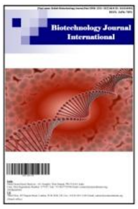

Biotechnology Journal International

25(4): 45-53, 2021; Article no.BJI.74590
ISSN: 2456-7051
(Past name: British Biotechnology Journal, Past ISSN: 2231–2927, NLM ID: 101616695)

Anti-bacterial Effect of Aqueous and Ethanolic Extracts of Onion, Garlic and Cinnamon on *Xanthomonas* Species

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJI/2021/v25i430148
Editor(s):
(1) Dr. Chan Yean Yean, Universiti Sains Malaysia, Malaysia.
(2) Dr. Pankaj Kumar, H. N. B. Garhwal Central University, India.
(3) Prof. Antar El-Banna, Kafrelsheikh University, Egypt.
Reviewers:
(1) S. Selvarani, Thiagarajar College, India.
(2) Lalu Zulkifli, University of Mataram, Indonesia.
Complete Peer review History: https://www.sdiarticle4.com/review-history/74590

Original Research Article

Received 09 August 2021
Accepted 18 October 2021
Published 28 October 2021

ABSTRACT

Onion *(Allium cepa*), garlic (*Allium sativum*) and cinnamon (*Cinnamomum zeylanicum*) are edible herbs but they contain many chemicals which can be used as medicine. These herbs also contain many antibiotic molecules that are capable to inhibit or reduce growth of many types of microbes including bacteria. Due to the development of resistance of bacteria against synthetic antibiotic now there is a need to have natural antibiotic which must be harmless, cheap and easily available. To know the effectiveness of garlic, onion, and cinnamon on disease causing bacteria such as *Xanthomonas campestris pv. malvacearum, Xanthomonas axonopodis pv. citri, Xanthomonas oryzae pv. oryzae*, extracts of each sample was made in water and ethanol. Antibacterial potential of plant extracts was observed at two different stages: at the time of inoculation and after growth of pathogen on media. Results showed extracts applied on all the bacteria before growth inhibited

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maximum bacterial growth as compared to applied after growth. Moreover, cinnamon extract in ethanol effectively control bacterial growth than onion and garlic extracts. Maximum inhibition zone was shown by ethanolic extract of cinnamon before growth of *Xanthomonas oryzae pv. oryzae* (7.333 mm), *Xanthomonas campestris pv. malvacearum* (7.83 mm) and *Xanthomonas axonopodis pv. citri* (5.1 mm). After 24 hours, maximum growth of *Xanthomonas oryzae and Xanthomonas axonopodis* was inhibited by ethanolic extracts of cinnamon (2.82 and 3.35 mm, respectively) while *Xanthomonas campestris* was inhibited by ethanolic extract of onion (6.55 mm). Extract diluted from 66mg/ml to 0.01mg/ml showed different minimum inhibitory concentration against pathogens. As concentration decreases inhibition of bacteria also decreases. Ethanolic extracts of cinnamon showed maximum MIC against *Xanthomonas campestris pv. malvacearum* (3.5 mm to 1.5 mm) and *Xanthomonas oryzae pv. oryzae* (5 mm to 2 mm) while for *Xanthomonas axonopodis pv. citri* ethanolic extract of garlic (3 mm to 1 mm) was effective ranging between 66mg/ml to 33 mg/ml. This study highlighted that natural products possess ability to inhibit pathogenic bacterial growth and would also be helpful in medicinal field for further study.

Keywords: Antibacterial activity; ethanolic extract; aqueous extract; spices.

1. INTRODUCTION

Many important crops are infected by bacteria such as *Xanthomonas* species which damages more than 50% percent production of rice, cotton and citrus *etc*. [1]. *Xanthomonas* (from the Greek xanthos, meaning, "yellow" and monas, meaning entity) is associated with plant yellow pigment bacteria which belong to large genus of gram-negative bacteria. *Xanthomonas* has the largest species that affect nearly four hundred plants and belong to class of Gamma proteobacteria. *Xanthomonas* damages most important economical crops such as rice, citrus, banana, cabbage, tomato, pepper and bean. It is host specific bacteria and infects plants with contaminated seed, rainwater, or aerosols. Bacteria remain on the surface of leaf or stem then enter into parenchyma cells through stomata, wounds and hydathodes. Approximately all species of *Xanthomonas* contain chromosomes ranging from 4-5.3 mega base pairs with single circular and GC content nearly 70%out of hundred almost all genes are same but in genomic sequences large number of inversions, insertion, translocation, deletion [2].

