The Effect of *Cinnamomnum zeylanicum* Extract on *Pseudomonas aeruginosa* Isolated from Burns and Purulent Wounds

Hameedah Hamzah Ajeel
Department of Microbiology, Collage of Veterinary Medicine, AL-Qasim Green University, Babylon, Iraq.
Corresponding E-mail: hameedahhamza@vet.uoqasim.edu.iq

Rawaa Adday Ali
Department of Microbiology, Collage of Veterinary Medicine, AL-Qasim Green University, Babylon, Iraq.

Haider Truky Mousa Al-Mousawi
Collage of Biotechnology, AL-Qasim Green University, Babylon, Iraq.

Abstract

The presented study, here, was conducted to evaluate the antimicrobial characteristics of the extract of *cinnamomnum zeylanicum* against *Pseudomonas aeruginosa*. From burns and suppurative wounds, 120 swab samples were collected from patients, who attended Al-Hyshama Hospital and Al-Qasim Hospital, Al-Qasim City, Babel, Iraq. The samples were subjected to traditional steps of bacterial cultivation and detection of *P. aeruginosa* via colony, microscopic, and biochemical properties. The bacteria were then used to grow biofilm and perform the antimicrobial sensitivity test for the evaluation of the *cinnamomnum zeylanicum* extract against the bacterial growth in comparison with some standard antibiotics. The *P. aeruginosa* biofilm production was 21 (60%). The *P. aeruginosa* isolates were highly sensitive to the extract of *cinnamomnum zeylanicum* at high concentrations. The percentages of the sensitive and resistant isolates varied, such as 100% of resistance against amoxicillin-clauvanic acid and 38.09% of sensitivity against amikacin and Imipenem. The *cinnamomnum zeylanicum* extract may be beneficial for the treatment of *Pseudomonas aeruginosa* infected burns and wounds.

Keywords: *Antimicrobial resistance, Cinnamomnum zeylanicum, FTIR, Pseudomonas aeruginosa*.

Introduction

The bacterium; *P. aeruginosa*, is a potential etiology of both healthcare-associated and community-acquired infectious illnesses [1]. *P. aeruginosa* is a major contributor of the genus *Pseudomonas* and the family Pseudomonadeceae [2]. It is motile Gram-negative rods. *P. aeruginosa* infections are treated with antibiotics, although evidence suggests that drug resistance has emerged. Existence of the carbenicillin intrinsic resistance is one of *P. aeruginosa* many primary resistance types [3]. *P. aeruginosa* accounts for around 8% of healthcare facility infections acquired in the United States, and 13% of those strains are multidrug resistant (MDR) [4]. Antibiotic therapy has been a cornerstone in the battle against bacterial illnesses and has vastly improved public health since centuries past. Antibiotic therapy has come a long way, but sadly, the number of pathogenic resistant to antibiotics is rising at a troubling rate [5]. Plants used for medical

purposes are used both in developed and poor nations. In addition to their traditional uses, medicinal herbs are increasingly being included into the food industry as a means of prolonging shelf life, enhancing taste, and warding off illness. Because of their antibacterial qualities, the bioactive compounds found in plants are employed for the cure of a wide range of conditions. In the same way as molecules of plant chemicals inhibit the development of bacteria and cure a particular illness, in which microbes also gain resistance to most antibacterial drugs [6]. Ancient Chinese literature from 4000 years ago [7] make reference to *Cinnamomum zeylanicum*, making it the world's oldest documented herbal remedy. The cinnamon tree, or cinnamomum, is an effective contributor of the Lauraceae family of evergreens. Cinnamon is a tree whose leaves and bark have several cooking and medicinal uses [8].

The presented study, here, was conducted to evaluate the antimicrobial characteristics of the extract of *cinnamomnum zeylanicum* against *Pseudomonas aeruginosa*.

Materials and Methods:

Collection samples

From burns and suppurative wounds, 120 swab samples (60 samples each) were collected from patients, who attended Al-Hyshama Hospital and Al-Qasim Hospital, Al-Qasim City, Babel, Iraq, during October, 2021 to February, 2022. The samples were immediately transported to a microbiology laboratory, and there, were nutrient-agar-cultivated for 24hrs at 37°C. Then, the resulted colonies were sub-cultivated using MacConkey, blood, and pseudomonas agars, under 37°C for 24-48hrs. Gram stain, selective and differential cultivation, and biochemical based identification were made to identify the bacterial species according to Bergey's [9].

Biofilm Production:

A qualitative tube method was employed for the evaluation of the biofilm production as described by [10].

Antibiotic susceptibility testing:

P. aeruginosa growth on Mueller-Hinton agar (CM0337-OXOID) and the Clinical and Laboratory Standards Institute (CLSI) were followed for the identification of antimicrobial sensitivity test according to the manufacturer's company. The media was sterilized using an autoclave at 121°C for 15mins. Following cooling down, the media at 25ml was poured into a sterile Petri-plates (90mm diameter and 4mm depth). Even spread of the bacterial inoculum was performed on the plates. Dryness of the contents was assured before sterile-forceps-inserting the discs into the media, and, then incubated at 37°C for 18hrs in inverted brace. After getting done with the incubation, the diameter reads for the zone of inhibition were collected.

