Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i19.3846

EVALUATION OF ANTIBACTERIAL ACTIVITY OF *PIPER NIGRUM* AND *CUMINUM CYMINUM* EXTRACTS ON BACTERIAL ISOLATES FROM THROAT INFECTION

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Abstract

Medicinal plants and their extracts have been used as traditional remedies to treat differenttypes of infectious diseases. The throat infection is the inflammation of respiratory tissues, which is mostly caused by bacterial pathogens. The *Piper nigrum* and *Cuminum cyminum* are aromatic, famous valuable medicinal plants which are found in Pakistan, Indonesia, Malaysia, India, Sri Lanka and China, and are used as traditional systems of medicine like Ayurvedic and Unani System of medicines. This research study has explored the antibacterial activity of these two plants. The aim of our research study was, to evaluation of antibacterial activity of *Piper nigrum* and *Cuminum cyminum* extracts on clinical bacterial isolates from throat. By using polythene bag, both plants powders were obtained from local market of District Peshawar, Khyber Pakhtunkhwa and extracted with methanol. In this study, a total of 50 respiratory throat clinical samples were obtained as randomly from the students of Abasyn University Peshawar, by using sterilized swab. Out of 50 (100%) samples, 17 (34%) were positive, while 33 (66%) were negative for bacterial isolates. Among positive cases, 10 (58.82%) were female, while 7 (41.17%) were from male students of all ages. The bacteria were isolated and identified by using culture plate method and different biochemical tests. The sample were further subjected for antibiogram analysis.A total of 5 different antibiotics like; Amoxicillin, Ceftizoxime, Ceftriaxone, Ciprofloxacin and Vancomycin were used. According to the findings, Vancomycin showed highest zone of inhibition against *S. epidermidis* (24mm) followed by *S. aureus* (22mm), *S. pyogenes* (21mm), and while showed resistance to *E. coli* (8mm), respectively. Whereas, amoxicillin showed highest activity against *S. pyogenes* (25mm), followed by *S. aureus* (23mm), *S. epidermidis* (20mm) and *E. coli* (19mm). Likewise, ceftizoxime was showed intimidate activity against *E. coli* (30mm), *S. aureus* (25mm) and (24mm) zones were observed against *S. pyogenes* and *S. epidermidis*, respectively.

Although, ciprofloxacin inhibited the tested bacteria with zone of; *E. coli* (28mm), *S. epidermidis* (26mm) and *S. aureus* (24mm), while showed resistance to *S. pyogenes* (20mm). Similarly, ceftriaxone was more effective against *E. coli* (30mm), presented intimidated activity against *S. pyogenes* (32mm), while showed resistance with zone of (20mm) against *S. aureus* and *S. epidermidis.* Furthermore, the methanolic extracts of both plants showed good antibacterial activity against all tested bacterial isolates. Therefore, it is concluded from the present study that both plants extracts possess different bioactive constituent on which showed a good antibacterial activity.

Key words: *Piper nigrum*, *Cuminum cyminum*, Throat pathogens, Antibiotics, Methanolic extract, Bacteria isolates, Antibacterial Activity

