.031	0.122	0.08	0.105	0.031	0.98	0.11	0.041	0.038	0.172	0.029
6	0.788	0.63	0.702	0.57	0.115	1.176	0.657	0.907	0.11	0.537	0.649	0.211
8	0.83	0.694	0.827	0.877	0.121	1.134	0.719	1.003	0.221	0.646	0.842	0.312
10	0.801	0.701	0.801	0.892	0.126	1.135	0.723	1.006	0.222	0.658	0.846	0.432
12	0.741	0.701	0.78	0.891	0.128	1.135	0.711	1.008	0.216	0.711	0.846	0.578
14	0.698	0.699	0.774	0.890	0.129	1.135	0.701	1.007	0.221	0.775	0.845	0.588
16	0.655	0.699	0.714	0.988	0.13	1.133	0.652	1.007	0.209	0.831	0.701	0.591
18	0.64	0.678	0.411	0.988	0.131	1.133	0.601	1.007	0.204	0.871	0.625	0.589
20	0.544	0.672	0.323	0.983	0.129	1.132	0.6	1.005	0.204	0.87	0.603	0.589

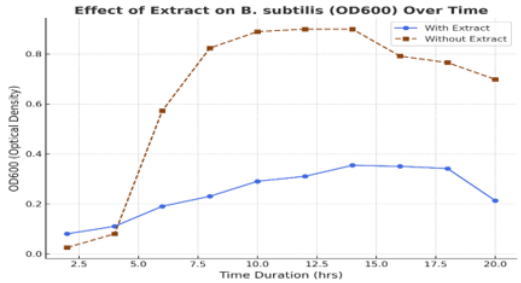

Fig No. 05: Antibacterial activity of plant extract against a *Bacillus*

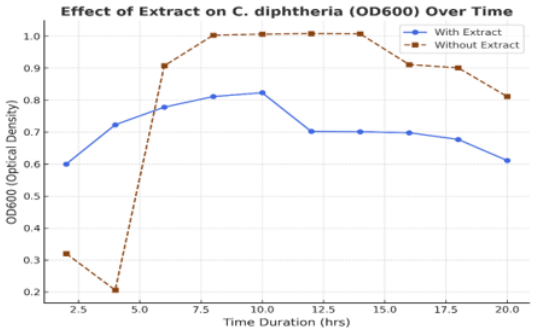

Fig No. 06: Antibacterial activity of plant extract against a *C.diphtheria*

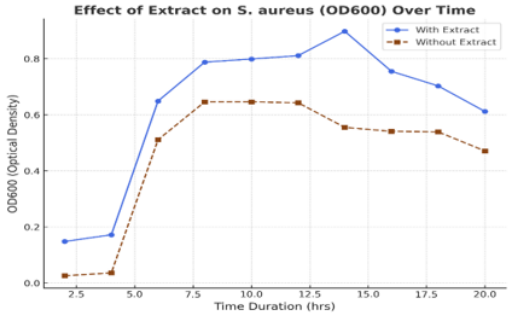

Fig No. 07: Antibacterial activity of plant extract against a *S.aureus*

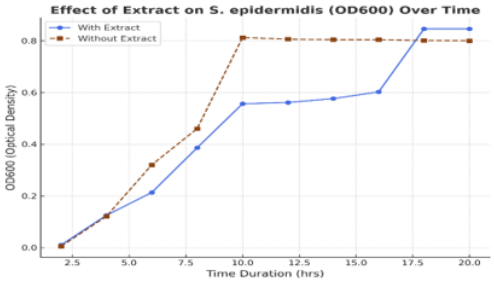

Fig No. 08: Antibacterial activity of plant extract against a *S.epidermidis*

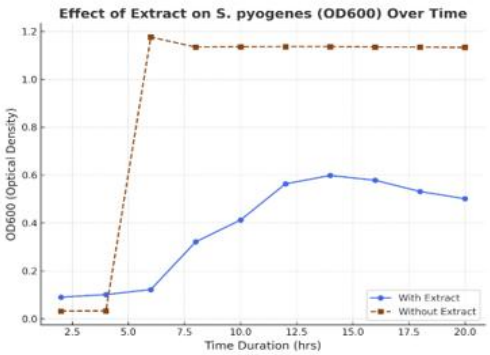

Fig No. 09: Antibacterial activity of plant extract against a *S.pyogenes*

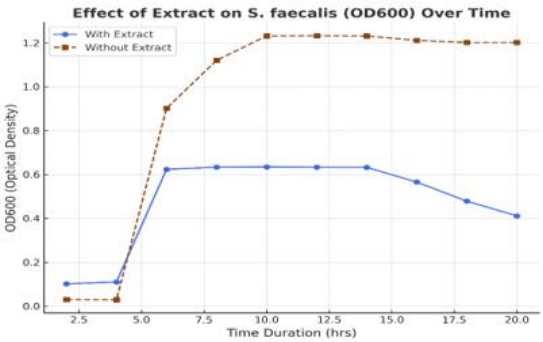

Fig No. 10: Antibacterial activity of plant extract against a *S.faecalis*

This graph shows the effects of an extract, most likely from plant pods, on Bacterial strains growth over a 20-hour period as determined by optical density (OD_{600}).

Fig No. 05 represents the development of bacteria when the extract is present. At every time point, growth is markedly inhibited. Restricted bacterial proliferation is shown by the OD_{600} values, which stay low and plateau at 0.35. OD_{600} even slightly decreases after 18 hours, which could be the result of stagnant growth or bacterial mortality.
In the without extract B. subtilis growing normally without developing any resistance. The OD_{600} shows strong bacterial growth, rising quickly and peaking at 0.89.

After 14 hours, growth seems to level or slightly fall, which is normal when nutrients in the culture run out.