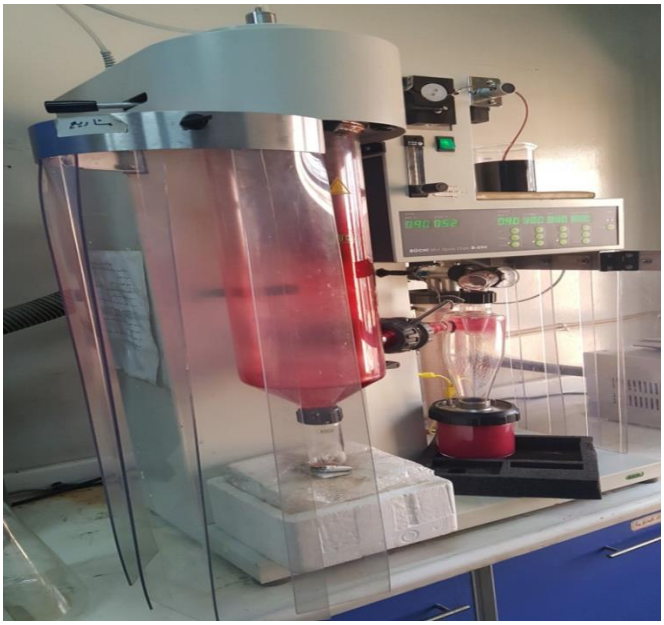
Well diffusion method

The well diffusion method [11] was followed for evaluating the antibacterial activity of the extract against the growth of the bacterium. The bacterial inoculum at 0.1ml was evenly spread onto nutrient agar (NA) plates. Uniform wells were made on the NA surface. Then, 100µl of each concentration of the extract was poured into the wells. Finally, 37°C-24hrs incubation for the plates was completed. After getting done with the incubation, the diameter reads for the zone of inhibition were collected.

Plant extracts

For aqueous extraction, put 40 g of the leaves of the *cinnamomnum zeylanicum* in a 1-liter conical flask with 300 ml of distilled water and put it in a 37°C-6hrs based shaking incubator, filter the solution and pass it on several layers of soft cloth (medical gauze) to get rid of impurities using a spray dryer as shown in fig. (1), store the extract 6 g in an opaque, airtight container until use.

Figure 1: Spray dryer B 290 (Buchi/ Switzerland).