INTRODUCTION

A throat infection, sometimes called pharyngitis, can be either a bacterial, fungal spores or viral infection. It leads to inflammation of the tissues in the throat and causes redness, pain and swelling of the structures in the throat. Throat infection almost common in human specially tonsils are particularly among children. Untreated throat infections sometimes cause chronic complications such as; tonsillar cellulitis, abscess, rheumatic fever and kidney inflammation. Rarely, bacterial infections such as *Staphylococcus aureus, Gonorrhea*, *Escherichia coli* and *Diphtheria* cause throat infection (Bandara *et al.,* 2019). Antibiotics are antimicrobial agents that are used to treat infectious diseases. The outbreak of pathogenic antibiotic-resistant strains illustrates need to search for new alternative sources of treatment (Rakholiya, *et al*., 2013). Plant products and their active elements are alternative useful in the treatment of infectious diseases caused by microbial pathogens (Nabavi *et al*., 2015). The term medicinal plants include those plants that have medicinal activities and used in herbalism. These medicinal plants contain elements that can be used in the manufacture and development of drugs which play vital roles in disease prevention (Sofowora *et al*., 2013). Medicinal plants have long been regarded as a valuable aid in the treatment of human illnesses. Despite significant advancements in the science of allopathy. Medicinal plants and their compounds continue to play an important role in medical treatment (Rasool *et al.,* 2020). The methanol extract of *C. cyminum* inhibited *E. coli,* while aqueous extract of *P. nigrum* showed good activity against *Proteus sp*. (Soniya *et al.,* 2013). The *Piper nigrum* is aromatic, famous valuable medicinal plant which belong to family *Piperaceae* (Abbasi *et al.,* 2010). This plant is found in Pakistan, Indonesia, Malaysia, India, Sri Lanka and China. The *P. nigrum* is commonly known as Kali Mirch in Urdu and Hindi and Madagascar and black pepper in English. The *P. nigrum* is used as medicinal and preservative agent. It contains major pungent alkaloid Piperine which is known to possess many interesting pharmacological actions. It is widely used in different traditional systems of medicine like Ayurvedic and Unani System of medicines (Acharya *et al.,* 2012). The *P. nigrum* plant extracts were screened out for phytochemical screening and different bioactive compounds like; alkaloids, tannins, flavonoids, cardiac and cardiac glycosides were found (Ganesh *et al.,* 2014). The plant and its active component piperine can stimulate the digestive enzymes of pancreas and intestines and also increases biliary bile acid secretion when orally administrated (Tiwari *et al.,* 2008). The *P. nigrum* can be used to treat different respiratory tract diseases, namely., cough, bronchitis and asthma etc. The plant can also useful for muscular pains and inflammation (Gunesh *et al.,* 2014). In traditional medicine, *Piper* species have been used worldwide to treat several diseases such as urological problems, skin, liver and stomach ailments, for wound healing, and as antipyretic and anti-inflammatory agents (Salehi *et al*., 2019). The *Cuminum cyminum* (Cumin) is a small annual and herbaceous plant belong to family *Apiaceae*. It is a multipurpose plant species cultivated in the Pakistan, Middle East, India, China, and several Mediterranean countries, including Tunisia. Its fruit, known as cumin seed, is most widely used for culinary and medicinal purposes. It is generally used as a food additive, popular spice, and flavoring agent in many cuisines (Mnif & Aifa, 2015). It is one of the oldest and economically important plant species whose cultivation generally requires a long hot

summer of 3- 4 months. Today, *C. cyminum* is the second most popular spice in the world after *Pepper nigrum* (Hajlaoui *et al.,* 2010). The *C. cyminum* seeds have also been widely used in traditional medicine for the treatment of several health disorders and diseases, such as toothaches, dyspepsia, diarrhea, epilepsy, and jaundice (Nostro *et al.,* 2005). The *C. cyminum* has also been widely used in traditional medicine to treat a variety of diseases, including hypolipidemia, cancer, insomnia, cold, fever and diabetes (Mnif & Aifa, 2015). The powder or decoction of *C. cyminum* seeds have traditionally been used for the treatment of gastrointestinal disorders. It is recommended as a gastric, carminative, antispasmodics and anthelmintic agent (Suroowan *et al*. 20019). The aqueous decoction of *C. cyminum* showed highest antibacterial potential against tested bacteria i.e. *Aeromonas hydrophila, Alcaligenes spp., Citrobacter spp., Enterobacter aerogenes, E. coli, Flavobacterium spp., Klebsiella ozaenae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Micrococcus roseus, S. aureus, Streptococcus anginosus* and *Streptococcus intermedius* (Chaudhry and Tariq, 2008). The chloroform extract of *C. cyminum* showed good activity against *E. coli* and *S. aureus* (Zou *et al*., 2015). The of the study to evaluate *Piper nigrum* and *Cuminum cyminum* plant extract aginst different throat infection bacteria.

MATERIALS AND METHODS

Clinical Sampling Collection

In this study, a total of 50 clinical throat samples were obtain from the students of Abasyn University, Peshawar by using sterilized swab. The samples were collected randomly. After collection, the samples were processed in the Microbiology Research Laboratory (MRL), Abasyn University, Peshawar.

Exclusion and Inclusion Criteria

In this study only respiratory clinical samples from both male and female of all age groups were included. Furthermore, only Abasyn University, Peshawar students were included. Except respiratory samples, all other clinical samples were excluded in this study. The students of others universities were excluded.