According to the fig No.06 growth of C. diphtheria is moderately inhibited by the extract. Although there is initial development (OD600 increases from around 0.60 to about 0.82 by 10 hours), it plateaus and then starts to drop, which suggests suppression. By 20 hours, the extract's inhibitory impact may have caused bacterial death or a static phase, as the OD_{600} declines to about 0.61.

Bacterial growth, reaching a peak between 10 and 14 hours with an OD_{600} of 1.0. After 14 hours, there is only a slight decrease, most likely as a result of nutrient depletion. In the absence of any inhibiting agents, this is the anticipated natural growth curve.

Exhibits rapid bacterial growth, reaching a peak between 10 and 14 hours with an OD_{600} of 1.0.After 14 hours, there is only a slight decrease, most likely as a result of nutrient depletion. In the absence of any inhibiting agents, this is the anticipated natural growth curve.

According to the fig No. 7 shows the typical growth pattern of *S. aureus* under control conditions. By 14 hours, the OD_{600} rises from about 0.15 to a peak of about 0.89. A slow decline thereafter indicates the start of the stationary or death phase, which is caused by waste build up or nutrient exhaustion.

In the presence of extract shows that the pod extract was exposed to S. aureus. The OD_{600} peaks at about 0.65, and growth is slower and more constrained. The bacteria don't grow as much as the untreated group, and this plateau happens sooner. After 12 hours, a lower trend indicates early inhibition or bacterial death brought on by the extract.
The outcomes make it abundantly evident that the pod extract inhibits *S. aureus*. In comparison to untreated bacteria, the treated bacteria (brown line) exhibit slower growth, a lower peak OD_{600}, and an earlier decline phase. This suggests that the extract might have antibiotic chemicals that effectively inhibit the growth of *S. aureus*.
According to the fig No, 08 without extract lines displays the proliferation of S. epidermidis. By 10 hours, there is a sharp rise to about 0.81 OD_{600}, after which it plateaus, indicating that the bacteria enter a stationary phase early.

As per the fig No. 08 with extract the growth that was initially slower than that of the untreated group. After 18 hours, the OD_{600} gradually increases to ~0.85, surpassing the control. This spike in the late stages indicates that either the bacteria adapted or the extract's impact waned with time.