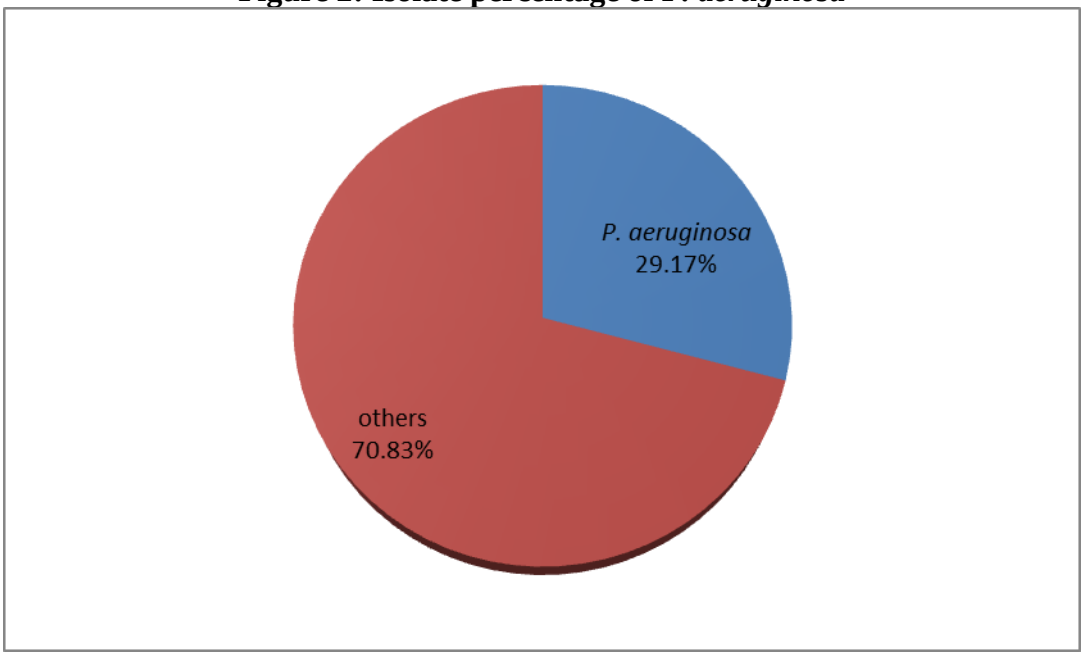


Results

P. aeruginosa based isolation and identification

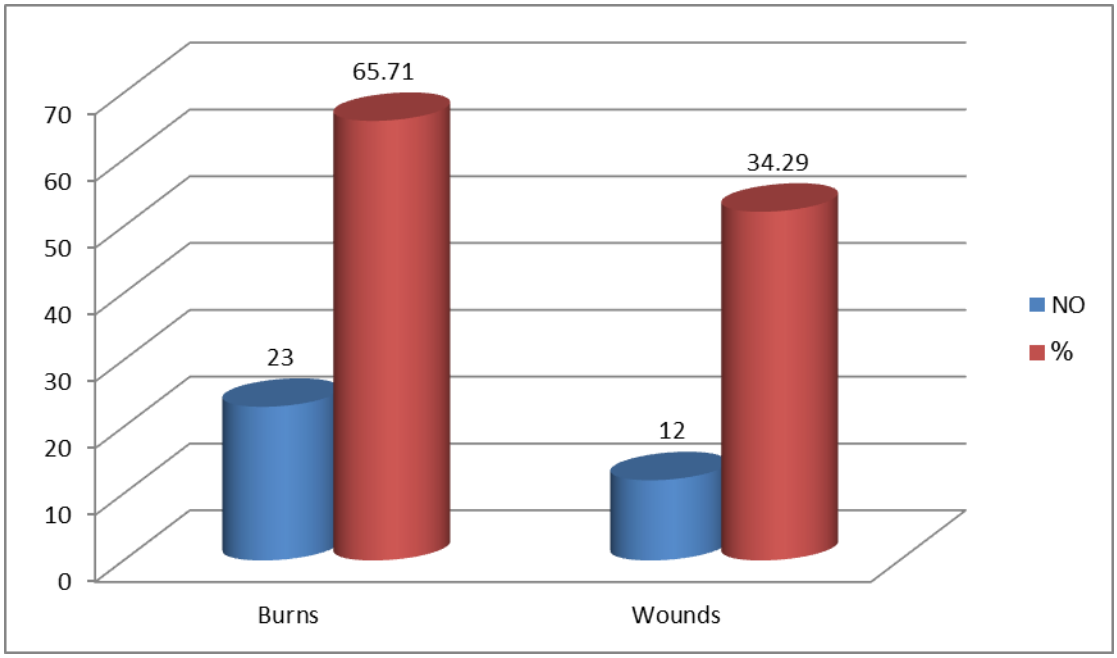
Out of 120 samples, only 35 (29.17%) of the isolates were *P. aeruginosa*, figure 2.

Figure 2: Isolate percentage of *P. aeruginosa*



The most of these isolates *P. aeruginosa* were obtained from burns 23(65.71%) and purulent wounds 12(34.29%), figure 3.

Figure 3: Numbers and Percentages of *P. aeruginosa*



Following the 24-hour period of cultivation and incubation at 37°C, colonies with typical characteristics on Pseudomonas base, MacConkey, and blood agars were determined as *P. aeruginosa*, and the resulted characteristics for the colonies are demonstrated in the table 1 and figure 4.

Table 1: Colony characteristics on Pseudomonas base agar, Blood agar, MacConkey Agar.

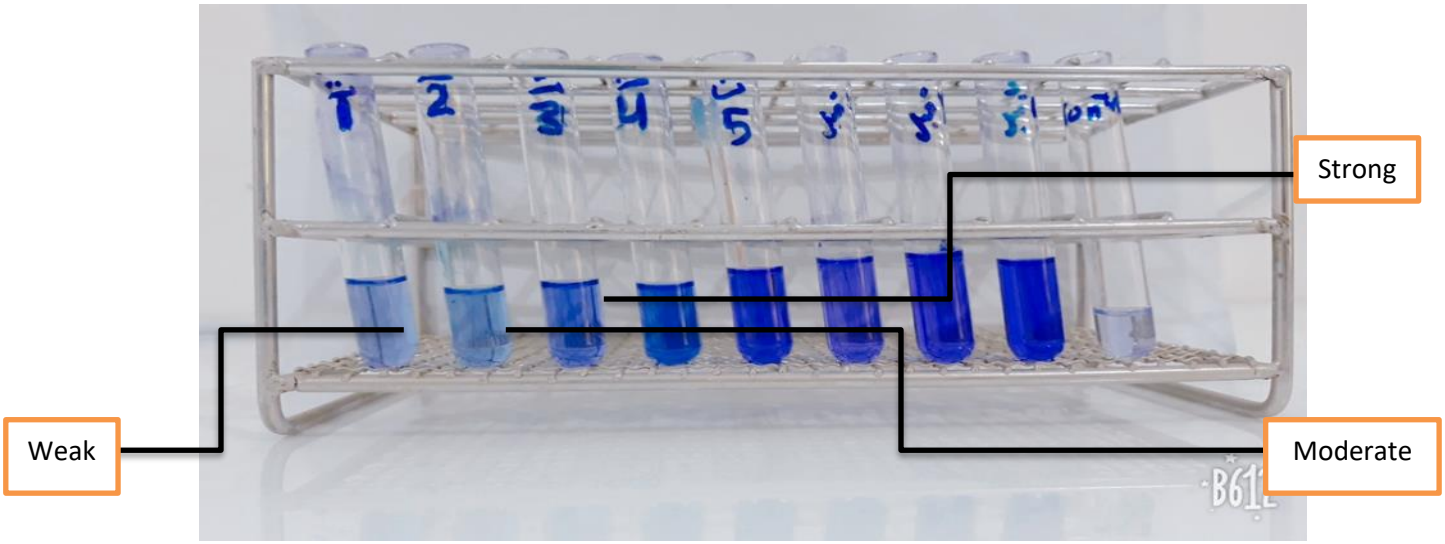
Cultivation media	**Colony morphological features**
Pseudomonas base agar	Glossy, cloudy, convex, greenish to yellow colored, light blue color of medium.
Blood agar	Hemolysis type (β)
MacConkey agar	Mucoid, smooth, and cloudy colonies

