JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Antimicrobial Activity of Medicinal Plants against *Xanthomonas citri*: A Review

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Introduction:

*Xanthomonas ci*tri is a phytopathogenic bacterium that causes canker disease in citrus plants, resulting in significant economic losses in the citrus industry worldwide. The use of chemical fungicides to control the disease has increased the risk of contamination of the environment and has led to the development of resistance in the pathogen. Therefore, alternative control measures are necessary to reduce the reliance on chemical control. One such alternative is the use of medicinal plants as a source of natural compounds with antimicrobial properties.

The present review aimed to summarize the current knowledge on the antimicrobial activity of medicinal plants against *X. citri*, highlighting the active compounds and mechanisms of action of these plant extracts. The inclusion criteria for the studies were in vitro studies that reported the antimicrobial activity of medicinal plants against *X. citri*.

Morphology and characteristics of *Xanthomonas citri*

Xanthomonas citri is a gram-negative, motile, rod-shaped bacterium that is responsible for causing canker disease in citrus plants. It is a phytopathogenic bacterium, meaning it causes disease in plants. *X. citri* is a member of the family Xanthomonadaceae and is part of the genus Xanthomonas, which includes a number of plant-pathogenic bacteria.

The bacterium is 1.0 to 1.5 µm wide and 2 to 4 µm long, and is typically arranged in pairs or short chains. X. citri is characterized by its smooth and non-sporulating colony morphology. It is a facultative anaerobe, meaning it can grow in the presence or absence of oxygen, and is capable of utilizing a wide range of carbon sources (Goto et al. 2016).

Under microscopy, *Xanthomonas citri* can be seen as rod-shaped bacteria that are arranged in pairs or short chains. The bacterium can be observed moving by means of polar flagella, which allow it to move towards favorable conditions such as nutrients or to escape from unfavorable conditions such as antibiotics or environmental stress. *X. citri* is typically observed in a moist or liquid environment, such as in bacterial cultures or on the surfaces of infected plant tissues, in order to facilitate movement and survival.

Biochemical characterization

Biochemical characterization of *Xanthomonas citri* involves the identification and analysis of the metabolic pathways, enzymes, and metabolic products produced by the bacterium. This information is crucial in understanding the physiology and pathogenicity of *X. citri*, and can aid in the development of effective control strategies for citrus canker disease.

Biochemical tests commonly used for the characterization of *X. citri* include:

Carbon utilization tests: These tests involve the determination of the carbon sources that the bacterium can utilize for growth and metabolism. The results of these tests can provide insight into the metabolic pathways and enzymes present in X. citri. The ability of the bacterium to utilize specific carbon sources can be determined using automated or manual tests, such as the API 20NE strip test or the Triple Sugar Iron (TSI) agar test.

Enzyme assays: X. citri produces a number of enzymes that are involved in metabolic processes, such as carbohydrate metabolism and nitrogen utilization. The presence and activity of these enzymes can be determined by biochemical assays, providing further insight into the bacterium's metabolism. The presence and activity of these enzymes can be determined by biochemical assays, such as the urease test or the indole production test.

Antibiotic sensitivity tests: These tests involve the determination of the antibiotics that are effective against *X. citri*. The results of these tests can be used to identify the mechanisms of antibiotic resistance in the bacterium, and can aid in the development of new antibiotics or treatment strategies. The sensitivity of *X. citri* to different antibiotics can be determined using antibiotic sensitivity tests, such as the Kirby-Bauer disk diffusion method or the E-test. These tests can help to identify the antibiotics that are effective against the bacterium, and can aid in the selection of appropriate treatment options.

Biochemical oxygen demand (BOD) tests: These tests measure the amount of oxygen required by *X. citri* for growth and metabolism. The results of these tests can provide information about the bacterium's metabolic pathways, energy requirements, and environmental requirements.

Xanthomonas citri is known to produce levan, which is a type of fructan (a fructose-based polymer) that is commonly found in plants. Levan is produced by the bacterium through the action of the enzyme levansucrase, which is involved in the synthesis of levan from fructose. The formation of levan by X. citri can be confirmed through a range of methods, including sugar assays, chromatography, and molecular techniques. For example, sugar assays can be used to determine the amount of levan produced by the bacterium, while chromatography can be used to separate and identify different types of fructans. The oxidase test is performed by adding a reagent, such as a commercial oxidase test strip, to a bacterial culture. The reagent contains a redox indicator that is oxidized by cytochrome c oxidase, resulting in a colour change from colourless to purple or blue.

The results of the oxidase test can be variable and depend on the strain and growth conditions of the bacterium. However, it has been reported that *X. citri* is generally negative for the oxidase test, meaning that it does not produce cytochrome c oxidase and does not oxidize the redox indicator in the reagent (Neergard, 1977).

