In vitro Antifungal Activity of Cinnamon Extracts against Plant Pathogenic fungi

Ayad Ismael Khaleel[1]; Fadhal A.Al-Fadhal[2]
[1]Department of Horticulture, Faculty of Agriculture, Al Qasim Green University, Babylon, Iraq.
[2]Department of Plant Protection, Faculty of Agriculture, Kufa University, Iraq.
Email: ayad.altaee@agre.uoqasim.edu.iq , engayad78@gmail.com

Abstract
The possibilities of *Cinnamomum* genus as a biological control agent against plant pathogenic bacteria. Cinnamon sticks powder were extracted by steeping using ethanol, *n*-hexane, and methanol. For the *in vitro* antibacterial activity, species of plant pathogenic bacteria: *Pseudomonas syringe* pv. *Tomato* selected. Four different concentrations, 10, 25, 50, and 75 mg/ml were achieved through the cup plate agar diffusion technique. Streptomycin sulphate at 30 µg/ml concentration was set as the positive control, whereas every respective solvent used in the sticks powder extraction was set as the negative control. The results have shown that, only ethanol extract verified antibacterial activity when tested on the plant pathogenic bacteria. The highest diameter of inhibition zones was observed in *P. syringae* pv. *tomato,* at all range of concentrations. The least methanol extract concentration utilised in determination of minimum inhibitory concentration (MIC) assay was at 2.5 mg/ml, inhibiting *P. syringe* pv. *Tomato.* The phytochemical screening of *Cinnamon* ethanol extracts the presence some major compound known as secondary metabolites determine. It showed the positive results for the presence of alkaloid, flavonoid, saponin, tannin, terpenoid and phenol.
Keyword: Plant Pathogenic Bacteria; plant pathology; Cinnamon; plant extract

Introduction

Herbs and spices used since olden times, because of their antimicrobial things increasing the safety and shelf life of food crops by acting against foodborne pathogens and rot bacteria[1]. Plants over all used in old-style medicine as sources of natural antimicrobial elements for the treatment of infective disease[2]. Thus, much courtesy has been paid to medicinal plants as a source of other antimicrobial plans. Furthermore, due to the growing mandate for preserving free cosmetics, antimicrobial activity of herbal extracts newly used in the cosmetic manufacturing to decrease the risk of reactions connected to the presence of methyl-parabens[3]. More or less species going to the genus *Cinnamomum*, usually used as flavors, contain several antibacterial compounds[4]. In addition, a brief summary of the history, traditional uses, phytochemical constituents, and clinical impact of cinnamon is provided[5].

Materials and Methods

Preparation of Cinnamon sticks extract

The fresh Cinnamon sticks have been obtained from the Alhillah market,Babylon, Iraq. Then, The dried sticks were ground to a powder with the help of a cross beater mill (SK100, Retsch) machine and sieved through a sieve having a pore size of 0.50 mm. Solvents, with differing polarities (methanol, hexane and ethanol), were used for extraction. Firstly, for determining the finest solvent, extraction was carried out using small amounts (20 g/200ml solvent)[6]. The solvent and powder mixture was soaked in 250 ml flasks and then sealed with an aluminum foil and reserved on a rotary shaker at 30°C for 48 h. This slurry passed through a Whatman No. 1 filter paper, at the end of 48 h. The solvent was vanished with the help of a rotary evaporator (Hahn Shin Scientific Co., Taiwan), at a rotator speed 150 rpm and a temperature of 40°C till complete dryness[7]. The dried extracts were placed in the chiller at 4°C till further use.

Preparation of Bacterial Culture

Pseudomonas syringe pv. tomato (Accession number KR261604) the causal agents of tomato bacterial speck respectively were obtained from the culture collection of Plant pathology Laboratories, Faculty of Agriculture, University Kufa. A loopful of the 24 h old bacterial culture, cultured on agar plates, was moved to 30 ml test tubes, which contained sterile distilled water and was mixed by vortex. The bacterial density was attuned to reach a value of Optical Density (OD) = 0.1 measured at 660 nm wavelength[8].

In Vitro Evaluation of Antibacterial Activity

Efficacy of the Cinnamon sticks extracts was determined *in vitro* by the agar well diffusion assays, as described before with slight modifications[9]. The assay was conducted using 9 cm petri plates that contained 20 ml of the Muller-Hinton Agar (MHA) culture medium. 50 µl of the bacterial cell suspension was pipetted out onto the agar plates and then spread on the solid media using an L-shaped glass rod, for ensuring a homogeneous spread of the culture. The whole spread plate experiment was carried out in the laminar flow to prevent any kind of

doi.org/10.31838/hiv23.03.200 Received: 23.11.22, Revised: 13.12.22, Accepted: 19.01.23.

contamination. Once the plates has dried out, 6 wells were bored in the plated using a sterile cork borer (0.4 mm). 50 µl of the different plant extracts were added to every well. The concentration of the extracts was (10, 25, 50 and 75 mg/ml). 2 wells filled with 50 µl of the streptomycin sulphate (30 µg/ml) solution, which represented as a positive control. The plates were left to dry for 3 h. Then, the plates were inverted and were incubated for 24 h at 30˚C. The same test was performed in 4 different replicates. The bacterial growth was estimated by calculating the diameters of the zone of inhibition which was seen around the wells, using a ruler graduated in millimetres (mm).