As per the fig No. 09 the *S. pyogenes* grows extremely quickly and highly; after six hours, its OD_{600} has increased to about 1.17 and has remained there for the whole twenty-hour period.

This is an example of unchecked, exponential bacterial growth, which rapidly approaches a plateau when the culture achieves its maximum density.

In fig No. 09 The pod extract significantly inhibits growth. OD_{600} only gradually rises, reaching a peak of about 0.60 after 14 hours, before slightly declining. Growth is almost halved in comparison to the untreated group, indicating a strong antibacterial activity of the extract.

According to fig No. 10 *S. faecalis* grows rapidly, reaching OD_{600} ~1.24 by 10 hours. Growth remains stable at this high level through 20 hours. This is normal exponential bacterial growth, followed by a plateau phase (stationary phase).
When compared to the untreated group, the pod extract dramatically slows down *S. faecalis* growth. The extract's antibacterial potential is demonstrated by the treated cells' (blue line) lower maximum OD600 and quicker growth fall. After 14 hours, the trend started to decline, which could indicate a loss of viability or long-term inhibition.

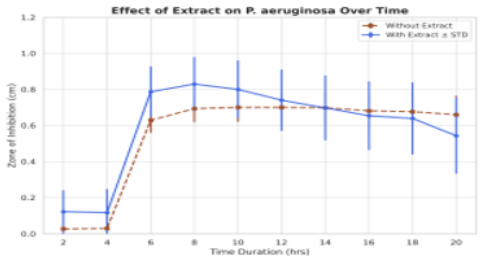

Fig No. 11: Antibacterial activity of plant extract against a *P.aeruginosa*

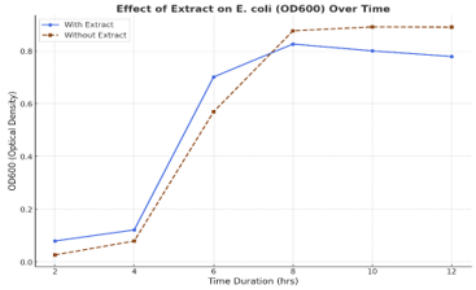

Fig No. 12: Antibacterial activity of plant extract against a *E.coli*

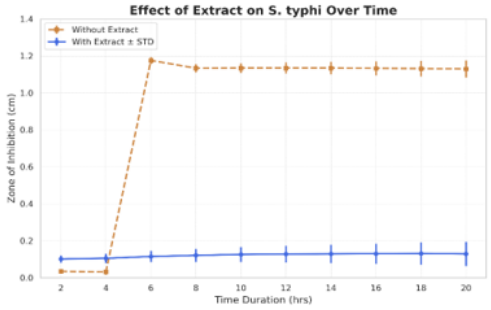

Fig No. 13: Antibacterial activity of plant extract against a *S.typhi*

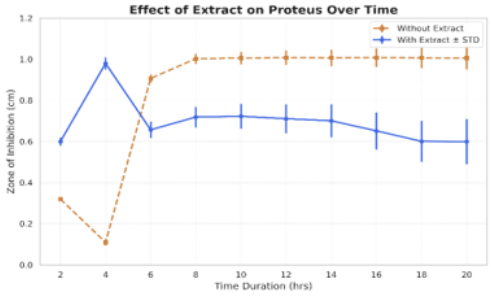

Fig No. 14: Antibacterial activity of plant extract against a *Proteus*

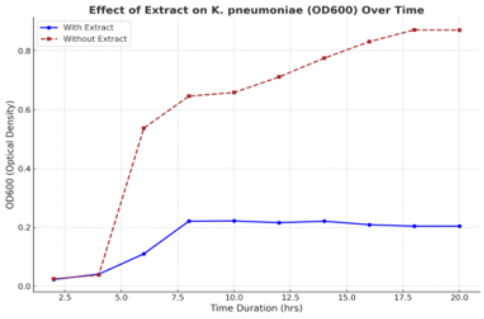

Fig No. 15: Antibacterial activity of plant extract against a *K.pneumoniae*

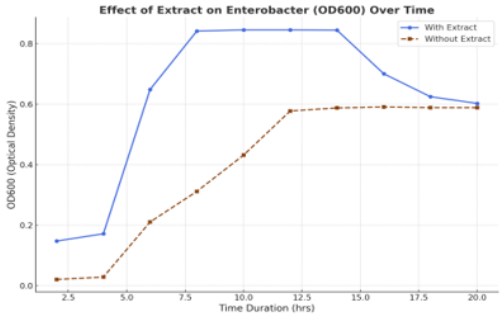

Fig No. 15: Antibacterial activity of plant extract against a *Enterobacter*

Fig No. 11 shows the growth of pseudomonas after being treated with the extract. Up to eight hours, the OD_{600} rises rapidly, reaching a value of 0.83. Growth starts to slow down after ten hours, which could indicate the start of inhibition or a stress reaction. This suggests that the extract might, through its potential antibacterial qualities, develop resistance to bacterial development.

In without extract symbolises untreated, spontaneous Pseudomonas growth. After ten hours, the OD_{600} gradually increases and reaches a plateau at 0.70. The progression through the lag, exponential, and stationary phases without suppression is indicative of a typical bacterial growth curve.

Perhaps as a result of nutritional depletion or the activation of inhibitory chemicals, the extract first seems to allow growth before suppressing or stopping additional proliferation. The extract's effectiveness in regulating the Pseudomonas population at later stages is demonstrated by the untreated culture's typical growth.