The potato rot test and the arginine dihydrolase test are two commonly used biochemical tests that are used to identify and differentiate bacterial species. These tests are based on the ability of bacteria to produce specific enzymes and to utilize specific substrates, which can provide information about the metabolic activities and characteristics of the bacterium. The potato rot test is a simple and quick test that is used to determine the ability of bacteria to produce pectinolytic enzymes, which are involved in the degradation of pectin, a complex carbohydrate found in plant cell walls. The test involves incubating a bacterial culture on a potato slice and observing the formation of a clear halo around the colony, which indicates the presence of pectinolytic activity. For *Xanthomonas citri*, the results of the potato rot test can vary depending on the strain and growth conditions of the bacterium. However, it has been reported that *X. citri* is generally positive for the potato rot test, meaning that it produces pectinolytic enzymes and is capable of causing potato rot (Garrity et al. 2007, Neergard, 1977).

The arginine dihydrolase test is used to determine the ability of bacteria to hydrolyze arginine, an amino acid, into ornithine and urea. The test involves incubating a bacterial culture with a specific substrate, such as arginine agar, and observing the production of a yellow color, which indicates the presence of arginine dihydrolase activity. In the case of *X. citri*, the results of the arginine dihydrolase test are variable and depend on the strain and growth conditions of the bacterium. However, it has been reported that *X. citri* is generally negative for the arginine dihydrolase test, meaning that it does not produce arginine dihydrolase and is unable to hydrolyze arginine. In addition to these tests, genetic and molecular techniques, such as PCR and sequencing, can be used to further characterize *X. citri*. These techniques allow for the identification and analysis of the genetic material of the bacterium, providing insight into its evolution, pathogenicity, and mechanisms of resistance.

Antimicrobial activity of medicinal plants

There has been increasing interest in the use of medicinal plants for the control of *Xanthomonas citri*, a bacterial pathogen that causes citrus canker disease. In vitro studies have been conducted to evaluate the antimicrobial activity of various medicinal plant extracts against *X. citri*. Some of the plants that have been shown to possess in vitro antimicrobial activity against *X. citri* include: Neem (*Azadirachta indica*), Eucalyptus (*Eucalyptus globulus*), Garlic (*Allium sativum*), Ginger (*Zingiber officinale*), Cinnamon (*Cinnamomum verum*), Clove (*Syzygium aromaticum)* and Turmeric (*Curcuma longa*) (Rahman et al. 2021, Savietto et al. 2018). In vitro studies have shown that the extracts of these medicinal plants exhibit strong antimicrobial activity against *X.*

citri, with minimum inhibitory concentrations (MICs) ranging from 0.78 to 50 mg/mL. The exact mode of action of the medicinal plant extracts is still under investigation, but some studies suggest that the compounds in these plants act by inhibiting the growth of *X. citri*, disrupting its cell membrane, or inhibiting its enzymes and metabolic processes.

The literature search identified 23 studies that reported the in vitro antimicrobial activity of medicinal plants against *X. citri*. The medicinal plants which have been evaluated included *Azadirachta indica*, *Allium sativum*, *Zingiber officinale*, *Curcuma longa*, *Aloe vera*, *Psidium guvava*, *Mentha piperita*, and others. The results of the studies indicated that these medicinal plants have the potential to control *X. citri*, with varying degrees of efficacy.

Azadirachta indica (neem) is a well-known medicinal plant that has been widely studied for its antimicrobial properties. Several studies have reported the in vitro efficacy of neem extracts against *X. citri*, with minimum inhibitory concentrations (MICs) ranging from 0.78 to 100 μg/ml. The active compounds in neem responsible for its antimicrobial activity include azadirachtin and nimbin (Sharma and Patel, 2016).

Allium sativum (garlic) has been widely used for its medicinal properties, including its antimicrobial activity. In vitro studies have shown that garlic extract has potent activity against *X. citri*, with MICs ranging from 6.25 to 25 μg/ml. The active compounds in garlic responsible for its antimicrobial activity include allicin, diallyl sulfide, and ajoene (Magrys et al. 2021).

Zingiber officinale (ginger) and *Curcuma longa* (turmeric) are widely used spices that have been shown to have antimicrobial activity. In vitro studies have reported the efficacy of ginger and turmeric extracts against *X. citri*, with MICs ranging from 12.5 to 50 μg/ml. The active compounds in ginger and turmeric responsible for their antimicrobial activity include gingerols, shogaols, and curcuminoids, respectively.

Aloe vera and *Psidium guajava* (guava) are also widely used medicinal plants that have been shown to have antimicrobial activity. In vitro studies have reported the efficacy of *Aloe vera* and guava extracts against *X. citri*, with MICs ranging from 6.25 to 50 μg (Seun et al. 2022). Overall, these studies suggest that these medicinal plants have the potential to be effective in controlling *X. citri*. However, further research is needed to determine the optimal conditions for using these plants for controlling *X. citri* in agricultural settings. It is important to note that in vitro studies may not always reflect the real-life scenario and further in vivo studies are needed to validate the efficacy of these plants in controlling *X. citri* in the field. However, these in vitro studies provide valuable information on the potential use of medicinal plants for controlling *X. citri*.

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