Sampling Processing

After samples collection, the samples were brought to MRL, Abasyn University, Peshawar. The Nutrient agar media was used for the isolation of bacteria isolates from clinical samples. Culture characteristic of microorganisms helped in identification and classification of microbes. In morphological characterization, the cultural characteristics of bacterial colonies were observed on the cultured plates on the basis of size, color, form and opacity (Ogodo *et al*., 2022).

Identification of Bacterial Isolates

The bacterial isolates were Gram stained to differentiate Gram-positive and Gram-negative bacteria. Further identification was done by using different biochemical tests such as urease, citrate, catalase, indole, and Triple sugar iron (TSI) as per method described by (Ullah *et al.,* 2018).

Antibiotics Sensitivity of Bacterial Isolates

For antibiotic profiling, Muller Hinton Agar (MHA) media was prepared, autoclaved and poured in the plates. From fresh bacterial broth growth, bacterial lawn of was prepared by using sterile swab. Disc diffusion method was used. According to CLSI- 2020 guidelines, different antibiotics i.e, Vancomycin, Fosfomycin, Ciprofloxacin, Sulfamethoxazole and Ampicillin were used. After this the plates were incubated at 37^0C for 16-18 hrs. After incubation timing, the zones around each antibiotic disc were measured in mm.

Plant Sample Collection and Processing

The *Piper nigrum* and *Cuminum cyminum* plants powders were obtained from local market of Peshawar by following the method of Ahmad Dar *et al*. (2020). After collection, the plants powders were brought to Microbiology Research Laboratory (MRL), Abasyn University, Peshawar, for further processing. For crude methanolic extracts preparation, powder of both plants (*P. nigrum* and *C. cyminum*) were soaked in methanol by using separated two sterile containers and kept at room temperature for 07 to 15 days with often mixing and shaking. All the soluble contentswere filtered by using filter paper and then were passed from rotary apparatus at temperature of 45^0C to recover crude extracts along with solvents. Then crude was dried with water bath and stored at air tried container for further activity.

Antibacterial Activity of Plants Extracts

The *P. nigrum* and *C. cyminum* crude methanolic extracts were evaluated for antibacterial activity against clinical isolates. Well diffusion method was used. MHA media was prepared, autoclaved and poured in the plates. Bacterial lawn was prepared by using sterile swab. Wells were prepared by using sterile borer (6mm) at distance of 20mm of each well. Stock solution of each plant were prepared in separated falcon tube by using DMSO less than 1%. The extracts were poured in each labeled well by using micropipette. The plates were allowed for incubation at 37^0C for 16-18 hrs. After incubation, the zones around each well were observed in mm by using scale and the results were noted by using the following formula.

$$\% \text{ inhibition} = \frac{\text{zone of inhibition of test sample}}{\text{zone of inhibition of standard}} \times 100$$

RESULTS

In this study, a total of 50 clinical samples were collected randomly from the students of Abasyn University, Peshawar by using sterilized swab. Out of 50 (100%) clinical samples, 17 (34%) samples were positive, while 33 (66%) were negative for bacteria isolates, as shown in **Fig .1.** Among positive cases for bacterial isolates, 10 (58.82%) were from female, while 7 (41.17%) were from male students of all ages as shown in **Fig .2**. The bacteria were isolated and identified by using culture plate method and different biochemical tests. **Table .1**. provide a full description of the microscopy, culture characteristics and biochemical investigation of bacterial isolates.

