Detection of the Active Compounds of the Hot Aqueous Extract of *Cinnamomum cassia* using FTIR Technology and Testing as Antibacterial and Antioxidant

Afnan I. Abdulwahab Department of Applied Science, University of Technology, IRAQ

Corresponding Author: 100252@uotechnology.edu.iq

ABSTRACT

The results of the FTIR analysis of the hot aqueous extract of bark indicated that it contains many groups and active compounds, and the results of the bacterial tests conducted by digging and spreading method showed a high inhibitory activity of the hot aqueous extract of cinnamon against all pathogenic bacterial strains and at different concentrations. It was observed that the highest efficacy of the extract was against bacteria an inhibition diameter of 35 mm at concentration 800 mg/mL in Bacillus sp. while the bacterial strains were resistant to most of the antibiotics, in the study (Amoxicillin, Gentamycin, Ampicillin, Erythromycin, Tetracycline) except for gentamicin, which showed inhibition of bacteria Staphylococcus aureus with a diameter 19mm and Serratia sp. in diameter17mm. We conclude from the study that the cinnamon plant contains many active compounds and that the hot aqueous extract of Cinnamomum cassia (cinnamon) bark has a high inhibitory ability for different bacterial strains, which exceeded the inhibitory ability of antibiotics.

Keywords- Antibacterial, Antioxidant, Resistant, Extract, Active Compounds.

I. INTRODUCTION

Plants are a natural treasure that man has used and benefited from for thousands of years. The human use of medicinal plants dates to the beginning of human civilizations. Human use of medicinal plants dates to the Sumerian, Akkadian, Babylonian and Assyrian populations of Iraq, thousands of years BC. They used plants. In the treatment of many diseases, these tablets and this clay form that contains the oldest therapeutic recipes can be considered the oldest medical constitution and symbol in the world, and perhaps reducing the lifespan of civilized man is due to his forgetting this treasure that God Almighty gave for him. It should be noted that medicinal plants still play an important role in maintaining health in third- world countries ⁽¹⁾. Microbial multidrug resistance (MDR) is a major cause of human suffering and economic losses. Without healthy organisms, human survival would be jeopardized in the realm of microbe-human symbiosis, and there would be no way to withstand the rise of multidrugresistant bacteria. Consequently, antibiotics were the greatest option from a health point of view.

Antimicrobial resistance (AMR) is a major source of worry, since it results in the highest loss of individual and society financial resources (2). Antimicrobial resistance is expected to kill ten million people per year by 2050, at a cost of one hundred trillion dollars. Today, the fast evolution of MDR in microorganisms is causing worldwide health concerns and posing a challenge to scientifically produced infectious disease medicines (3). Many researchers have studied different plants and their medicinal importance as antibiotics as observed in the Hibiscus sabdariffa ^{(4),} Mentha crispate ⁽⁵⁾, Cordia myxa ⁽⁶⁾ and in many other plants and their active groups. Cinnamon is a ubiquitous spice that has been used by various civilizations around the world for ages. Cinnamomum zeylanicum (CZ) and Cinnamon cassia (CC) (also known as Cinnamomum aromatic/ Chinese cinnamon) are really the two main variations of the genus Cinnamomum, a tropical evergreen plant having two main types. Cinnamon is a cure for respiratory, digestive, and gynecological disorders in Ayurvedic medicine, in addition to its culinary purposes. Cinnamon has medicinal and culinary uses in almost every part of the tree, including the bark, leaves, flowers, fruits, and roots. The chemical makeup of volatile oils extracted from the bark, leaf, and root barks varies greatly, implying that their pharmacological effects may also differ (7). The major ingredients of the plant, including as cinnamaldehyde (bark), eugenol (leaf), and camphor (root), are found in variable amounts in different regions of the plant ⁽⁸⁾. As a result, cinnamon produces a variety of oils with distinct properties, each of which determines its 'worth to various sectors. In contrast to the leaf and bark, the root, which contains camphor as its major ingredient, has little problems. In this commercial value. It is this chemical variety that is most prone to cause research, I will study the medical importance of the Cinnamomum cassia as an anti-bacterial and an antioxidant and what it contains of effective groups.

II. MATERIAL AND METHOD

Sample collection

Cinnamomum cassia bark was collected and purchased from the market of the city of Baghdad, after which it was washed with D.W, air dried and then ground into a powder.