Figure 4: Colonies of *P. aeruginosa*. A: Pseudomonas base agar. B: Blood agar.

A **B**

Biofilm Production

Figure 5: Biofilm Pseudomonas aeruginosa (tube method) without treated by essential oil

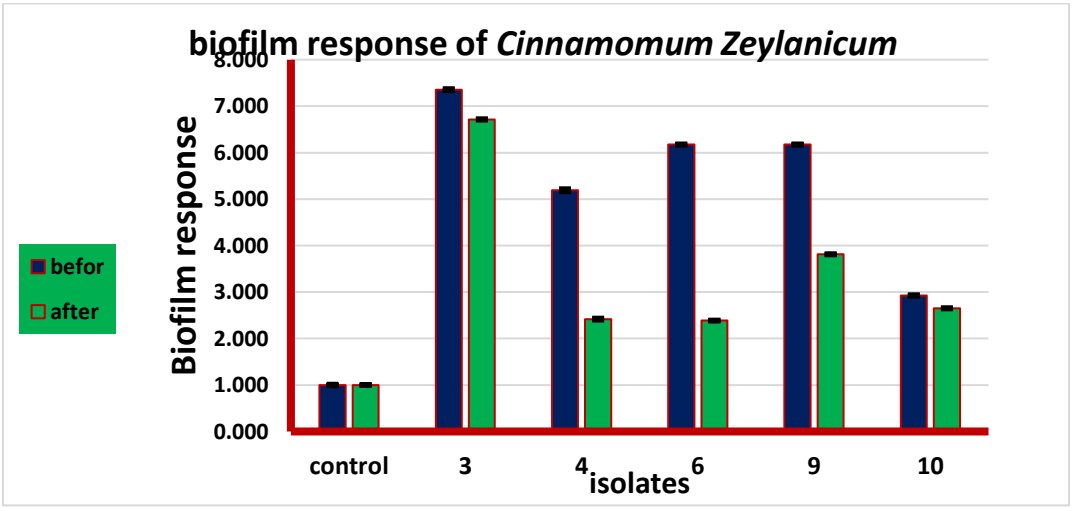


In results of presents study, the density of biofilms formed was measured as weak/no, moderate, high/strong , shown in fig.5 on the other hand, showed the biofilms response in control was weakly adherent while the formation of biofilms of *P. aeruginosa* isolate with treated by *cinnamomnum zeylanicum* that is strongly adherent. Shown in fig.6

Figure 6: Biofilm *Pseudomonas aeruginosa* (tube method) after treated by essential oil

The current study was detected the slime production that is responsible for the production of biofilms in the isolates of *P. aeruginosa*. out of 35 (100%) isolates 21 (60%) were slime layer positive, as figure 7, showed the biofilm production of *P. aeruginosa* with and without treated by essential oil.

Figure 7: Explain biofilm before and after used *Cinnamomum zeylanicum* extract on *Pseudomonas aurogenosia*.



Sensitivity of Pseudomonas isolates to antibiotics

Pseudomonas aeruginosa ten (10) antibiotics were examined for antimicrobial susceptibility on isolates from various sources (burns and wounds): (amoxicillin- clauvanic acid, Piperacillin-tazobactam, Gentamicin, Levofloxacin, Ceftazidime, ciprofloxacin, Cefepime, Imipenem, Amikacin and Meropenem).

In this study, 21 (60%) isolates were with biofilm activity, and the antimicrobial susceptibility profile of *P. aeruginosa* against various antibiotics. Table (2) indicates that 100% *P. aeruginosa* isolates were resistance for amoxicillin-clauvanic acid and also depicts that 80.95% of isolates of *P. aeruginosa* were resistance to Cefepime, 38.09% isolates sensitive to Amikacin, Imipenem and Levofloxacin. 42.85% isolates sensitive to Piperacillin-tazobactam and Ceftazidime. while 71.42% isolates *P. aeruginosa* were resistance to ciprofloxacin and Meropenem.

