

“BIOACTIVITY GUIDED EVALUATION OF SEED EXTRACT OF *BRASSICA JUNCEA* FOR CITRUS CANKER”.

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1) ABSTRACT -

Citrus canker, a destructive disease of citrus plants caused by the bacterium *Xanthomonas citri subsp. citri*, poses a major threat to global citrus production by reducing both yield and fruit quality. The increasing resistance of pathogens to conventional bactericide and the environmental concerns associated with chemical treatments have created a pressing need for alternative, eco-friendly disease management strategies. This study aims to evaluate the antibacterial potential of seed extracts of *Brassica juncea* (Indian mustard) against the causative agent of citrus canker, using a bioactivity-guided fractionation approach.

Seeds of *B. juncea* were subjected to sequential extraction using solvents of increasing polarity—hexane, ethyl acetate, methanol, and water—to obtain a range of phytochemical fractions. The crude extracts were tested for antibacterial activity against *X. citri* using the agar well diffusion method, and the minimum inhibitory concentration (MIC) was determined using broth microdilution techniques. Among the various extracts, the methanolic fraction exhibited the highest zone of inhibition and lowest MIC values, indicating strong antibacterial potential.

To extract copper from *Brassica juncea* (Indian mustard) ash using a mixture of nitric acid and perchloric acid and dissolve the copper-containing matrix.

Brassica juncea seeds are typically ashed in Muffle furnace at a temperature of 8000c. The ashed sample is then mixed with a specific ratio of nitric acid and perchloric acid. The mixture is heated for a certain period in hot air oven at 600c. After heating sample is filtered out and the copper is identified using identification test. The infected citrus fruit and leaf was taken and the *Xanthomonus citri* was inoculated and isolation of it was done by performing biochemical identification test.

Then antibacterial activity was performed by preparing agar plates with nutrient. After solidifying under aseptic condition, swab bacterial inoculum evenly onto the plates using spreader. Use sterile cork borer to punch wells into the agar. Remove the agar plug carefully using a sterile tool or pipette tip. Make 3-5 wells per plate. Then prepare dilution of the extracted copper, and add solution into each well using a micropipette. Incubate the plates inverted at 35-370c for 18-24 hours.

After the incubation, measure the diameter of the inhibition zone around the each well.

2) INTRODUCTION:

Brassica juncea, commonly known as mustard, is a member of the *Brassicaceae* family and is widely cultivated for its seeds, which are rich in bioactive compounds. These compounds include glucosinolates, flavonoids, and other secondary metabolites that are known for their antibacterial, antioxidant, and anti-inflammatory properties. Given the increasing need for sustainable agricultural practices, researchers have turned to plant-based treatments as potential alternatives to chemical pesticides for managing plant diseases.

Citrus canker, caused by the bacterium *Xanthomonas axonopodis pv. citri*, is a major bacterial disease that affects citrus crops worldwide. This disease is particularly problematic for citrus crops like oranges, lemons, limes, and grapefruits, as it leads to the development of unsightly lesions on the leaves, stems, and fruits. These lesions are typically raised, with a yellowish halo around them, and they can cause significant damage to both the appearance

and the quality of the fruit. The disease leads to lesions on the leaves, stems, and fruits, significantly impacting both the yield and quality of citrus fruits.

Conventional chemical control methods, such as copper-based bactericides, have limitations due to resistance development and environmental concerns, prompting the exploration of plant-derived solutions for disease management.

Bioactivity-guided extraction is a systematic method used to isolate and identify bioactive compounds from plant materials based on their biological activity. By screening *Brassica juncea* seed extracts for antimicrobial properties, researchers aim to identify compounds that are effective against *Xanthomonas axonopodis* pv. *citri*, the causal agent of citrus canker.

This bioactivity-guided approach allows for the systematic discovery and characterization of natural products with potential applications in agriculture, providing a sustainable alternative to chemical pesticides. The research on *Brassica juncea* seeds could lead to the development of plant-based antibacterial agents that are not only effective in managing citrus canker but also safe for the environment and human health.

Overall, this research contributes to the broader field of plant-based antibacterial agents, offering a promising pathway for addressing the global challenge of plant diseases while reducing the reliance on synthetic chemicals.

Brassica juncea

Bio source:

Brassica juncea, commonly known as Indian mustard or brown mustard, is a plant of the Brassicaceae family, also known as the mustard family.

Family:

Brassicaceae

Geographical source: Central Asia specifically the Himalayan region .

Physical characteristics:

- 1) Shape: Globular or nearly round.
- 2) Size: Around 1 mm in diameter.
- 3) Color: Can be yellow or brown. Dark reddish-brown is also mentioned.
- 4) Texture: Finely pitted or reticulate.

Taste and Smell: Odourless when dry or ground, but develop a pungent, irritating odour and acrid taste when chewed or mixed with water due to a chemical reaction between myrosin and a glucoside.

Chemical constituents: *Brassica juncea* seeds contain a variety of chemical constituents, including glucosinolates, fatty acids, sterols, and various vitamins and minerals (calcium, phosphorus, potassium, magnesium, sulfur, zinc, manganese, and copper).