95

International Journal for Research in Applied Sciences and Biotechnology

https://doi.org/10.31033/ijrasb.8.6.16

www.ijrasb.com

Plant crude extract

weighed 50 mg of powder and placed in 300 ml of water and placed on a low heat and after boiling for 15 minutes, it was removed from the fire and cooled at room temperature and filtered by 0.4-micron filter papers under sterile conditions and left to dry at a temperature of 39 $^\circ$ C $^{(9)}$.

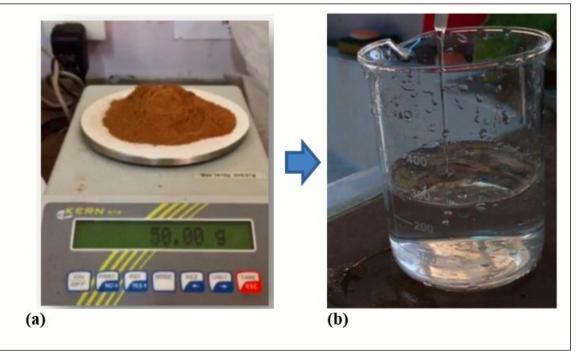


Figure 1: a- Weight 50 mg of plant bark powder, b- Measure the volume of 300 ml of distilled water

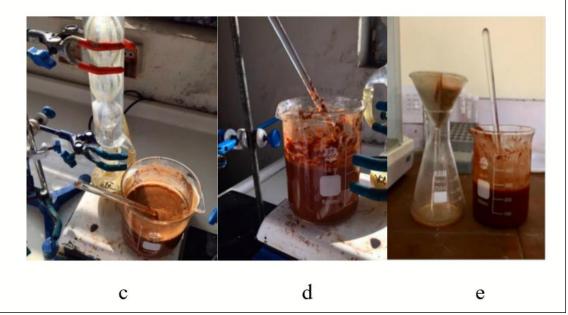


Figure 2: c-The extract while being heated over a low heat, d-Stir the extract to mix it, e-Filter the extract to get the filtrate.

Detection of active groups using Fourier transforms infrared (FTIR) spectroscopy.

Analysis (FTIR) of the aqueous extract was carried out using a FTIR-8400S device from SHIMADZU company, and this was done by taking a very small amount of the sample and crushing it well after adding potassium bromate salt to it in a ceramic slurry (Mortar) then We take the mixture and put it in a piston to obtain a disk of the substance KBr then the disk is placed in the place designated for the sample in the

https://doi.org/10.31033/ijrasb.8.6.16

device and then instructions are given to measure the model and within seconds the result appears and then takes the form Chart $^{(10)}$.

Strains of bacteria

The laboratory of biotechnology was supplied of more than one strain of bacteria (*Proteus spp., Bacillus* spp. *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa*).

Antioxidant Activity

To assess the antioxidant activity, several concentrations of *Cinnamonum cassia* (80, 60, 40 g/ml) were studied. The absorbance at 517 nm was calculated using the method below $^{(6)}$.

Antibacterial Activity

E.coli, P. aeruginosa, Bacillus spp., Proetus sp., and *Staph aureus* were used to test the activity against *Cinnamomum cassia* bacteria. CFU 10^5 ml was used to cultivate the bacterial cultures overnight. The antibacterial activity was measured using the disc diffusion technique. Muller Hinton agar plates were used to examine the test. To expand the inoculum on agar plates, a sterile Hi-media cotton swab was utilized. The plates were incubated at 37° C overnight, and the width of the inhibitory zone (mm) was measured after 24

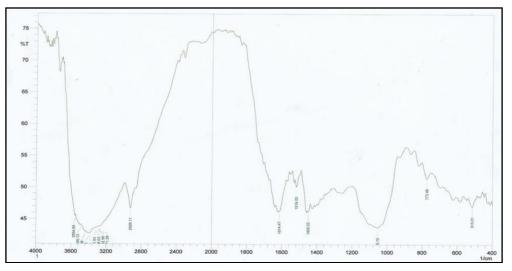
hours. All samples were examined three times. Solvent without plant extract was used as a control ⁽¹¹⁾.