Fig No. 12 illustrates how an extract affects *E. coli* growth over time using OD_{600} (optical density at 600 nm), a metric that is correlated with bacterial concentration.

Reflects E. coli growth in the presence of the extract. Growth increases significantly until 8 hours (peaking at ~0.83), then starts to decline gradually. This trend suggests that while initial growth was not severely hindered, the extract begins to exert inhibitory effects over time, possibly due to

cumulative stress or bacteriostatic action. The decline after 8 hours indicates reduced viability or slowed replication, implying the extract can resist or suppress bacterial growth.
The extract's presence affects the dynamics of *E. coli* growth. Although the two cultures begin similarly, the treated group peaks earlier and shows a slight drop, whereas the untreated group grows steadily. This suggests that the extract may have antibacterial or stress-inducing qualities.

In fig No. 13 shows the growth of *S. typhi* after being treated with the extract. Very little growth is seen, as the OD_{600} steadily rises from about 0.10 to about 0.13 over the course of 16 hours. This low, flat growth curve shows that the extract has a potent bacteriostatic or inhibitory action, greatly reducing the growth of *S. typhi*.

Denotes normal bacterial proliferation without extract. Demonstrates a significant rise in OD_{600} from 4 to 6 hours, reaching a peak of around 1.17, and subsequently stabilizes. This indicates standard, unrestricted bacterial proliferation, adhering to a conventional exponential growth trajectory that stabilizes during the stationary period.

The substantial difference in OD_{600} values between the treated and untreated groups indicates that the extract successfully suppresses *S. typhi* growth. The extract's antibacterial ability against S. typhi is confirmed by the contrast between the two curves.

In fig No. 14 how *Proteus* grows when the extract is present. The OD_{600} increases rapidly in the first four hours (from 0.6 to 0.98), then stabilizes and gradually drops back to 0.6 by the twentieth hour. This implies that while initial growth may take place, the extract soon begins to prevent bacterial activity or reproduction. The downward trend suggests that Proteus growth is being inhibited or resistant as a result of the extract's antibacterial qualities.
According to the fig No. 14 untreated Proteus growth. Following a brief decrease at 4 hours, OD_{600} rises quickly and stays high (~1.0) for the next 6 hours. This indicates consistent and typical bacterial growth, which is a feature of an untested environment.
Following an initial growing period, the extract shows a bacteriostatic or inhibitory action on *Proteus*. The extract's antimicrobial potential against *Proteus* is confirmed when the treated group exhibits decreased long-term proliferation whereas the control group develops unhindered.