Table 2: Antibiotic susceptibility of *Pseudomonas aeruginosa*.by Kirby pure method

No. antibiotic	No. of isolates		Total
	Sensitivity rate %	Resistance rate %	
Amoxicillin-clauvanic acid	0(0)	35(100%)	21
Piperacillin-tazobactam	9(42.85%)	12(57.14%)	21
Levofloxacin	8(38.09%)	13(61.90%)	21
Ceftazidime	9(42.85%)	12(57.14%)	21
ciprofloxacin	6(28.57%)	15(71.42%)	21
Cefepime	4(19.05%)	17(80.95%)	21
Gentamicin	6(28.57%)	15(71.42%)	21
Amikacin	8(38.09%)	13(61.90%)	21
Imipenem	8(38.09%)	13(61.90%)	21
Meropenem	6(28.57%)	15(71.42%)	21

Antibacterial of *Cinnamomnum zeylanicum* extract by agar well diffusion test.

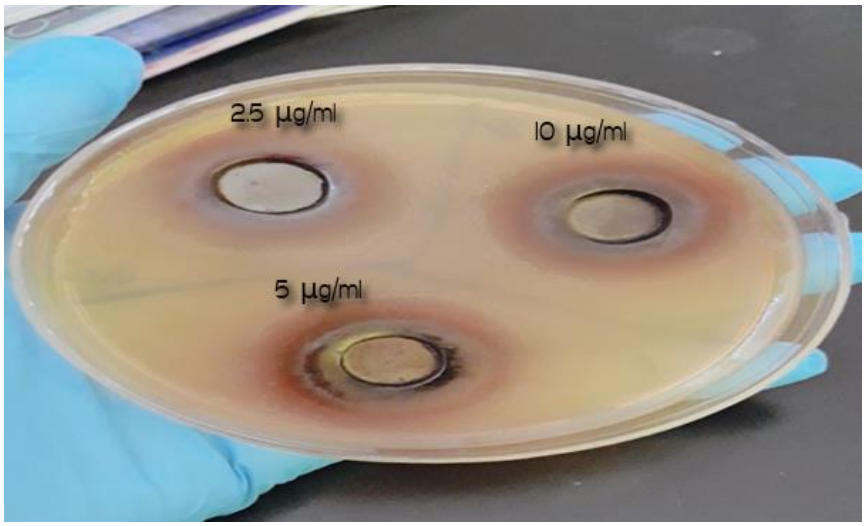
The table (3) and figure (8) showed antibacterial effects of different concentration of *cinnamomnum zeylanicum* on growth of *P. aeruginosa* isolates with well diffusion method , In results of presents study, the inhibition zone of *cinnamomnum zeylanicum* for *P.aeruginosa* isolate wounds samples in concentration 2.5 µg/ml has been to be (0-12), While its inhibition zone for concentration 5 and 10 µg/ml *cinnamomnum zeylanicum* has been significantly increased to reached (12-20, 21-25) mm. In *P.aeruginosa* isolate of burn samples , the inhibition zone that occurred by concentration essential oil, *cinnamomnum*

zeylanicum (2.5, 5 , 10) µg/ml were reached to (3- 14, 11-19, 19-29) mm respectively.

Table 3: Antibacterial effects of different concentration of *cinnamomnum zeylanicum* on growth of *P.aeruginosa* isolates.

Isolates of *P. aeruginosa* (Source and No.)		Inhibition zone (mm)		
		2.5µg/ml	5µg/ml	10µg/ml
Wound Isolate	3	0-12	13-22	22-25
	5	4-12	12-20	21 -25
Burn isolate	3	0-8	11-19	20- 29
	4	9- 14	8-13	23- 27
	6	3-13	16	19- 28
Total	21			

Figure 8: Zone of bacterial growth inhibition according antibacterial activity in *P. aeruginosa*



Fourier transformed infrared spectroscopy (FTIR) analysis of essential oil of *Cinnamonum zeylanicum*