III. RESULTS AND DISCUSSION

Active (Beneficial) groups of the extract were determined by FTIR. Detection of effective aggregates using the FTIR device. This aspect aims to identify the products of Natural products and identification of the most important chemically active groups present in plant extract using FTIR technology. Effective aggregate was detected in the water extract Fault in infrared spectroscopy using FTIR technology, absorption array and position inference with the active groups present in the bark of Cinnamomum cassia (cinnamon) plant The most important characteristic active groups that appeared in the boiled water extract are as follows: The O-H folded package is (3200-3650cm⁻¹) and a mount back to H-C Appeared at position (2840-3000cm⁻¹) and a folded band back to C=O at area (1641.47 cm⁻¹) Also a folded bundle back to C=C at region (1518.03 cm⁻¹) and a folded bundle back to C-O near (1000-1300 cm⁻¹) And a curved beam belonging to the H-C also appeared in the area(1450.52cm⁻¹) In table 1 and figure 3.

Table 1: Infrared vibration frequencies of the effective groups of the study plants

pack type	The location of the appearance of the pack	plant name	
О-Н	3200-3650cm ⁻¹		
H-C	2840-3000cm ⁻¹		
C=0	1641.47 cm ⁻¹	hot aqueous extract	
C=C	1518.03 cm ⁻¹	For the bark of <i>Cinnamon cassia</i> plant	
C-0	1000-1300 cm ⁻¹		
H-C	1450.52cm ⁻¹		





International Journal for Research in Applied Sciences and Biotechnology

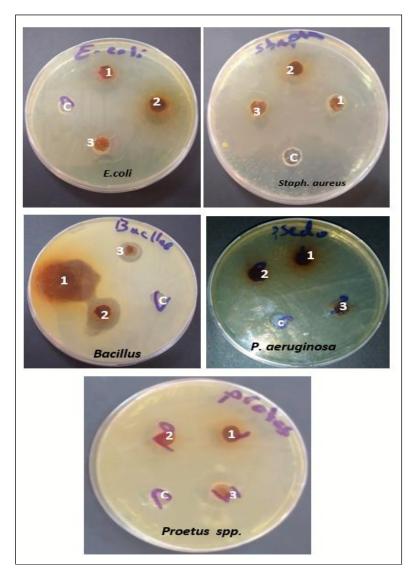
www.ijrasb.com

https://doi.org/10.31033/ijrasb.8.6.16

Based on these aggregates that appeared during specific regions in the FTIR spectrum of the studied plants, it is according to It has been confirmed that these active groups are due to the compound cinnamaldehyde, which is the active substance. Which gives the taste Cinnamon cassia and smell, and also using HPLC, it was observed during the study that the extract contained a plant Studying Cinnamon cassia on alkaloids and saponins, which are anti-fungal and anti-bacterial agents The polarity of the solvent used in preparing the extract plays an significant role in determining the inhibitory activity of the extract (12)referred to the solubility of some of the active compounds in one of the extracts without their solubility in the other extracts It may affect the effectiveness of the extract. For example, it was noted during the study that the ethyl extract of Cinnamon cassia bark contained Alkaloids and saponins, which are anti-fungal and anti-bacterial agents compared to other extracts The aqueous extract contained, based on other

research, for inference about the effective groups, which turned out to be from Carbohydrates, saponins, resins, tannins, and alkaloids using chemical detections ⁽¹³⁾. *Antibacterial activity*

Table (2) shows that the hot aqueous extract of *Cinnamon cassia* bark had a high inhibitory activity against each of the bacteria used in the study in a different way. The effect of the hot aqueous extract of the plant ranged between inhibition zones with a diameter of 2 mm to 35 mm. The percentage of inhibition against bacilli at concentration $800\mu g/ml$ for a diameter of 35 mm, as shown in Figure 4 where the diameters of inhibition of extract from *Bacillus* spp. ranged from 2-15 mm, *E. coli* 21-30 mm, *Proteus* 10-25 mm, *Staph* bacteria from 21-29 mm. And this indicates the great effectiveness of the hot aqueous extract of the plant against bacterial isolates and its high behavior as an antibiotic and this agrees with ^(14, 15, 16).





www.ijrasb.com

Microorganisms	Control	Concentratio	Concentration of extract			
		0.1%	0.05%	0.025%		
P. aeruginosa		15mm	12mm	2mm		
S. aureus		29mm	25mm	21mm		
E. coli		30mm	25mm	21mm		
Bacillus sp.		35mm	25mm	15mm		
Proetus sp.		25mm	19mm	10mm		

Table 2. Antibacterial activity of Cinnamon cassia extract

Test the inhibitory effect of some antibiotics against bacterial strains in the study

Five types of antibiotic tablets (Amoxicillin, Gentamycin, Ampicillin, Erythromycin, Tetracycline) Tetracycline were taken and the inhibitory effect of these antibiotics on the bacteria used in this research was studied Five types of antibiotic tablets were taken and the inhibitory effect of these antibiotics on the bacteria used in this research was studied.