In the fig. No. 15 bacterial growth in the extract's presence is shown by this line. Throughout the experiment, the increase stays steady and rather modest. Effective prevention of bacterial proliferation is shown by the OD_{600} values plateauing and slightly declining after an initial small increase that lasts for around 8 hours. The extract has a potent bacteriostatic action, inhibiting *K. pneumoniae* growth.
This line illustrates the bacteria's untreated natural growth. After 4–6 hours, there is a noticeable spike in OD_{600}, which continues to rise slowly until it reaches a stable value of 0.87 at 20 hours. In the absence of the inhibitory extract, this pattern indicates unchecked bacterial proliferation.

In comparison to without extract, the extract's presence dramatically decreased the proliferation of *K. pneumoniae*.
The treated group exhibits early growth inhibition beginning at 6 hours. Rapid bacterial multiplication, characteristic of log-phase growth, is seen in the untreated group.

In the fig, No. 16, depicts bacterial growth in the presence of the extract. Initially, the OD_{600} increases significantly, peaking at 8-10 hours (~0.85), indicating early bacterial development. However, after this moment, the optical density decreases, showing that the extract has a long-term suppressive or death impact on bacterial cells. The extract eventually has a bactericidal or growth-inhibitory effect, which is notably visible after 12 hours.

This line displays the development of bacteria in the absence of any extract. The *Enterobacter* growth entered a stationary phase without being impeded by any external factor, as evidenced by the OD_{600} rising gradually but steadily to a high of about 0.59 by 12–14 hours, after which it plateaus.

With a time-dependent effect, the plant extract shows moderate to strong antibacterial activity against *Enterobacter,* permitting initial development followed by a notable drop in OD600 values. These results imply that the extract might have bactericidal qualities that show up after a specific amount of exposure.
Secondary metabolite is responsible to inhibit the growth of bacteria (Hassan. *etal*, 2022)

Table 6: Phtochemical analysis of prppsipus jully flora

Metabolite	Phenolics	Flavonoids	Tannins	Terpenoids	Saponins	Alkolids	Steroid	Anthrainone
*+/-	+	+	-	+	+	-	+	-

Indication * +, Present. -, Absent

Conclusion:
The high phytochemical content and high antibacterial activity displayed by Prosopis juliflora pod extract against both Gram-positive and Gram-negative bacterial strains are strong evidence in favor of its traditional application in the treatment of bacterial infection. These results offer a strong justification for the possible therapeutic utilization of this plant in contemporary medicine. Yet, more studies are needed to isolate, purify, separate, and characterize the active bioactive constituents in the pods, as well as other plant parts.

Future research should involve systematic screening of the most active solvent fractions for identification and isolation of the principal bioactive constituents responsible for observed antibacterial activity. This may allow for the identification of new antibacterial compounds, which may result in the creation of new, successful therapies to fight bacterial pathogens, particularly with respect to the growing resistance to traditional antibiotics. Isolation and thorough analysis of these bioactive principles will be essential in furthering our knowledge of P. juliflora's pharmacological potential and its use in antimicrobial lead discovery.

Acknowledgements The Authors are thankful to Dr. Saima, Assistant Professor, Department of Microbiology, FUUAST, Karachi for providing necessary research facilities