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bacterial Concentration (MBC)

The MIC for the ethanol extract was studied using the broth micro dilution technique by the 2-fold serial dilution method[10]. The MIC assay was based on the lowest concentrations of the most active plant extracts, which inhibited the bacteria in the agar plate assay. Based on the results of the *in vitro* assay, the least concentration for the MIC estimation was considered 10 mg/ml. Eleven capped test tubes were also filled with 1ml of the Mueller Hinton Broth (MHB, Oxoid) and then sterilized in an autoclave at 121˚C for 20 minute. To the test tube 1, a concentrated plant extract (50 mg/mL) was added to obtain a final concentration of 10 mg/mL. Thereafter, a 2-fold dilution was carried out from the test tube 1 - 10. Test tube 11 contained only the MHB without any extract. Throughout the whole experiment, the bacterial density was kept constant at 0.1 OD 660. 100µl of the microbial cell suspension was also added to all the test tubes except test tube 10 and was mixed well. The test tube 10 containing no microorganisms but only the extracts and the broth served as a positive control, whereas the test tube 11 containing a mixture of the broth and microbes was the negative control for the assay. Thereafter, 50 µl of the aqueous solution of 2,3,5-TriphenlTetrazolium Chloride (TTC, stock of 2 mg/ml,Sigma) was also added to all the test tubes as an indicator dye to determine the microbial growth. These tubes then incubated for 24 h at 30˚C. The MIC represented the lowest concentration of the plant extract which did not show any color change[11]. The MBC values were evaluated by sub culturing the culture from the tube that did not show any color change, on sterilized MHA plates. The plates were incubated at 30˚C, overnight. MBC value was determined as the lowest concentration that did not show any visible growth on the agar plates

Protocol for Phytochemical Screening

The phytochemical screening of Cinnamon sticks ethanol extracts was done to find the presence of active chemical elements such as Phenolic, Flavonoids, Saponins, Alkaloids, terpenoids, and Tannins. The phytochemical screening was done based on previous procedures published somewhere else[12]

Statistical Analysis

SAS software version 9.2 was used to perform the Analysis of Variance (ANOVA) (Cary, NC: SAS Institute Inc. 2011). LSD test at the 0.05 probability level was used to determine the statistical differences among the different bacteria and fungi isolates.

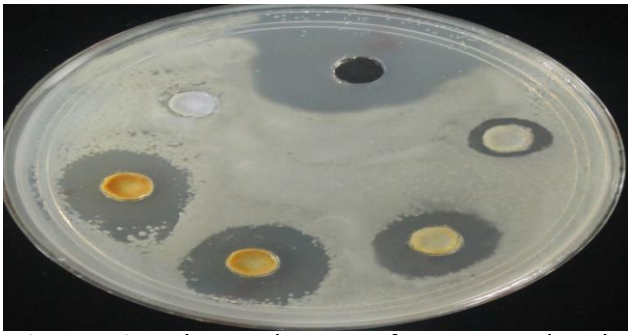
Result and Discussion

In Vitro Evaluation of Antibacterial Activity

The results showed as (**Table 1**) that the antibacterial activity of each crude extract sample was strongminded by the presence or the nonappearance of the inhibitory zone around the well. The *Cinnamon* methanol and the n-hexane extracts did not show any antibacterial activity against the pathogenic bacteria. However, the ethanol crude extract displayed antibacterial activity against the germs that were tested. The ethanol extracts displayed zones of inhibition ranging from a diameter of 10.6, 13.8, 16.5, 18.4 mm for concentration 10, 25, 50, and 75 respectively. Moreover, the diameter of the inhibition for the positive control of streptomycin sulphate demonstrated zone in the range of 19 mm. The negative control did not display any inhibition against the pathogenic bacteria. ethanolic extract, with the lowest concentration (10mg/ml) was able to inhibit the bacteria *P. syringae* growing. The assay indicated that the antibacterial activity increased with significantly differences (figure 1).

(Table 1) Antibacterial activity of *Cinnamon* ethanol extract against pathogenic bacteria.

Concentration (mg/ml)	Inhibition zone (mm)
10%	16.6 ± 0.4 d
25%	13.8 ± 0.2 c
50%	16.5 ± 0.6 b
75%	18.4 ± 0.5 b
Positive control	19 ± 0.5 a
Negative control	0 ± 0 e



(Figure 1) Antibacterial activity of Cinnamon ethanol extract against pathogenic bacteria.

Minimum Inhibitory Concentration (MIC), (MBC) and (IC50%)

The (MIC) for the tested bacterial isolate, the lowest concentration that still inhibited the plant pathogenic bacteria was at 2.5 mg/ml. For MBC, The

lowest concentration that showed no visible growth on the agar plates considered the MBC value 10 mg/ml recorded for *P. syringae*. The results of IC90 and IC50 values calculated by probit analysis using Polo plus Ver 2. Results specified that the chi square test for bacteria have significant heterogeneity in the test population as shown in (**Table 2**) and (**Figure 2**).