The results were as shown in the table (3) that most of the bacteria in the study showed resistance to the antibiotics used, except for the antibiotic gentamycin, which had an inhibitory effect on *S. aures* bacteria with an inhibition diameter of 19 mm and on bacteria *Proteus sp.* with an inhibition diameter of 17 millimeter

Table 3: Effect of using antibiotics on	bacterial isolates measured in milliliters
---	--

Antibiotics	S. aureus	E. coli	P. aeruginosa	Proteus sp.	Bacillus sp.
Amoxicillin (AM 10 µg)	S	S	S	S	S
Gentamycin (GN 10 µg)	19	S	S	17	S
Ampicillin (AM 10 µg)	S	S	S	S	S
Erythromycin (E 15 μg)	S	S	S	S	S
Tetracycline (TE 30 μg)	S	S	S	S	S

(S): Sensitive

The extract's antibacterial activity is attributed to phytochemicals such as alkaloids, flavonoids, phenols, and tannins that affect bacteria ^{(17).}

The inhibitory effect of aqueous extracts of cinnamon is due to the fact that it contains tannin, which contains some phenolic compounds such as gallic acid and tannic acid, which have a biological effect against many bacterial species due to the presence of hydroxyl groups. (OH) - which has the ability to form hydrogen bonds between the hydroxyl group of these compounds and water molecules in the bacterial cell, where water is 90% by weight, which leads to disruption of the vital work of the bacterial cell ⁽¹⁸⁾.

From the previous results we concluded two important things (the first) is the resistance of the bacterial species in this study to most of the antibiotics used, and this is an expected result due to the excessive and indiscriminate use of these antibiotics ⁽¹⁹⁾. Which

may cause the emergence of resistant bacterial strains, and this is what was shown by the results of the study in addition to the negative effects and side effects of using these antibiotics on human health in the long term, which indicates the necessity of plant therapy. Preparations that do not contain added chemicals, which have the same therapeutic effect and (secondly) the remarkable superiority of the hot aqueous extract of cinnamon in its inhibitory activity of the bacterial strains in this study compared to the antibiotics used because they contain the active natural compounds mentioned previously. Here the importance of plant extracts emerges as an effective therapeutic alternative for its effective ability to eliminate some pathogens.

Antioxidant activity

The DPPH was found to be proportional to the increase in concentration. The concentrations of 40,60, and 80 μ g/ml in figure5. gave the free radicals 50.40, 58.40, and 78.49.

International Journal for Research in Applied Sciences and Biotechnology

www.ijrasb.com

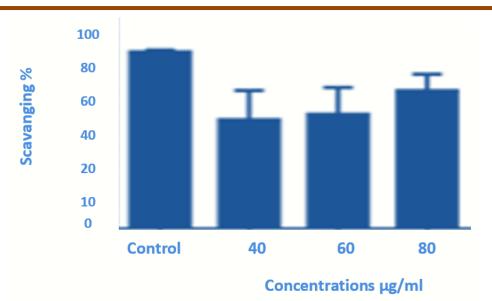


Figure 5: Antioxidant activity of Cinnamomum cassia Bark by DPPH.

REFERENCES

[1] Chandra, H.; Bishnoi, P.; Yadav, A.; Patni, B.; Mishra, A.P.; Nautiyal, A.R. Antimicrobial Resistance and the Alternative Resources with Special Emphasis on Plant-Based Antimicrobials—A Review. Plants 2017, 6, 16. [CrossRef] [PubMed]

[2] David, B.; Wolfender, J.-L.; Dias, D.A. The pharmaceutical industry and natural products: Historical status and new trends. Phytochem Rev. 2015, 14, 299–315. [CrossRef]

[3] Pye, C.R.; Bertin, M.J.; Lokey, R.S.; Gerwick, W.H.; Linington, R.G. Retrospective analysis of natural products provides insights for future discovery trends. Proc. Natl. Acad. Sci. USA 2017, 114, 5601–5606. [CrossRef]

[4] Raghad K. Maeah , Butheina A. Hasoon , Afnan I.Alwahab, Khalida F. F. AL-azawi, Wafaa Beed Allah Hameedi .2020. Biosynthesis of silver nanoparticles using Hibiscus sabdariffa and their biological application .Eurasia J Biosci 14, 3377-3383 .