Xanthomonas axonopodis pv. citri destroys most economical citrus fruits, 125 monocot and 268 dicot plants. This disease known over centuries but disease-causing agent was first noticed in India [3]. Citrus production in many countries of the world decreases because of this bacterial attack. *Xanthomonas axonopodis* or citrus canker develops lesions on all parts of the plant, due to this fruit quality and yield reduced. This disease prevails all over US as well as in Asia due to this [4]. Bacterial leaf blight (*Xanthomonas campestris pv. malvacearum*) affects all aerial parts of the cotton crop, causes premature defoliation, blockage of xylem phloem tissues and finally death of parenchyma tissues [5]. Pakistan's is the third biggest exporter of cotton and due to this the production affects badly that ultimately affects Pakistan economy [6]. Rice is most abundantly cultivated across the world in temperate regions but Asia produces rice 90% of the world. Pakistan is the second country for the production of rice in world. However, this most important crop is destroyed by bacterial leaf blight disease caused by *Xanthomonas oryzae pv. oryzae* all around the world. This disease may attack at any stage of crop development which results in immature grain and total yield lost [7].

Most of our agricultural industry treats crop diseases with different chemicals that are non-biodegradable and leach down in soil, move into water resources and their residue remains in edible parts of crop. These chemicals add toxic components to environment and are inhaled by human and animals causing different lethal diseases related to breathe, stomach heart and cancer. Contaminated water also causes difficulties for the survival of aquatic animals. In Pakistan, bacterial blight is being treated with strong and environmental toxic chemicals such as Copper- oxychloride which is expensive and its residuals pose danger to human health. Now a day's researchers are giving attention to environmental friendly and nontoxic methods for treating plant diseases. For this purpose, developing countries like Pakistan use plants extract for treating different diseases that do not affect the growth and production of crops. Interestingly about 20% of plants extract contain such metabolites that are used against bacterial diseases [7].

The use of medicinal plants or herbs has received greater attention due to its minimum or no adverse effects. Spices are substances used to increase food taste and aroma including leaves (coriander and mint), bulbs (turmeric and garlic), fruits (black pepper), flowers (clover), rhizomes (ginger) and stems (cinnamon) are some of the plant components utilized to improve taste. Several bioactive compounds produced by medicinal plants have antibacterial and antifungal properties [8]. Plants contain a wide range of secondary metabolites, including tannins, terpenoids, alkaloids, and flavonoids, all of which were shown to have antibacterial effects [7]. Numerous medicinal plants also include antioxidant and antimicrobial characteristics that defend the host against cellular oxidation processes and other infections, emphasizing the significance of exploring the natural antimicrobials [9]. The majority of bacterial infections found in foods are susceptible to plant extracts such as oregano, garlic, mustard, cinnamon and onion [10]. Among these garlic (*Alliums sativum*) is being used as medicine for long time due to its antibiotic, antifungal and antiviral properties [11]. Garlic is used as herbal antioxidant that reduces reactive oxygen species and low-density lipoprotein oxidize it containing allicin and sulfur compound that are highly active against microbes [12,13]. Onion (*Allium cepa)* grows worldwide and has strong antiseptic quality due to different chemicals in its skin and bulb such as quercetin, aglycone, flavonoids, phenols and polyphones. As an anti-bacterial agent its production has been increased around 25% in last 10 years [14]. Cinnamon (*Cinnamomum zeylanicum*) is a green spice plant that is grown in tropical region and also used as medicine in ancient and modern ages. Bark and leaves contains chemicals like cinnamte, cinnamic acid, and cinnamaldehyde that have anti-diabetic, anti-fungal, antioxidant, anti-inflammatory, anticancer, insecticidal, and nematocidal properties [15]. Based on the above literature, present study is planned to investigate the anti-bacterial activity of onion, garlic and cinnamon extract on *Xanthomonas* species causing damage to economically important crops.

2. MATERIALS AND METHODS

2.1 Sample Collection

Fresh onion, garlic and dry cinnamon were purchased from local market. Onion and garlic were disease free and without physical damage. *Xanthomonas campestris pv. malvacearum, Xanthomonas oryzae pv. oryzae, Xanthomonas axonopodis pv. citri* were collected from Department of Agriculture, Bahauddin Zakariya University, Multan, Pakistan.