References

1. Khandelwal P, Sharma RA, Agarwal M. Phytochemical analyses of various parts of Prosopis juliflora. Int J Curr Pharm Res. 2016;8(3):1-5. Available from: https://journals.indexcopernicus.com/api/file/viewByFileId/447249
2. Ibrahim M, Nadir M, Ali A, Ahmad VU, Rasheed M. Phytochemical analyses of Prosopis juliflora Swartz DC. Pakistan Journal of Botany. 2013 Dec 1;45(6):2101-4.
3. Sathiya M, Muthuchelian K. Investigation of Phytochemical Profile and Antibacterial Potential of Ethanolic Leaf Extract of Prosopis juliflora DC. Ethnobotanical leaflets. 2008;2008(1):167.
4. Malik SK, Ahmed M, Khan F. Identification of novel anticancer terpenoids from Prosopis juliflora (Sw) DC (Leguminosae) pods. Tropical Journal of Pharmaceutical Research. 2018;17(4):661-8.
5. Selvamohan T, Ramadas V, Kishore SS. Antimicrobial activity of selected medicinal plants against some selected human pathogenic bacteria. Advances in Applied Science Research. 2012;3(5):3374-81
6. Tajbakhsh S, Barmak A, Vakhshiteh F, Gharibi M. Invitro antibacterial activity of the Prosopis juliflora seed pods on some common pathogens. Journal of clinical and diagnostic research:

JCDR. 2015 Aug;9(8):DC13.WHO (2000). General guidelines for methodologies on research and evaluation of traditional medicine.
7. Thakur R, Singh R, Saxena P, Mani A. Evaluation of antibacterial activity of Prosopis juliflora (SW.) DC. leaves. African Journal of Traditional, Complementary and Alternative Medicines. 2014 Jul 10;11(3):182-
8. Tajbakhsh S, Barmak A, Vakhshiteh F, Gharibi M. Invitro Antibacterial Activity of the Prosopis Juliflora Seed Pods on Some Common Pathogens. J Clin Diagn Res. 2015 Aug;9(8):DC13-5. doi: 10.7860/JCDR/2015/13549.6370. Epub 2015 Aug 1. PMID: 26435943; PMCID: PMC4576534.
9. Qureshi H, Arshad M, Bibi Y. Invasive flora of Pakistan: a critical analysis. International Journal of Biosciences. 2014;4(1):407-24.
10. William K, Jafri L. Mesquite (Prosopis juliflora): Livestock grazing, its toxicity and management. Journal of Bioresource Management. 2015;2(2):7.Gayathri V, Kiruba D.
11. Gayathri V, Kiruba D. Preliminary phytochemical analysis of leaf powder extracts of Psidium guajava L. International Journal of Pharmacognosy and Phytochemical Research. 2014 Aug 25;6(2):332-4.
12. Dos Santos ET, Pereira ML, Da Silva CF, Souza-Neta LC, Geris R, Martins D, Santana AE, Barbosa LC, Silva HG, Freitas GC, Figueiredo MP. Antibacterial activity of the alkaloid-enriched extract from Prosopis juliflora pods and its influence on in vitro ruminal digestion. International journal of molecular sciences. 2013 Apr;14(4):8496-516.
13. Agarwal P, Agarwal N, Gupta R, Gupta M, Sharma B. Antibacterial activity of plants extracts against methicillin-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus faecalis. Journal of microbial and biochemical technology. 2016;8(5):404-7.
14. Ahmad A, Ali A, Basit A. Ethnomedicinal study of various plants in lone valley, district Chitral, KPK, Pakistan. Journal of Medicinal Plants. 2019;7(3):24-8.
15. Chandra H, Bishnoi P, Yadav A, Patni B, Mishra AP, Nautiyal AR. Antimicrobial resistance and the alternative resources with special emphasis on plant-based antimicrobials—a review. Plants. 2017 Jun;6(2):16.
16. Chew YL, Chan EW, Tan PL, Lim YY, Stanslas J, Goh JK. Assessment of phytochemical content, polyphenolic composition, antioxidant and antibacterial activities of Leguminosae medicinal plants in Peninsular Malaysia. BMC complementary and alternative medicine. 2011 Dec;11(1):1-0.
17. Hassan, K., Bano, R., Aziz, A., & Zuberi, M. H. (2022). PHYTOCHMENICAL STUDY OF DIFFERENT PART OF RICINUS COMMUNIS. *FUUAST Journal of Biology*, *12*(2), 53-56.