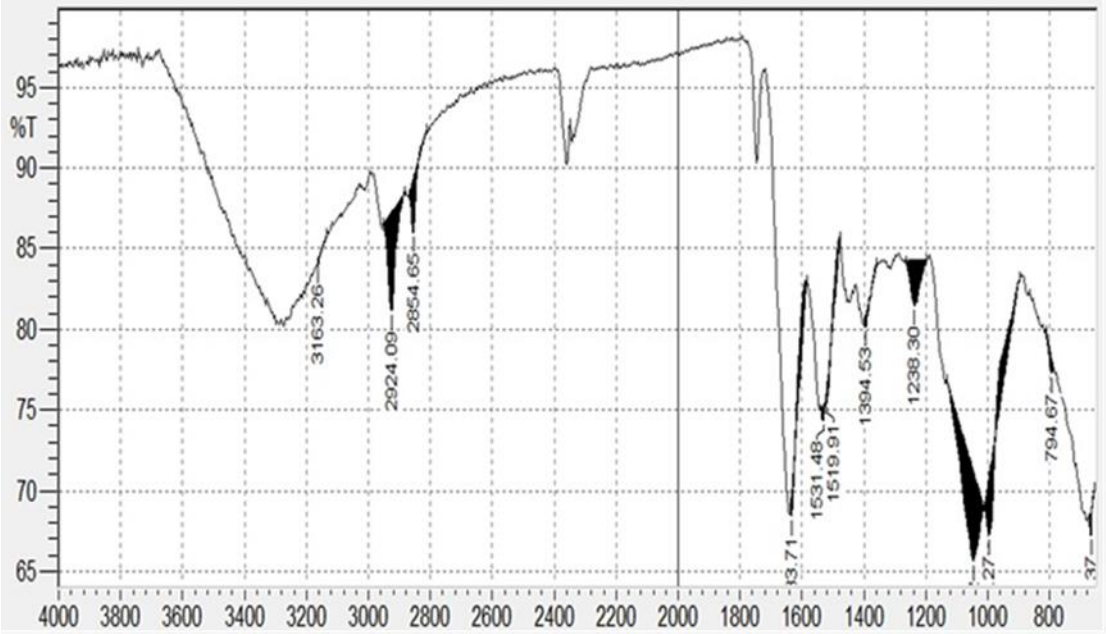
The result FTIR analysis in figure (9) reveals the characteristic peaks for the essential oil of the *C. zeylanicum* extract (600-4000cm).

In this study, potential bands (650-1000cm^{-1}) representing the functional group of =C–H bond for alkenes bending, While the signals that are appeared between 1000-1400cm^{-1} representing the functional group of C-F bond for *stretching alkyl halides* and 1400-1600cm^{-1} represents the functional groups of C=C bond for Aromatic stretching, While the signals range of (1550-1640 cm^{-1}) representing the functional groups of N-H amide stretching and 2850-3000cm^{-1} represents the functional groups of C-H bond for alkane stretching.

The absorption by the carbonyl stretching is among the potential absorptions of IR that can be beneficial for structure-detailing.

Wavenumber (cm-1)

Figure 9: FT-IR profile solid analysis of *Cinnamomnum zeylanicum*



Discussion

P. aeruginosa was revealed to be a Gram-negative, motile, aerobic with obligation, and with-positive-oxidase rods that live in a wide variety of environments, including feces, groundwater, soil, and human intestines. [12,13]. Possibilities for infection that arise only in those with compromised immune processes [13,14].

Noticeable films on the wall, bottom, and the liquid surface of the tube that act as a safe habitat for the bacterial community, were used to assess *P. aeruginosa* biofilm formation; the strength of the biofilm shaped was rated as weak (no film), moderate (some film), or strong (complete biofilm) [15,16].

This results agreed with a study by Pournajaf *et al* (2018) showed that (57.3%) of *P. aeruginosa* isolates produce slime layer on cultured test tube wall when empty and stained with safranin [17].

Current antiapoptotic drugs were applied during the beginning of the sixties of the previous century, and since that time, only a small number of newly discovered agents were certified for the use in the clinical settings [18 - 20].

According to the outcomes of the antimicrobial sensitivity test, the profile revealed that 100% amoxicillin-clauvanic acid, 80.95% cefepime, 61.90% of amikacin, imipenem, and levofloxacin, and 71.42% ciprofloxacin, gentamicin, and meropenem displayed greater levels of resistance than other commonly used antipseudomonal medications. In addition, we found that piperacillin-tazobactam and Ceftazidime were effective against 42.85% of the isolates. Antibiotic susceptibility profiles remained almost the same, with modest alterations except only for piperacillin-tazobactam, according to a research of the same kind conducted in this center in 2019. A contrast to our results [21], [22], only 33% of the isolated bacteria were found to be sensitive to piperacillin-tazobactam in that investigation. For the current work, Piperacillin-tazobactam and Ceftazidime are the favored antibiotics for these microorganisms over other anti-pseudomonal agents, as shown by the regulation on these shifting trends of the antibiotics susceptibility spectrum.

The oil from c. zeylanicum has anti-bacterial action towards Bacillus subtilis and Escherichia coli, as mentioned by Wong et al. (2014) [23,24]. Priyanga et al. (2013) cited research showing that c. zeylanicum has antibacterial properties against a wide variety of bacterial species and yeasts. These include: *Acinetobacter* spp., *Salmonella spp., Enterococcus faecium, Staphylococcus spp., Bacillus* spp., *Clostridium* spp., *Brucella melitensis, E. coli, Enterococcus faecalis, Helicobacter pylori, Mycobacterium tuberculosis, Streptococcus* spp, *Proteus mirabilis, Listeria monocytogenes, Klebsiella pneumonia, P. aeruginosa, and Saccharomyces cerevisiae* [23,25].