2.2 Preparation of Extract

For extract preparation, onion and garlic were peeled, washed and dried. All the clean samples were cut into small pieces and were grounded in mortar and pestle. Dry Cinnamons were grounded in electric grinder and powder was made. 40 g of each sample was taken and soaked in 400 ml of water and 400 ml ethanol in separate beakers. Each sample was left for 72 hours and stirred at 25°C using magnetic stirrer. These water and ethanol extracts were centrifuged at 3000 rmp for 10 minutes at room temperature. Each extract prepared was weighed *i.e.,* water and ethanol extract of cinnamon were 2.015 g and 3.595 g, respectively, water and ethanol extract of onion were 2.033g and 2.022 g, respectively, and water and ethanol extract of garlic was 2.031 g and 2.019 g, respectively.

2.3 Media Preparation and Bacterial Inoculation

Antibacterial potential of plant extracts was tested at two different stages of growth by applying the extract at the time of inoculation and after the growth of pathogen on media. For this purpose, pure culture of *Xanthomonas campestris pv. malvacearum, Xanthomonas oryzae pv. oryzae* and *Xanthomonas axonopodis pv. citri* were cultured in nutrient broth under highly sterile conditions following streaking on nutrient agar plates and were placed for 48 hours at 28°C. After that, these cultures were poured on nutrient agar plates and placed in incubator at room temperature for 24hours. All the activities were performed in laminar flow to prevent entrance of irrelevant microbes.

2.4 Extract Application before Bacterial Growth

Nutrient agar plates were taken and bacterial suspension was applied on petri-dishes evenly before placing extracts dip disc placed at different position on petri dishes. Paper discs were dipped in already prepared aqueous and ethanolic extract of garlic, cinnamon, and onion having the concentration of 100 mg/ml for two minutes and were placed in the culture plates for two days. For negative control group, water and ethanol dipped disc were placed on the culture plates while for positive control penicillin (30µg) was used and results were noticed. The experiment was repeated three times. A precise ruler was used to determine the zone of inhibition as strong (10-20 mm), moderate (5-10 mm) or weak (1-5 mm) based on the diameter of the inhibition zone [16].

2.5 Extract Application after Bacterial Growth

When bacterial growth appeared on culture plates, extract was applied by using disc diffusion method. 8 mm disc was dipped in ethanol and water extract of garlic, cinnamon, and onion having the concentration of 100 mg/ml for 2 minutes and was placed on culture plates of each bacterium. Extracts applied on the bacterial pathogen's samples were placed in incubator for 24 hours. For negative control group, water and ethanol dipped disc were placed on the culture plates while for positive control penicillin (30 µg) was used and results were noticed. The experiment was repeated three times. A precise ruler was used to determine the zone of inhibition as strong (10-20 mm), moderate (5-10 mm) or weak (1-5 mm) based on the diameter of the inhibition zone [16].

2.6 Minimum Inhibitory Concentration (MIC)

The broth dilution technique was used to determine the MIC [17]. The aqueous and ethanolic extracts were diluted in distilled water in different concentrations (66.6 to 0.01 mg/ml). In the test tubes, 500 µl of different concentrations aqueous and ethanolic extracts were introduced in sterile nutritional broth (2 ml) along with the test microorganisms (1 ml) following incubation for 24 hours at 37°C. The minimum inhibitory concentration was determined as the lowest extract concentration that prevented observable growth of each of the test microorganisms.

2.7 Statistical Analysis

All of the tests were repeated three times, with the findings reported as mean ± standard deviation. Data was examined statistically by Statistics 8.1 software using analysis of variance (ANOVA) approach at 5% probability. The least significant difference (LSD) test was used to compare means.

3. RESULTS AND DISCUSSION

Xanthomonas spp. damage most crops and fruits that are economically important. Their control with chemicals is more expensive which may reduce the quality of natural products likewise [1]. Control of these bacteria is very important for protecting crops and plants. Natural plants (cinnamon, onion, garlic) extracts are also used to control the growth of such bacteria. All extracts used in this study successfully controlled the growth of these bacteria.

3.1 Before Inoculum of Bacteria

Results for test bacterial growth inhibition zone before grow this shown in Fig. 1A to C. The penicillin used as a positive control showed significant activity on all the three isolates as compared to the aqueous and ethanolic extracts of all three spices, however, water and ethanol taken as negative control showed zero activity against isolates. Among all three spices, maximum inhibition zone was shown by ethanolic and aqueous extract of cinnamon against *Xanthomonas oryzae pv. oryzae* (7.333 mm and 3.5 mm), *Xanthomonas campestris pv. malvacerum* (7.83 mm and 7.33 mm) and *Xanthomonas axonopodis pv. citri* (5.1 mm and 2.333 mm) as shown in Fig. 1A. Thus, it can be concluded that cinnamon extract was more effective to control the growth than garlic and onion extracts. In comparison to aqueous extract, the cinnamon ethanolic extract has higher antibacterial activity. The reasoning behind this is that the antibacterial substance of cinnamon bark show more solubility in ethanol than in water as suggested in previous studies [18,19].