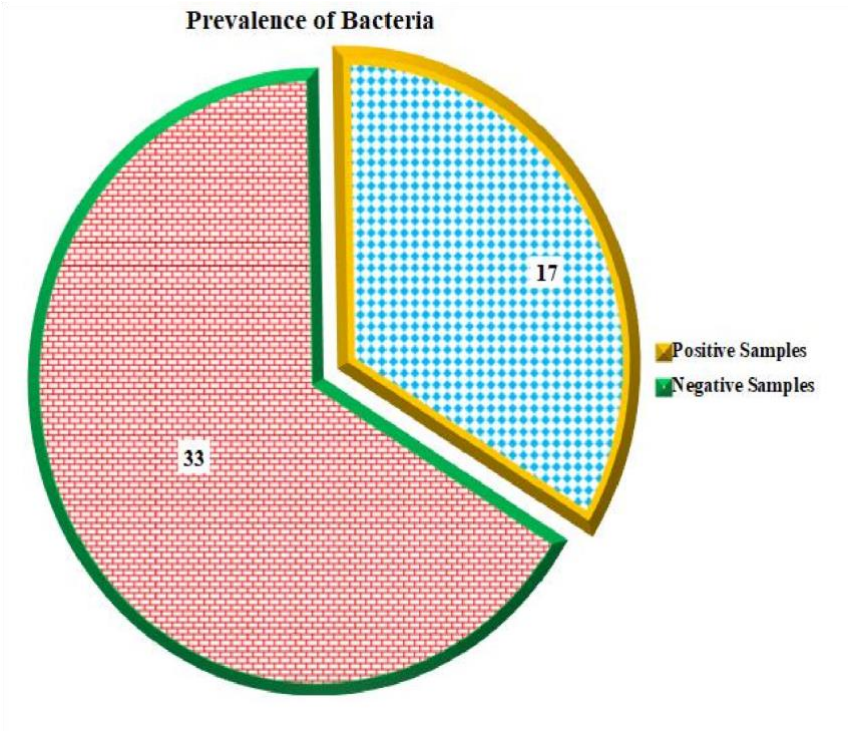

Figure .1. Prevalence of bacteria in collected Samples

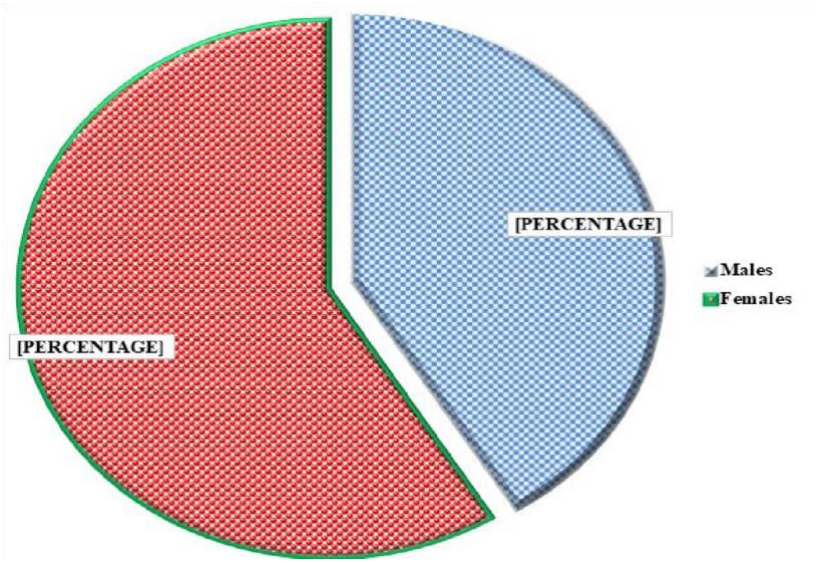

Figure .2. Gender Wise Distribution of Positive Samples

Table .1 Microscopy, Cultural characteristics different Biochemical Tests for bacterial isolates

Isolates	Microscopy			Catalase	Oxidase	Coagulase	Urease	Indole	TSI test	Identified Organisms
	Color	Shape	Gram's Rx							
1	Pink	Rod	-	+	-	-	-	+	K/A, AG	*Escherichia coli*
2	Purple	Spherical, grape	+	+	-	-	+	-	K/A, AG	*Staphylococcus epidermidis*
3	Purple	Round, Pair	+	-	-	-	-	-	K/A, AG	*Streptococcus pyogenes*
4	Purple	Spherical, clusters	+	+	-	+	+	-	NC	*Staphylococcus aureus*

Key: AG = Acid and gas; + = Positive; • = Negative; A = Acid production; K = alkaline reaction; NC = No change; H2S = Sulfur reduction; K/A = Red/yellow; K/NC = Red/no color change; K/A, H2S = Red/yellow with bubble and black precipitate; A/NC = Acid/no color change.

Antibiogram Pattern

The bacteria isolates were subjected to antibiogram analysis. A total of five different antibiotics (Amoxicillin, Ceftizoxime, Ceftriaxone, Ciprofloxacin and Vancomycin) were used for determination of susceptibility profile of isolated bacteria according to CLSI- 2020 guideline's Standard, Intermediate, Sensitive and Resistant values, which are shown in **Table .2**.