[5] Afnan I. Abdulwahab, Butheina A. Hasoon, Raghad K.Maeah and Khalida F. Al-azaw. 2021.Preparation of Mentha Crispata Extract and Detection of its Biological Application. Sys Rev Pharm ,12(1):1151-1155

[6] Butheina A. Hasoon, Afnan I. Abdulwahab,, Raghad Khwater Maeh, Khalida F. Al-azawi.: Preparation and Characterization of Silver Nanoparticle by Cordia myxa Extract and their Study Anticancer, Antioxidant, Antibacterial Activity Indian Journal of Forensic Medicine & Toxicology, July-September 2021, Vol. 15, No. 3

[7] Shen Q, Chen F, Luo J:Comparison studies on chemical constituents of essential oil from ramulus

cinnamomi and cortex cinnamomi by GC-MS.Zhong Yao Cai2002,25:257–258.

[8] Gruenwald J, Freder J, Armbruester N: Cinnamon and health. Crit Rev FoodSci Nutr2010,50:822–834.

[9] Gharaiben N., Elayan and Salhab,A.(1988).Hypoglycemic effect of Teucrium olium Ethnopharmacol,24(1):93-99.

[10] Silverstein, R. M., Webster, F. X. and Klemle, J .D.(2005). Sectrometic identification organic compounds.John Wiley &Sonc, Incl/New Yorc.

[11] Pepeljnjak, S., Kosalec, I., Kalođera, Z., & BLAŽEVIĆ, N. (2005). Antimicrobial activity of juniper berry essential oil (Juniperus communis L., Cupressaceae). Acta pharmaceutica, 55(4), 417-422.

[12] Mishra N, Upma K, Shukla D (2000), Antifungal activity of essential oil of Cinnamomum zeylanicum. J Essent Oil Res 3, 97–110. Mothana RA, Lindequist U (2005), Antimicrobial activity of some medicinal plants of the island Soqotra. J Ethnopharmacol 96, 177–181.

[13] Melvin Joe, M., Jayachitra J. and Vijayapriya M., (2009). Antimicrobial activity of some common spices against certain human pathogens. Journal of Medicinal Plants Research Vol. 3(11), pp. 1134-1136.

[14] Karada `gluo `glu, Ö. I.; Ulusoy, N.; Ba,ser, K.H.C.; Hano `glu, A.; ,Sık, 'I. Antibacterial activities of herbal toothpastes combined with essential oils against Streptococcus mutans. Pathogens 2019, 8, 20. CrossRef] [15] Chaudhari, L.K.; Jawale, B.A.; Sharma, S.; Sharma, H.; Kumar, C.D.; Kulkarni, P.A. Antimicrobial activity of commercially available essential oils against Streptococcus mutans. J. Contemp. Dent. Pract. 2012, 13, 71–74.[CrossRef]

[16] Abbaszadegan, A.; Dadolahi, S.; Gholami, A.; Moein, M.R.; Hamedani, S.; Ghasemi, Y.; Abbott, P.V.; Patil, S.Antimicrobial and cytotoxic activity of Cinnamomum zeylanicum, Calcium hydroxide, and www.ijrasb.com

https://doi.org/10.31033/ijrasb.8.6.16

triple antibiotic paste as root canal dressing materials. J. Contemp. Dent. Pract. 2016, 17, 105–113. [CrossRef] [17] Khuzaie R.F., Rashan J., and Al-Dori N., 1999, In Vitracts of some local Medicinal plants growing in Jordan ,J. J. Appli. Sci, 1(1): 46-52

[18] Allan G., Robert A., Denis St. J. Michael J. S. and James S. 1999,"An illustrated colour text clinical biochemistry ,ed. 2ed ,UK, p.106-114

[19] Bakken J.S.,Sander C.C.andThomson K.S., 1987.Selective ceftazidime resistance in E.coli association with changes in outer protein J.Inf.Dis.155:1220-1225.