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Research Article

Antimicrobial Potential of Mangroves: A Phytochemical Exploration

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Abstract

This study explores the antimicrobial potential of two mangrove species, *Avicennia marina* and *Excoecaria agallocha,* collected from Mumbai's coastal regions. Leaf extracts were prepared using cold maceration with methanol and ethyl acetate. Phytochemical screening revealed compounds such as flavonoids, tannins, and alkaloids. Antibacterial activity was tested against Xanthomonas campestris and Staphylococcus xylosus using the agar well diffusion method. Methanolic extracts, particularly of A. marina, showed significant inhibition zones, suggesting strong antibacterial activity. These findings highlight the potential of mangrove plants as eco-friendly antimicrobial agents for sustainable crop protection.

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KEYWORDS: Mangroves, antimicrobial properties, Phytochemical diversity, Therapeutic Potential, *Avicennia marina*, *Excoecaria agallocha*

1. INTRODUCTION

Background: Mangrove ecosystems host plant species adapted to saline, anaerobic conditions, often producing unique secondary metabolites. Among these, *A. marina* and *E. agallocha* have shown promising antimicrobial activity. Their use aligns with sustainable agricultural practices and offers alternatives to synthetic pesticides amid growing resistance in crop pathogens.

Significance: This research supports sustainable agriculture by evaluating mangrove-based antimicrobials as eco-friendly alternatives to synthetic pesticides. The use of natural extracts from *Avicennia marina* and *Excoecaria agallocha* may help

control crop pathogens while reducing environmental impact. Highlighting the agricultural value of mangroves also reinforces the need to conserve these ecosystems in urban coastal areas, preserving biodiversity and promoting ecological resilience.

2. OBJECTIVES

1. To collect, identify, and extract bioactive compounds from *A. marina* and *E. agallocha*.
2. To screen for phytochemicals and evaluate antibacterial efficacy against plant pathogens.
3. To compare antimicrobial activity between solvents and plant species.

3. MATERIALS AND METHODS

Plant Collection

- *A. marina* – Gorai Beach, Mumbai
- *E. agallocha* – Bhayandar Khadi, Thane

Pathogen Strain Procurement

Two authenticated phytopathogenic strains were selected for antimicrobial testing due to their relevance to mango and cashew crop diseases:

- *Xanthomonas campestris* and *Staphylococcus xylosus*

Standard laboratory strains were directly procured from culture collections, ensuring consistency and avoiding contamination risks associated with field samples.

Preparation for Antimicrobial Testing

Media Preparation:

- For bacteria: Nutrient Agar (Peptone 1g, Yeast Extract 0.3g, NaCl 0.5g, Agar 2.5g; pH 7.0–7.2)
- For fungi (if tested): Potato Dextrose Agar (Potato 20g, Glucose 1g, Agar 2.5g)

Inoculum: Revived strains were cultured under sterile conditions. As lab strains were used, no surface sterilization of infected samples was necessary.

Antimicrobial Assay

The agar well diffusion method was employed using sterile nutrient agar plates. Extracts (methanolic and ethyl acetate) were dissolved in DMSO and dispensed into 6 mm wells. The plates were incubated at 37°C for 24 hours.

Controls Used:

- Streptocycline as the antibacterial standard
- Diathane for antifungal reference (if fungal assays conducted)

Assessment

Zone of inhibition was measured in millimeters.

MIC (Minimum Inhibitory Concentration) Setup

MIC was determined using broth dilution in sterile test tubes:

Setup:

- Serial dilutions of plant extracts
- Inoculated with overnight broth cultures
- Incubated at 37°C for 18–24 hours

Controls:

- Streptocycline for bacterial strains
- Diathane for fungal strains

The MIC was identified as the lowest concentration with no visible turbidity.

Phytochemical Screening

Qualitative chemical tests were conducted on all crude extracts to detect secondary metabolites.

Key reagents included

Alkaloids: 1% HCl, Mayer's reagent
Flavonoids: 10% ammonia solution
Tannins and Phenols: Ferric chloride reagent
Saponins: Foam test
Steroids & Terpenoids: Chloroform, sulfuric acid, potassium hydroxide
Others: Acetone, glacial acetic acid
All tests followed standard phytochemical protocols and were performed in triplicate.

Collection of Plant Material

A) **Mangrove collection:** The extraction and characterization of active compounds from medicinal plants have resulted in the discovery of new drugs with high therapeutic value. Mangrove plants and mangrove associate species *Avicennia marina* & E. agallocha were collected from Gorai Creek area Mumbai & Bhayandar Khadi, Thane Respectively.