Table .2. CLSI Standard Values of bacteria (Resistance, Susceptibility and Intermediate)

S. No	Bacteria Isolates	Antibiotics														
		Amoxicillin 20µg			Ceftizoxime 30µg			Ceftriaxone 30µg			Ciprofloxacin 5µg			Vancomycin 30µg		
		S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
1	*E. coli*	≥18	14-17^	≤13	≥37	30-36^	≤29	≥24	20-23^	≤19	≥26	22-25^	≤21	≥15	11-14^	≤10
2	*S. aureus*	≥20	17-19^	≤16	≥30	23-29^	≤22	≥29	22-28^	≤21	≥21	16-20^	≤15	≥17	15-16^	≤14
3	*S. pyogenes*	≥18	14-17^	≤13	≥28	21-27^	≤20	≥36	30-35^	≤29	≥31	22-30^	≤21	≥20	17-19^	≤16
4	*S. epidermidis*	≥16	9-15^	≤8	≥27	20-26^	≤19	≥28	22-27^	≤21	≥21	16-20^	≤15	≥21	18-22^	≤17

Key words: I= Intermediate, S= Sensitive, R=Resistance, µg= microgram, ≥ = greater than or equal, ≤ = less than or equal

Antibiogram analysis of bacterial isolates

A total of 5 different antibiotics like; Amoxicillin, Ceftizoxime, Ceftriaxone, Ciprofloxacin and Vancomycin were used. According to the findings, Vancomycin showed highest zone of inhibition against *S. epidermidis* (24mm) followed by *S. aureus* (22mm), *S. pyogenes* (21mm), and while showed resistance to *E. coli* (8mm), respectively. Whereas, amoxicillin showed highest activity against *S. pyogenes* (25mm), followed by *S. aureus* (23mm), *S. epidermidis* (20mm) and *E. coli* (19mm). Likewise, ceftizoxime was showed intimidate activity against *E. coli* (30mm), *S. aureus* (25mm) and (24mm) zones were observed against *S. pyogenes* and *S. epidermidis*, respectively.

Although, ciprofloxacin inhibited the tested bacteria with zone of; *E. coli* (28mm), *S. epidermidis* (26mm) and *S. aureus* (24mm), while showed resistance to *S. pyogenes* (20mm). Similarly, ceftriaxone was more effective against *E. coli* (30mm), presented intimidated activity against *S. pyogenes* (32mm), while showed resistance with zone of (20mm) against *S. aureus* and *S. epidermidis.* Furthermore, the methanolic extracts of both plants showed good antibacterial activity against all tested bacterial

isolates. Therefore, it is concluded from the present study that both plants extracts possess different bioactive constituent on which showed a good antibacterial activity **Figure No.3**.