3.2 After Growth of Bacteria

When bacteria were grown for 24 hours and covered culture plates were treated with all three extracts mentioned above they showed different zone of inhibition as shown in Fig. 2A to 2C.The penicillin used as a positive control showed significant activity on all the three isolates as compared to the aqueous and ethanolic extracts of all three spices, however, water and ethanol taken as negative control showed zero activity against isolates. Maximum growth of *Xanthomonas oryzae* was inhibited by ethanolic (2.82 mm) and aqueous (1.65 mm) extracts of cinnamon as compared to garlic and onion extracts. However, maximum growth of *Xanthomonas campestris* was inhibited by ethanolic extract of onion (6.55 mm) and aqueous extract of garlic (3.75mm) as compared to other extracts. In the case of *Xanthomonas axonopodis* ethanolic extract of cinnamon (3.35 mm) and aqueous extract of garlic (2.50 mm) showed maximum growth inhibition. These results are similar to previous findings conducted in different bacterial species such as *E.coli* [19,20].

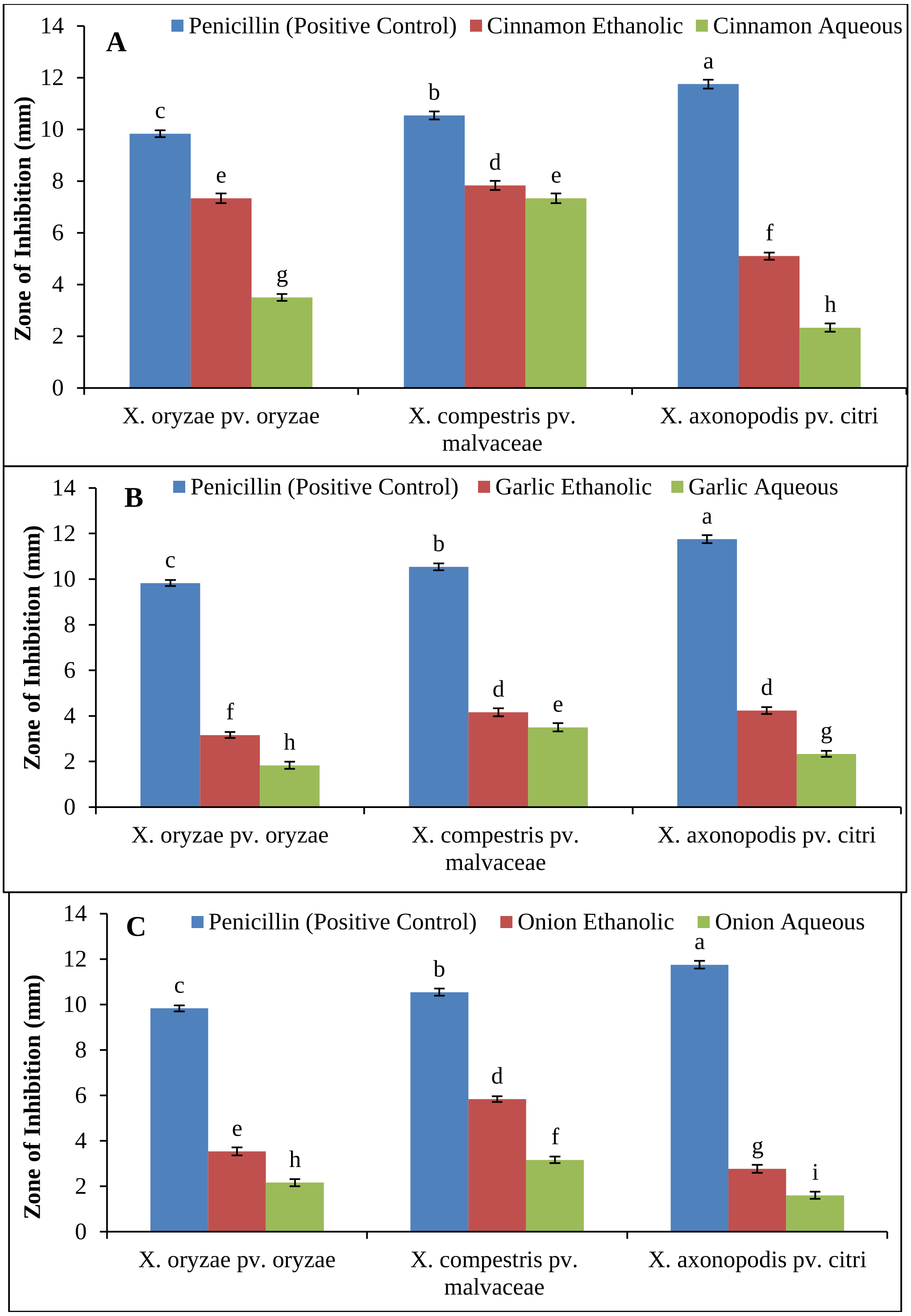

Fig. 1. Zone of inhibition of penicillin (positive control) and aqueous and ethanolic extracts of (A) Cinnamon, (B) Garlic and (C) Onion before growth of *Xanthomonas oryzae pv.*
oryzae, Xanthomonas campestris pv. malvacearum and Xanthomonas axonopodis pv. citri. Values are mean ± Standard Deviation. Letters a, b, c, d, etc. show significantly difference among different extracts