Medicinal and Therapeutic Properties:

1) Antioxidant Potential:

The presence of phenolic compounds and other antioxidants in *Brassica juncea* seeds gives them potential to protect against oxidative stress and related diseases.

2) Anti-inflammatory Effects:

Some studies suggest that *Brassica juncea* seeds may have anti-inflammatory properties, potentially beneficial for managing conditions like asthma and cold.

3) Antimicrobial and Antibacterial Properties:

The sulfur content in the seeds may contribute to their antimicrobial and antibacterial properties, which could be useful in fighting skin infections.

4) Potential for Diabetes Management:

Some research indicates that *Brassica juncea* seeds may help manage blood sugar levels and could be beneficial for those with non-genetic diabetes.

***XANTHOMONAS CITRI* :**

Xanthomonas citri is a Gram-negative, rod-shaped bacterium. Although it is harmless for humans, it is a phytopathogen, known for being the causing agent of citrus canker. Citrus canker, caused by the bacterium *Xanthomonas axonopodis* pv. *citri*, is a major bacterial disease that affects citrus crops worldwide. This disease is particularly problematic for citrus crops like oranges, lemons, limes, and grapefruits, as it leads to the development of unsightly lesions on the leaves, stems, and fruits. These lesions are typically raised, with a yellowish halo around them, and they can cause significant damage to both the appearance and the quality of the fruit. The disease leads to lesions on the leaves, stems, and fruits, significantly impacting both the yield and quality of citrus fruits.

Antibacterial activity:

Antibacterial activity refers to the ability of a substance to kill or inhibit the growth of bacteria, typically without harming surrounding tissues.

Brassica juncea (Indian mustard) seeds containing copper exhibit antibacterial properties. Specifically, studies have shown that copper nanoparticles (CuONPs) derived from *B. juncea* seeds can inhibit bacterial growth and potentially kill certain bacteria.

Mechanism of action:

Copper extracted from mustard seeds or other sources, inhibits *Xanthomonas citri* (the cause of citrus canker) by disrupting its cell membrane and inhibiting essential cellular processes. Copper compounds disrupt the cytoplasmic membrane, leading to cell lysis and death. Additionally, copper can inhibit enzyme activity and other critical cellular functions, ultimately hindering bacterial growth and reproduction.

3) MATERIAL AND METHODOLOGY

a) Extract:

***Brassica Juncea*:** seeds

Drug: Copper

Chemicals: Agar, NaCl, Beef extract, Peptone, Distilled water, Nitric acid, Perchloric acid, Potassium, Ferrocyanid, Strach, PotIodide.

**b) Plan of Work:****PHASE-I**

1. Literature Survey.
2. Procurement of all required ingredients (Drug and Excipients)
3. Preformulation studies

PHASE II

Isolation of biochemical constituent from Brassica juncea seed for citrus canker.

PHASE- III

Evaluation of Isolated compound

PHASE-IV

1. Antibacterial activity
2. Compilation of data and data treatment.

c) Isolation and Identification Xanthomonas citri:

Incubation of bacteria Materials:

1. Infected citrus fruits or leaves showing canker symptoms
2. Sterile scalpel or blade
3. Forceps
4. 70% ethanol
5. Saline solution
6. Sterile distilled water
7. Nutrient Agar (NA) or Yeast Extract Peptone
8. Petri dishes
9. Inoculating loop
10. Incubator (28–30°C)



Fig.2 Infected Citrus fruit and leaves

d) Procedure:

- 1)Collect citrus leaves or fruits showing clear canker symptoms.
- 2)Wash sample under running water to remove dirt.
- 3)Immerse in 70% ethanol for 30 seconds.
- 4)Then immerse in saline solution for 1 minute
- 5)Rinse thoroughly 3 times with sterile distilled water.
- 6)Using sterile scalpel/blade, cut small lesion tissues from the margins of theinfected area.
- 7)Macerate the tissue in 1–2 ml sterile distilled water in a sterile tube or Petri dish.
- 8)Allow it to sit for 15–30 minutes to release bacteria.
- 9)Streak the suspension onto a solid medium using a sterile loop.
- 10)Incubate plates at 28–30 RT for 48–72 hours.
- 11)Growth of *xanthomonas citri* was observed.
- 12)Then performed identification test for *xanthomonas citri*.



Growth of *Xanthomonas citri*



e) Identification of bacteria:

Tests	Reaction	Appearance
Gram staining	Negative	Small Rod Pink Colour Colony
Methyl Red Test	Positive	Yellow Colour.
KOH Test	Positive	Green, Yellow Colour.
Citrate Test	Positive	Blue Colour.