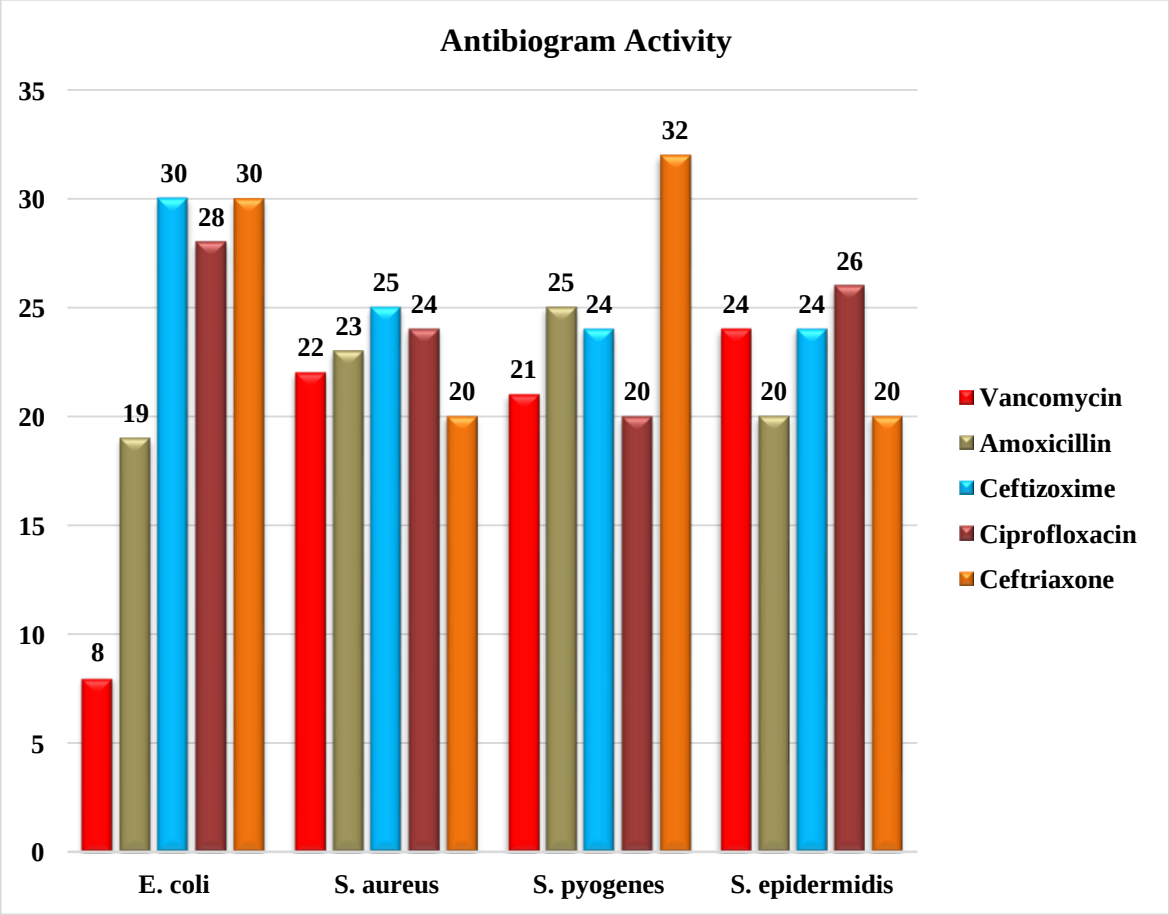

Fig No .3: Antibiogram analysis of bacterial isolates

Antibacterial Activity of *P. nigrum* and *C. cyminum* extracts

The crude methanolic extracts of *P. nigrum* and *C. cyminum* with 100 µl of the stock solutions were used against isolated bacteria. Both plant extracts showed good antibacterial activity against tested samples. Among the extracts, the *P. nigrum* showed highest zone of inhibition against *S. pyogenes* (20mm), *E. coli* (18mm), *S. aureus* (15mm) and *S. epidermidis* (13mm). While, *C. cyminum* showed zones of inhibition against *S. pyogenes* (18mm), *E. coli* (16mm), *S. epidermidis* (14mm) and *S. aureus* (15mm), respectively. The DMSO (<1%) was used as negative control while Ceftizoxime was used as positive control **Figure. 4** show the outcomes.