Both Gram-positive and Gram-negative bacterial microorganisms are growth-slowed-down by the extract of *C. zeylanicum*. This activity could be due to cinnamon's antibacterial properties [26]. This inhibition was reported to be due to the *C. zeylanicum* extract, as mentioned by Mohammed et al. (2005) .*P. aeruginosa, E. coli, P. mirabilis, B. subtilis, S. aureus, and S. typhi* were all shown to be inhibited by *C. zeylanicum* extract. It was found that *C. zeylanicum* extract had a significant inhibitory effect on *P. aeruginosa* [27].

In this investigation by correlating the vibration signals of the FT-IR spectrophotometer, the functional clusters contained in the essential oil of *C. zeylanicum* extract were recognized. The current study findings are supported by those of a previous FTIR spectroscopic investigation of *C. zeylanicum* essential oil [28] by confirming the presence of the expected functional groups within the oil, including CHx, C=O, C=C, and C-F.

Conclusion

Results from this research indicate that *Cinnamomum zeylanicum* extract may be useful for a variety of medical purposes. Because it reduces the proliferation of *P. aeruginosa* during experimental isolation, it is useful for treating burns and purulent wounds. If handled correctly, *Cinnamomum zeylanicum* extract has the power to act as a supply of effective antimicrobial agents for the development of medications to treat *P. aeruginosa* illnesses. Antibiotic-resistant bacterial strains may also be suppressed in this way. Because this knowledge is foundational to the creation of effective pharmaceutical drugs, more research is needed to identify the plant's biologically active components, determine how it functions against isolated bacteria, and clarify the process of synergistic activity.

Acknowledgements

We appreciate the collaboration and support of all lab employees and other technical professionals.

Disclosure and Conflict of Interest

No conflict of interest is found.

Reference

Yadav V, Kiran RV, Jaiswal MK, Singh .,(2017). A study of antibiotic sensitivity pattern of *Pseudomonas aeruginosa* isolated from a tertiary care hospital in South Chhattisgarh, ,vol.6 issue3.

Pathmanathan SG, Samat NA, Mohamed R. (2009). Antimicrobialsusceptibility of clinical isolates of *Pseudomonas aeruginosa* from a Malaysian Hospital. Malays J Med Sci.;16(2):27-32.2Centers for Disease Control .

Laleh Babaeekhou, Hossein Karshenasan, Leila Pishkar., .(2018). Antibiotic Resistance in Clinical Isolates of *Pseudomonas aeruginosa*: A New Viewpoint for Antibiotic Prescription. Antibiotic Resistance in Clinical Isolates of *Pseudomonas aeruginosa*: A New Viewpoint for Antibiotic

Prescription. Avicenna Journal of Clinical Microbiology and Infection;5(3):55-60.

Centers for Disease Control and Prevention (CDC). (2013). Antibiotic resistancethreats in the United States,. CDC, Atlanta. *Available at* http://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013- 508.pdf.

Polly S X Y, Beow C Y, Hu C P and Swee Hua E L. (2014). Essential Oils, A New Horizon in Combating Bacterial Antibiotic Resistance. The Open Microbiology Journal, , 8, 6-14.

Slam, M. M. (2021). A review on medicinal properties somecommonly used culinary agents of Bangladesh.Journal of Medicinal Plants,9(3), 111-117.

Elumalai, S.; Kesavan, R.; Ramganesh, S. and Murugasen, R. (2011). Isolation, purification and identification of the antidiabetic components from

Cinnamomum zeylanicum and Cinnamomum cassia bark oil extracts. J. Curr. Bot. 2(2): 12-17.

Schmidt, E.; Jirovetz, L.; Buchbauer, G.; Eller, G. A.; Stoilova, I. and Krastanov, A. (2008). Composition and Antioxidant Activities of the

Essential Oil of Cinnamon (*Cinnamomum zeylanicum* Blume) Leaves from Sri Lanka. J. Jeobp, 9(2): 170-182.

Buchanon UR, Gibbons NE. (1974.). Bergery's Manual of Determinative Bacteriology. 8th ed. Baltomore: The Willisms and Wilkins Company;

Christensen, G. D., Simpson W. A., Bisno A. L., Beachey E. H. (1982).

Adherence of slime producing strains of *Staphylococcus epidermidis* to smooth surfaces. *Infect Immun*. 37:318-26.

Kivanc M, Kunduhoglu B.(1997). Antimicrobial Activity of Fresh Plant Juice on the Growth of Bacteria and Yeasts. Journal of Qafqaz University;1:26-53.

Brooks GF, Butel JS, Moore SA.(2004) . Medical Microbiology. 23rdedition. Lange medical Books/McGraw Hill medical publishing, New York.