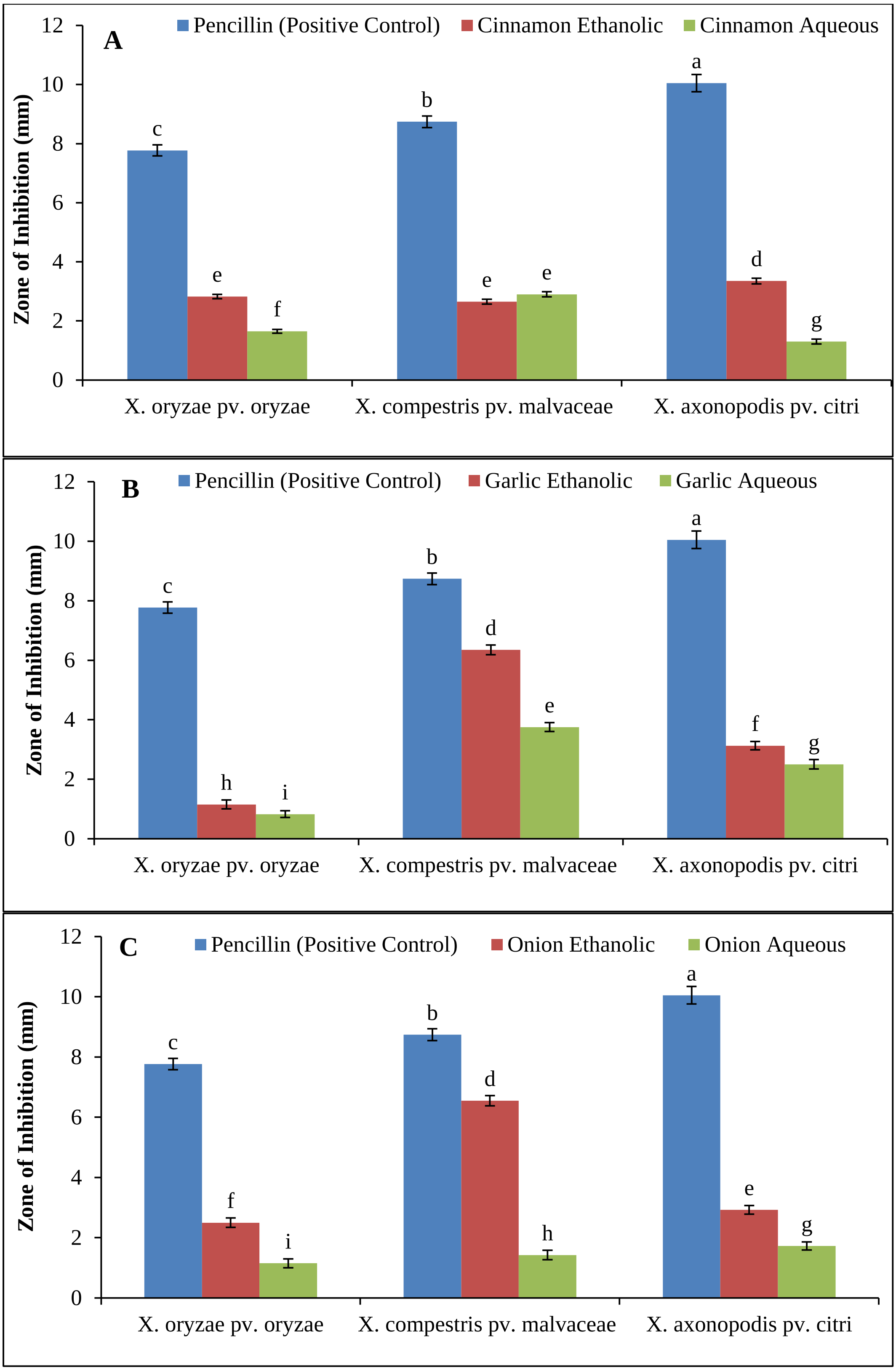

Fig. 2. Zone of inhibition of penicillin (positive control) and aqueous and ethanolic extracts of (A) Cinnamon, (B) Garlic and (C) Onion after growth of *Xanthomonas oryzae pv.*
oryzae, Xanthomonas campestris pv. malvacearum and Xanthomonas axonopodis pv. citri. Values are mean ± Standard Deviation. Letters a, b, c, d, etc. show significantly difference among different extracts

Table 1. MIC for bacterial pathogen

Sr. No.	Plant Name	Extract Type	*Xanthomonas campestris pv. malvacearum*			*Xanthomonas axonopodis pv. citri*			*Xanthomonas oryzae pv. oryzae*		
			Concentration mg/ml			**Concentration mg/ml**			**Concentration mg/ml**		
			66	**33**	**0.01**	**66**	**33**	**0.01**	**66**	**33**	**0.01**
1	Cinnamon	Ethanolic	3.5	1.5	0.0	2	1	0.0	5	2	0.0
		Aqueous	3	1	0.0	1.5	1	0.0	1.5	0.5	0.0
2	Onion	Ethanolic	3	1	0.0	2	0.5	0.0	2	0.5	0.0
		Aqueous	1.5	0.5	0.0	1	0.0	0.0	1	0.0	0.0
3	Garlic	Ethanolic	1.5	1	0.0	3	1	0.0	1.5	0.0	0.0
		Aqueous	1	0.0	0.0	2	0.5	0.0	1	0.0	0.0

3.3 Minimum Inhibitory Concentration (MIC)

As the concentration of extract decreases effectiveness of garlic, cinnamon, and onion also decreases to control growth of bacteria. All extracts used have minimum inhibitory concentration between 66 mg/ml to 33 mg/ml. Ethanolic and aqueous extracts of cinnamon showed maximum MIC against *Xanthomonas campestris pv. malvacearum* between 66 mg/ml to 33 mg/ml ranging from 3.5 mm to 1.5 mm and 3 mm to 1 mm, respectively. For *Xanthomonas axonopodis pv. citri* ethanolic (3 mm to 1 mm) and aqueous (2 mm to 0.5 mm) extracts of garlic showed maximum MIC (Table 