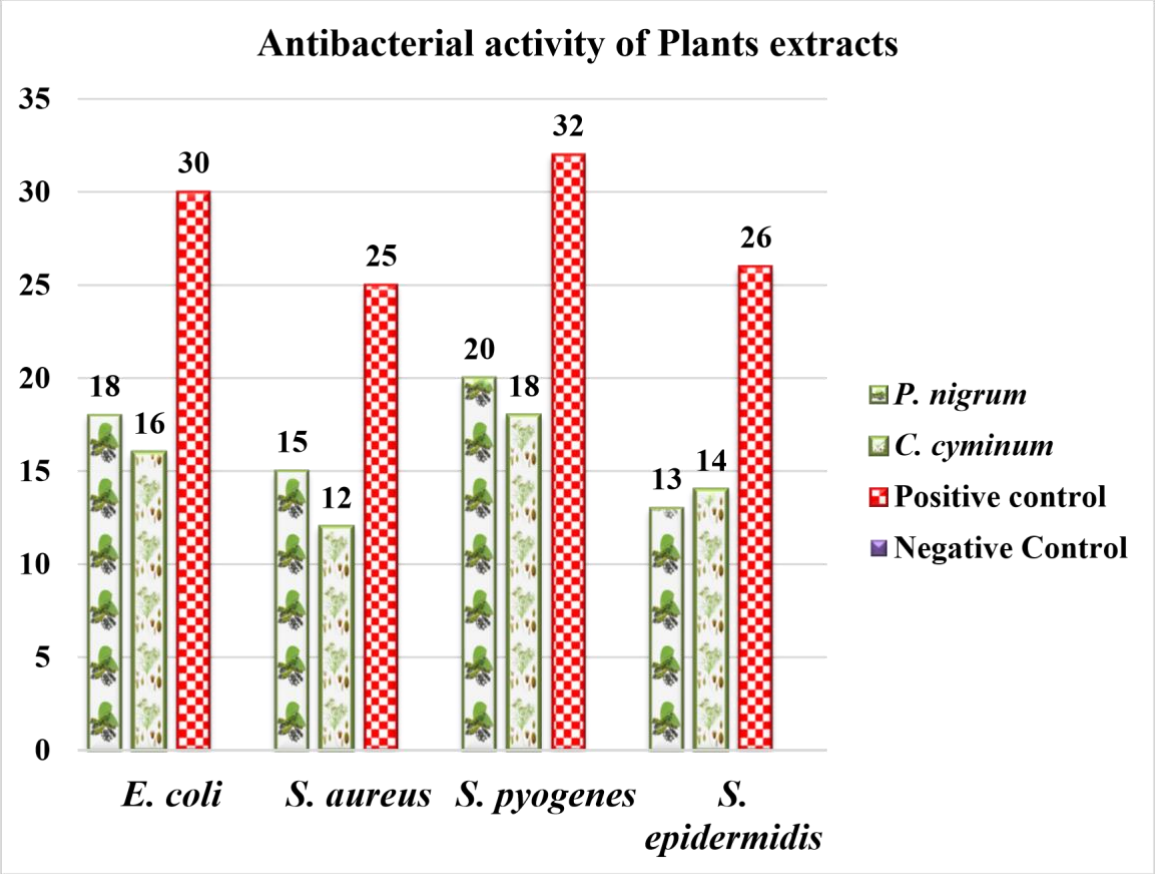

Figure No.4. Antibacterial activity of crude methanolic extracts of *P. nigrum* and *C. cyminum*

DISCUSSION

Disease is defined as any harmful deviation from the normal structure of an organism, generally associated with certain signs and differing in nature from physical injury (Park *et al.*, 2022). Throat infection is the inflammation of the tissues in the throat which causes redness, pain and swelling. The throat infection, sometimes called pharyngitis, can be either a bacterial, fungal spores or viral infection. It's mostly caused by bacterial infections such as *Staphylococcus aureus, Gonorrhea*, *Escherichia coli* and *Diphtheria* cause throat infection (Bandara, *et al.*, 2019). Mostly antibiotics are resistance to throat infection due to misuse of antibiotics (Hasan and Al-Harmoosh, 2020). Plants products and their active constituents are alternative and useful in the treatment of infectious diseases caused by various microbial pathogens (Nabavi *et al.*, 2015). The term medicinal plants included those plants that have medicinal properties and can use in herbalism which are contain bioactive compounds that can be used in the manufacture and development of drugs which play a vital role in disease prevention (Sofowora *et al.*, 2013). Medicinal plants and their compounds continue to play an important role in medical treatment (Rasool *et al.*, 2020). Phytochemicals are natural bioactive compounds which are found in medicinal plants. These bioactive compounds having antimicrobial activity (Altemimi *et al.*, 2017). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great consequence in therapeutic treatments (Khalil *et al.*, 2020). The *Piper nigrum* is aromatic, famous valuable medicinal plant which belongs to family *Piperaceae*. This plant is found in Pakistan, Indonesia, Malaysia, India, Sri Lanka and China which is used as medicinal and preservative agent (Abbasi *et al.*, 2010). The *P. nigrum* is commonly known as Kali Mirch in Urdu and Hindi and Madagascar and black pepper in English (Achary *et al.*, 2012). The *Cuminum cyminum* (Cumin) is a small annual and herbaceous plant belong to family *Apiaceae*. It is a multipurpose plant species cultivated in the Pakistan, India and China. Its fruit, known as cumin seed, most used for culinary as