Table (2) Minimal inhibitory concentration (MIC) and minimum bactericidal Concentration (MBC) and Probit regression line parameter and inhibition concentration (IC) of *Cinnamon ethanol* extracts against plant pathogenic bacteria by macro-broth dilution methods

	P. syringae
MIC (mg/ml)	2.5
MBC (mg/ml)	10
Regression equation	Y = 1.570 - 2.625 X
Chi-square (df)	0.063 (1)
IC50 with fiducial limits	46.971 (25.8 - 68.4)
IC90 with fiducial limits	307.64 (167.7 - 1622.8)

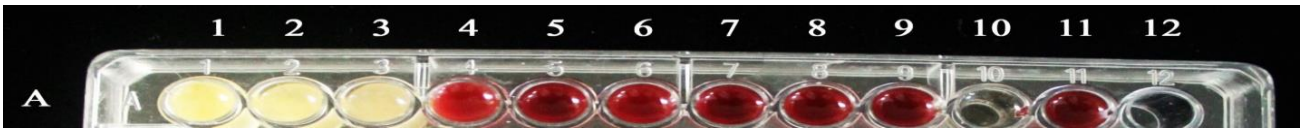


Figure (2) Minimal inhibitory concentration (MIC) Cinnamon ethanol extracts against plant pathogenic bacteria

Phytochemical Screening of Cinnamon ethanol extracts against plant pathogenic bacteria

The phytochemical screening of *Cinnamon* ethanol extracts the presence some major compound known as secondary metabolites determine. It showed the positive results for the presence of alkaloid, flavonoid, saponin, tannin, terpenoid and phenol. As determined by the color change. Table 3

Chemical Constituents	Positive Results	Cinnamon ethanol extracts
Saponin	Small bubbles (foam)	+
Tannin	Blue-green color	+
Alkaloid	Creamish precipitate	+
Terpenoid	Reddish brown coloration	+
Phenol	Dark blue	+
Flavonoid	Dark yellow	+

References

1. Du, W. X. *et al.* Effects of allspice, cinnamon, and clove bud essential oils in edible apple films on physical properties and antimicrobial activities. *J. Food Sci.* (2009). doi:10.1111/j.1750-3841.2009.01282.x
2. Hamidpour, R., Hamidpour, M., Hamidpour, S. & Shahlari, M. Cinnamon from the selection of traditional applications to its novel effects on the inhibition of angiogenesis in cancer cells and prevention of Alzheimer's disease, and a series of functions such as antioxidant, anticholesterol, antidiabetes, antibacterial, antifungal, nematicidal, acaracidal, and repellent activities. *Journal of Traditional and Complementary Medicine* (2015). doi:10.1016/j.jtcme.2014.11.008
3. Chen, C. *et al.* Transcriptome analysis and identification of genes related to terpenoid biosynthesis in Cinnamomum camphora. *BMC Genomics* (2018). doi:10.1186/s12864-018-4941-1
4. Husain, I. *et al.* Phytochemical characterization and biological activity evaluation of ethanolic extract of Cinnamomum zeylanicum. *J. Ethnopharmacol.* (2018). doi:10.1016/j.jep.2018.02.001
5. Hügel, H. M. *Natural Compounds as Therapeutic Agents for Amyloidogenic Diseases. Advances in Experimental Medicine and Biology* (2015).
6. Mickymaray, S., Al Aboody, M. S., Rath, P. K., Annamalai, P. & Nooruddin, T. Screening and antibacterial efficacy of selected Indian medicinal plants. *Asian Pac. J. Trop. Biomed.* (2016). doi:10.1016/j.apjtb.2015.12.005
7. Zaidan, M. R. *et al.* In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method. *Trop. Biomed.* (2005).
8. Suwanmanon, K., Rachtanapun, C., Surapat, S., Chiu, C. H. & Guu, Y. K. Inhibitory effects of plant extracts on the growth of five pathogenic bacteria. *Taiwan. J. Agric. Chem. Food Sci.* (2010). doi:10.6578/TJACFS.2010.024
9. Khaleel, A. I., Sijam, K., Rashid, T. S. & Bin Ahmad, K. Phytochemical Determination and Antibacterial Activity of <i>Punica granatum</i> Peel Extracts against Plant Pathogenic Bacteria. *Am. J. Plant Sci.* **07**, 159–166 (2016).
10. Pierce-Hendry, S. A. & Dennis, J. Bacterial culture and antibiotic susceptibility testing. *Compend. Contin. Educ. Vet.* (2010).
11. Ristivojević, P. *et al.* Antimicrobial activity of Serbian propolis evaluated by means of MIC, HPTLC, bioautography and chemometrics. *PLoS One* (2016). doi:10.1371/journal.pone.0157097
12. Obadoni, B. O. & Ochuko, P. O. Phytochemical Studies And Comparative Efficacy Of The Crude Extracts Of Some Haemostatic Plants In Edo And Delta States Of Nigeria. *Glob. J. Pure Appl. Sci.* (2002). doi:10.4314/gjpas.v8i2.16033