1). However, for *Xanthomonas oryzae pv. oryzae* minimum inhibitory concentration was shown by ethanolic (5 mm to 2 mm) and aqueous (1.5 mm to 0.5 mm) extracts of cinnamon. Indu et al. [20] suggested that the effectiveness of garlic extract against different bacterial stereotypes can be due the presence of antibiotic compounds such as nalidixic acid.

The hydrogen and hydrophobic bonding of phenolic compounds with membrane proteins, disruption of the system of electron transport, membrane disintegration, and cell wall disintegration are all aspects of the antibacterial activity of different spices [21]. Anionic components including nitrate, thiocyanate, sulphates, and chlorides, as well as several other chemicals naturally found in plants, might be responsible for the antibacterial action of all these aqueous extracts [22]. When ethanolic extracts were assessed with aqueous extracts, the ethanol extract performed better because they dissolve larger organic molecules, leading to the discharge of more active antimicrobial substances [23].

4. CONCLUSION

In the present study, aqueous and ethanolic extracts of cinnamon, garlic and onion showed significant anti-bacterial activity against different species of *Xanthomonas* including *Xanthomonas campestris pv. malvacearum, Xanthomonas oryzae pv. oryzae, Xanthomonas axonopodis pv. citri.* Thus, it can be concluded that extracts of plants can control the growth of pathogenic bacteria and can be utilized to protect plants against pathogenic attacks in an environment-friendly manner.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle4.com/review-history/74590