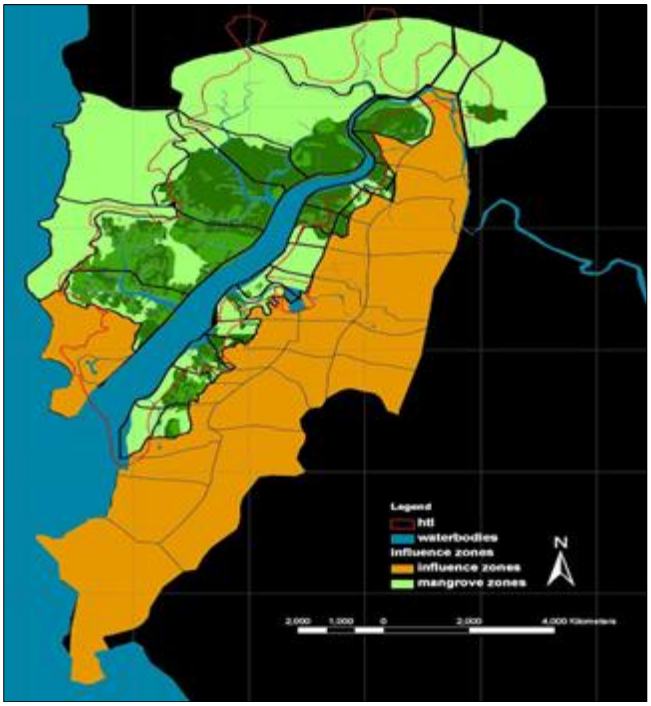


Fig 1: Mangrove collection

Collected plant leaves of Mangrove leaves
Avicennia marina

- **Kingdom:** Plantae
- **Subkingdom:** Tracheobionta – Vascular plants
- **Superdivision:** Spermatophyta – Seed plants
- **Division:** Magnoliophyta – Flowering plants
- **Class:** Magnoliopsida – Dicotyledons
- **Subclass:** Asteridae
- **Order:** Lamiales
- **Family:** Verbenaceae – Verbena family
- **Genus:** *Avicennia*

Species: *Avicennia marina*

Excoecaria agallocha

- **Kingdom:** Plantae
- **Subkingdom:** Tracheobionta – Vascular plants
- **Superdivision:** Spermatophyta – Seed plants
- **Division:** Magnoliophyta – Flowering plants
- **Class:** Magnoliopsida – Dicotyledons
- **Subclass:** Euphorbiidae
- **Order:** Euphorbiales
- **Family:** Euphorbiaceae – Spurge family
- **Genus:** *Excoecaria*
- **Species:** *Excoecaria agallocha*

B) Host Pathogen Selection
Two authenticated phytopathogenic strains were selected for antimicrobial testing due to their relevance to mango and cashew crop diseases:

- *Xanthomonas campestris* and *Staphylococcus xylosus* Standard laboratory strains were directly procured from culture collections, ensuring consistency and avoiding contamination risks associated with field samples.

C) Extraction Method: Cold Maceration
Plant materials (leaves) from *Avicennia marina* and *Excoecaria agallocha* were collected, washed, shade-dried, and powdered. Ten grams of each powder were soaked in 100 mL of either methanol (polar) or ethyl acetate (moderately polar) for 72 hours at room temperature with intermittent shaking. The extracts were filtered and concentrated using a water bath maintained below 50°C. Dried residues were stored at 4°C until use.

Pathogen Selection and Revival
Two authenticated bacterial strains were used:

- *Xanthomonas campestris* (NCIM 5028)
- *Staphylococcus xylosus* (MTCC 7441)

Strains were revived in nutrient broth and maintained on Nutrient Agar. These strains are relevant to mango and cashew crop diseases.

Antimicrobial Assay: Agar Well Diffusion Method
Media Composition:

- **Nutrient Agar (NA):** Peptone 1g, Yeast extract 0.3g, NaCl 0.5g, Agar 2.5g per 100 mL; pH adjusted to 7.0–7.2
- Sterilized by autoclaving at 121°C for 15 minutes.

Inoculum Preparation:

- A 0.5 McFarland turbidity standard (~1–2 × 10^8 CFU/mL) was used.
- Bacterial lawns were prepared on NA plates using sterile swabs.

Well Preparation and Extract Application:

- Wells (6 mm diameter) were punched into solidified agar.
- 50 µL of plant extract (25 mg/mL in DMSO) was loaded into each well.
- Plates were pre-diffused at 4°C for 15 minutes, then incubated at 37°C for 24 hours.

Controls Used:

- **Positive control:** Streptocycline (10 µg/mL)
- **Negative control:** Solvent blanks (methanol or ethyl acetate)

Reading and Interpretation:

- Zones of inhibition were measured in millimeters.
- All tests were performed in triplicate. Results were recorded as mean ± SD.

Minimum Inhibitory Concentration (MIC) – Broth Dilution Method

Although optional, MIC was determined to quantify antimicrobial potency.

Procedure:

- Serial dilutions of plant extracts were prepared in nutrient broth.
  0.1 mL of test bacteria was inoculated into each dilution.
- Tubes were incubated at 37°C for 18 hours.
- The lowest concentration showing no turbidity was recorded as MIC.
- A tube incubated at 4°C overnight served as a negative growth control.