Mahmudullah Bhuiya , Mohammad K. I. Sarkar , Mehadi H. Sohag , Hafij Ali, Chapol K. Roy , Lutfa Akther and Abu F. Sarker. (2018). Enumerating Antibiotic

Susceptibility Patterns of Pseudomonas aeruginosa Isolated from

Different Sources in Dhaka City. The Open Microbiology Journal, 12, 172 180. DOI: 10.2174/1874285801812010172.

. Moolenaar RL, Crutcher JM, San Joaquin VH, et al. (2000). A prolongedoutbreak of *Pseudomonas aeruginosa* in a neonatal intensive care unit: Did staff fingernails play a role in disease transmission? Infect Control Hosp Epidemiol; 21(2): 80-5. [http://dx.doi.org/10.1086/501739] [PMID:10697282.

Hassan A1, Usman J, Kaleem F, Omair M, Khalid A, Iqbal M. (2011).

Evaluation of different detection methods of biofilm formation in theclinical isolates.Braz J Infect Dis. Jul-Aug; 15(4):305-11.

Costa EM, Silva S, Pina C, Tavaria FK, Pintado M. (2014) Antimicrobial

Effect of Chitosan against Periodontal Pathogens Biofilms. SOJ Microbiol Infect Dis 2(1): 1-6.

Pournajaf A, Razavi S, Irajian G, Ardebili A, Erfani Y, Solgi S, et al. (2018). Integron types, antimicrobial resistance genes, virulence gene profile, alginate production and biofilm formation in Iranian cystic fibrosis Pseudomonas aeruginosa isolates. Infez Med.;26(3):226–36.

Bassetti M, Merelli M, Temperoni C, Astilean A.(2013) . New antibioticsfor bad bugs: where are we? Annals of Clinical Microbiology and Antimicrobials 12:22 DOI 10.1186/1476-0711-12-22.

Monnet DL, Giesecke J. (2014) . Public health need versus sales of antibacterial agents active against multidrug-resistant bacteria: a historical perspective. Journal of Antimicrobial Chemotherapy 69:1151–1153 DOI10.1093/jac/dkt478 .

Liew , Siew Mun , Ganeswrei Rajasekaram , SD Ampalam Puthucheary and Kek Heng Chua.(2019). Antimicrobial susceptibility and virulence genes of clinical and environmental isolates of Pseudomonas aeruginosa. PeerJ 7:e6217 http://doi.org/10.7717/peerj.6217.

A. Lamichhane, K.K. Nakarmi, P. Dahal, S.J. Basnet, P.B. Pokharel, S.

Bhattarai, et al. (2019). Bacteriological profile of burn patients and antimicrobial susceptibility pattern of their wound isolates at nepal cleft and burn centerJ. College Med. Sci.-Nepal, 15 (3), pp. 160-166.

PalisthaShrestha,SaraswatiShrestha, SriyaThapa, SanjitShrestha, Niroj

ManAmatya. (2021) . Detection of biofilm formation among *Pseudomonas aeruginosa* isolated from burn patients. Burns Open 5 , 125–129.

Wong, Y.C.; Ahmad, M. Y. and Wan-Nurdiyana, W.A. (2014). Extractionof Essential Oil from Cinnamon (*Cinnamomum zeylanicum*). Orient. J. Chem. 30(1), 37-47 .

Hili, P.; Evans, C. S. and Veness, R. G.. (1997). Antimicrobial action ofessential oils: the effect of dimethylsulphoxide on the activity of cinnamon oil," J. Letters App. Microbio. 24(4): 269–275, 1997.

Priyanga, R.; Shehani P.; Sirimal P.; Priyadarshani G.; Godwin R. C. and Prasad K. (2013). Medicinal properties of „true" cinnamon (*Cinnamomum zeylanicum*): a systematic review. J. Compl. Altern. Med. 13:275-285

Al-Ani, I. M.; Hayder Y.; Mohammad O. S. and Hameed M. G. (2011).

Histological findings of ascending urinary tract infections in experimental rats caused by Proteus mirabilis. J. Biomed. Res. 22 (4): 425-430.

Mohammed, J. M.; Omer M. R. and Gaidar S.J. (2005). Study the effect of efficacy cynamaldehyde that separated from Cinnamomoun

Zeylanicum in growth of numbers of gram negative and positive microorganism. J. Edu. Sci. 17(4): 67-77.

Kamath, J.V.; Rana A.C. and Chowdhury A.R. (2003). Pro-healing effect of Cinnamomoun Zeylanicum bark. J. Phytoth. Res. 17 (8) : 970- 972.

Boughendjioua, Hicham , Nadia Amoura and Zahra Boughendjioua . (2017). Purity Specifications of Constituents of Cinnamon Essential Oil by Fourier Transformed Infrared Spectroscopy Analysis. Indian J. Pharm. Biol. Res. 2017; 5(2):36-40.