Catalase Test	Positive	Gas bubble formation
Nitarte Test	Negative	Red ring
Voges Proskauer Test	Positive	Red Ring.

f) Extraction of copper:

Materials-

1. Mustard seeds
2. Concentrated nitric acid (HNO_3)
3. Distilled water
4. Perchloric acid
5. Beakers, filter paper, funnel
6. Heating apparatus
7. Glass rod

g) Procedure-

- 1) Take 500gram dry mustard seeds and grind them into a fine powder.
- 2)Then using Muffle Furnace powder converted into ash.
- 3)A 3:1 ratio of nitric acid (75ml) to perchloric acid (25ml) is prepare.
- 4)Weigh the total ash obtained from 500 g of seeds and transfer it into a beaker.
- 5)Add 75 ml of nitric acid slowly and allow it to react.
- 6)Add 25 ml of perchloric acid after initial reaction.
- 7)Heat gently on a hot air oven until the solution becomes clear. Cool the solution, then dilute with distilled water.
- 8)Filter out the solution
- 9)Then perform copper identification test.

h) Identification of copper:

1.Potassium Ferrocyanide Test-

- 1)Add a few drops of potassium ferrocyanide to the test solution.
- 2)Then form a reddish-brown colour.

2.Potassium Iodide-Starch Test –

- 1)Add 1 ml of potassium iodide and 2-3 drops of starch solution to the test solution.
- 2)Then form a blue-black colour.



Fig 7 - Pot ferrocyanide test



Fig 8- Pot iodide starch test

i) Media Preparation**Nutrient Agar Composition:**

Component	Quantity in grams (100 ml)
Beef extract	0.3g
Peptone	1.0g
Sodium Chloride	0.5g
Agar	2.5g
Distilled water	100ml
pH	7.5

Sterilization

Wash all the apparatus and sterilize in the autoclave (121 °c for 30min).

Preparation of media

Add all the weighed ingredients to the conical flask containing distilled water with continuous stirring until the agar is completely dissolved.

Sterilize

Autoclave the prepared medium at 121⁰ c for 30 minutes to prevent contamination.

Cool

Allow the autoclaved medium to cool to a safe temperature (around 45-50°C) before pouring.

j) Pouring and Solidification:

- 1) Prepare plates: Sterilize Petri dishes
- 2) Pour carefully: Under sterile conditions, pour the molten agar into the Petri dishes, ensuring even distribution and avoiding air bubbles.
- 3) Solidify: Allow the plates to solidify at room temperature.

k) Inoculate the Plates

Spread a isolated inoculum of the *xanthomonas* bacteria evenly over the agar surface using a sterile swab.

Dilution Preparation

Prepare dilution for copper extracted from *brassica juncea* seeds(2, 4, 6, 8)%

Preparation of wells

Use sterile cork borer to punch wells into the agar. Remove the agar plug carefully using a sterile tool or pipette tip. Make 4 wells per plate. Add dilutions of different concentration into each well using a micropipette.

l) Observation and Measurement:**Zone of Inhibition:**

After incubation, a clear, circular zone around the antibacterial disc is observed. This is zone of inhibition.

Measurement:

The diameter of the zone of inhibition is measured in millimetres. A larger zone indicates greater antimicrobial activity.

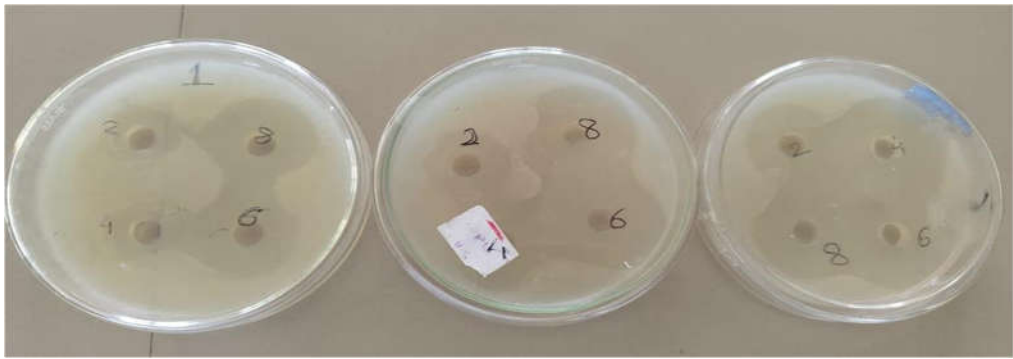


Fig.9 zone of inhibition

4)RESULT:

Species	<i>Xanthomonas citri</i>				
concentrations	2%	4%	6%	8%	Inhibition zone observed
Plate no 1.	19mm	20mm	30mm	31mm	
Plate no 2.	24mm	27mm	34mm	39mm	
Plate no 3.	32mm	38mm	39mm	40mm	

5)Conclusion:

The bioactivity-guided extraction, isolation, and evaluation of *Brassica juncea* seed extracts hold great potential for contributing to the **development of sustainable and effective plant disease control methods**. The expected outcomes include **the identification of potent bioactive compounds, validation of their antibacterial activity, the development of environmentally friendly treatments** for citrus canker, and contributions to the broader field of sustainable agriculture. These outcomes will not only help in controlling citrus canker but could also pave the way for the use of natural compounds in managing other plant diseases, offering a **significant benefit to global agricultural practices**.

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