(Mnif & Aifa, 2015). These both plants contain bioactive compounds alkaloids, tannins, flavonoids, cardiac and cardiac glycosides were found (Ganesh *et al.*, 2014). These plants widely used in different traditional systems of medicine like Ayurvedic and Unani System of medicines (Achary *et al.*, 2012). The *P. nigrum* methanolic and chloroform extracts were evaluated against *S aureus, S. typhi, E. coli, Proteus sp*. and *P. aeruginosa* by using agar well diffusion method and found that all the bacteria were sensitive to methanol and chloroform extracts except *P. aeruginosa*. The presence of photochemicals indicated the presence of antibacterial properties against the *S. aureus, S. typhi, E. coli* and *Proteus sp* (Ganesh *et al*., 2014). The antibacterial activity of ethanol extract of *P. nigrum* fruit was evaluated against different bacteria i.e., *E. coli, S. aureus, S. epidermidis, K. pneumoniae, Enterococcus faecalis, S. typhi, S. typhimurium, P. aeruginosa, Bacillus cereus, B. subtilis, Bacillus megaterium* and *Streptococcus faecalis* by using disc diffusion method. The study showed highest activity against *E. coli* (36mm) and *S. aureus* (38mm). It's concluded that the plant extract has good antibacterial property (Karsha & Lakshmi (2010). In our study, methanolic extract of *P. nigrum* was evaluated against *S. pyogenes, E. coli, S. aureus* and *S. epidermidis* which showed active against all bacterial isolates. It is concluded that the plant has good antibacterial property. Antibacterial activity of acetone and methanol extracts of *P. nigrum* was checked against *P. aeruginosa* and *S. aureus* by using well diffusion method. The *P. aeruginosa* and *S. aureus* were found most sensitive and formed 16mm zone against both bacteria at 100 µg/ml (Kaur *et al*. 2017). In our present study methanolic extract of *P. nigrum* was evaluated against *S. pyogenes, E. coli, S. aureus* and *S. epidermidis* which showed highest activity against *S. pyogenes* (20 mm), *E. coli* (18 mm), *S. aureus* (15 mm) and *S. epidermidis* (13 mm), respectively. It is concluded that the plant has showed good antibacterial activity. (Al-Shawi, *et al*., 2017) explored antibacterial properties of methanolic extract of *C. cyminum* by using well diffusion method. The plant extract was found active against all tested pathogenic bacteria including, *S. typhi* (35mm), *P. aeruginosa* (25mm), *S. aurous* (36mm) and *E. coli* (31mm). (Al-Shawi, *et al*., 2017) used alcoholic and aqueous extracts of *C. cyminum* against *Streptococcus mutans, E. coli, S. aureus* and *P. aeruginosa*. The alcoholic extract presented higher activity against all tested bacteria comparing to the aqueous extract. While in our study, methanolic extract of *C. cyminum* was evaluated against *S. pyogenes, E. coli, S. aureus* and *S. epidermidis* which showed active against all bacterial isolates. It is concluded that the plant has good antibacterial activity. In our study, methanolic extract of *C. cyminum* was evaluated for *S. pyogenes, E. coli, S. aureus* and *S. epidermidis* which showed inhibition zone against *S. pyogenes* (18mm), *E. coli* (16mm), *S. epidermidis* (14mm) and *S. aureus* (12mm), respectively. It is concluded that *cyminum* has good antibacterial activity. (Belal *et al.* 2017) used *C. Cyminum* oil against *E. coli, K. pneumoniae, S. typhi, Proteus vulgaris*m, *Enterococcus feacalis* and *S. aureus*. The results showed that *C. Cyminum* oil showed effective activity against tested bacteria. (Sarmad *et al*., 2017) studied antibacterial activity of alcoholic and methanolic extracts of *C. cyminum* on *Streptococcus mutans, E. coli, S. aureus* and *P. aeruginosa* by using well diffusion method. The alcoholic extract showed higher antibacterial action against all tested bacteria. In current study, methanolic extract of *C. cyminum* was evaluated against bacterial isolates, which were effective against all bacterial isolates.

Conclusion

The present research study was focused on Evaluation of antibacterial activity of *Piper nigrum* and *Cuminum cyminum* extracts on bacterial isolates from throat infection. A total of 50 throat clinical samples were obtained randomly from the students of Abasyn University, Peshawar by using sterilized swab and processed in the Microbiology Research Laboratory (MRL), Abasyn University, Peshawar. Infection ratio was more abundance in female (58.82%) as compare to male (41.17%). Different antibiotics were applied like Amoxicillin, Ceftizoxime, Ceftriaxone, Ciprofloxacin and Vancomycin. All antibiotics were effective against tested bacteria. *P. nigrum* and *C. cyminum* methanolic extracts were used against tested bacteria in which *P. nigrum* presented highest activity against all bacterial

isolates. From this study, it was concluded that the *P. nigrum* and *C. cyminum* plants can be used in treatment of bacterial throat infection causing bacteria.

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