Observations

1. **Collection and Identification of Plant Species**
   Mangrove species *Avicennia marina* and *Excoecaria agallocha* were collected from Gorai Beach and Bhayandar Khadi respectively. Identification was performed using standard taxonomic keys and verified through comparison with herbarium specimens and published flora.
3. **Extraction of Leaves Cold Maceration (*Avicennia marina* & *Excoecaria agallocha* – Current Study):**
   Ten grams of dried leaf powder were soaked in 100 mL of methanol or ethyl acetate at room temperature for 72 hours. After filtration and evaporation (below 50°C), semi-solid extracts were stored in sterile containers at 4°C.
4. **Pathogen Sources**
   Standard Culture Procurement): Two authenticated bacterial strains were used:

- *Xanthomonas campestris* (NCIM 5028) and *Staphylococcus xylosus* (MTCC 7441)
  Strains were revived in nutrient broth and maintained on Nutrient Agar. These strains are relevant to mango and cashew crop diseases.These were revived and maintained under lab conditions on Nutrient Agar and used directly in antimicrobial assays.

4. **Phytochemical Screening**
   All extracts underwent qualitative screening using standard reagents to detect alkaloids, flavonoids, tannins, phenols, terpenoids, and saponins. Methanolic extracts showed a richer phytochemical profile, especially in *Avicennia marina*.

Table 1: Phytochemical Screening

Phyto-chemicals	*A marina* Leaves (Methanol)	*A marina* Leaves (Ethyl Acetate)	*E. agallocha* Leaves (Methanol)	*E. agallocha* Leaves (Ethyl Acetate)
Alkaloids	+	+	+	+
Flavonoids	+	+	+	+
Tannins	+	–	+	–
Saponins	+	–	–	–
Phenols	+	+	+	+
Terpenoids	+	+	+	+
Steroids	+	–	+	–

4. Antimicrobial Testing

The agar well diffusion method was used to assess antimicrobial efficacy. Extracts at 25 mg/mL were loaded into 6 mm wells on inoculated plates. Inhibition zones were measured after 24-hour incubation at 37°C.

- Methanolic extract of *Avicennia marina* showed the highest activity.
- *Excoecaria agallocha* extracts showed moderate inhibition.
- Streptocycline served as a positive control; DMSO was used as solvent control.

Observed Zones of Inhibition

The zones of inhibition (in mm) were measured for each extract against both test organisms. Streptocycline was used as the positive control, while respective solvents (methanol or ethyl acetate) served as negative controls to ensure that observed activity was due to the plant extract alone.

Table 2: Zone of inhibition (in mm) of mangrove leaf extracts against Xanthomonas campestris and Staphylococcus xylosus

Plant Species	Solvent	Test Organism	Zone of Inhibition (mm)
A. marina	Methanol	*X campestris*	18.2 ± 0.5
A. marina	Methanol	*S. xylosus*	17.0 ± 0.6
A. marina	Ethyl Acetate	*X.campestris*	14.6 ± 0.8
A. marina	Ethyl Acetate	*S. xylosus*	14.2 ± 0.7
E. agallocha	Methanol	*X.campestris*	16.5 ± 0.4
E. agallocha	Methanol	*S. xylosus*	16.8 ± 0.5
E. agallocha	Ethyl Acetate	*X. campestris*	13.5 ± 0.4
E. agallocha	Ethyl Acetate	*S. xylosus*	13.5 ± 0.5
Positive Control	Streptocycline	*X. campestris*	22.0 ± 0.3
Positive Control	Streptocycline	*S. xylosus*	20.0 ± 0.4
Negative Control	Methanol	*X.campestris*	0 mm
Negative Control	Ethyl Acetate	*S. xylosus*	0 mm

Observation for MIC Values

Minimum Inhibitory Concentration (MIC) testing was conducted only for *Avicennia marina*, as it demonstrated greater antimicrobial activity in preliminary well diffusion assays compared to *Excoecaria agallocha*. This selective approach allowed for focused quantitative analysis on the more promising species.

MIC of Streptocycline and Dithane

Table 3: MIC (Minimum Inhibitory Concentration) of Antibiotics

Name of Organism	Streptocycline (µg/ml)	Dithane (µg/ml)
Xanthomonas campestris	0.78 – 1.56	NT
Bacillus boroniphillus	1.56 – 3.12	NT

MIC values of both Ethyl Acetate and Methanolic extracts of *Avicennia marina* and for the pathogens listed:

Table 4: MIC values (mg/mL) of *Avicennia marina* extracts against phytopathogenic bacteria

Avicennia marina	Organism name	MIC
MIC of Ethyl Acetate Extracts (mg/ml)	Xanthomonas campestris	1.56 - 3.12
MIC of Methanolic Extracts (mg/ml)	Bacillus boroniphillus	6.25 - 12.5

5. RESULTS AND DISCUSSION

1. Collection and Identification of Mangrove Plants

- **Plant Species:** *Avicennia marina*, *Excoecaria agallocha*
- Collected from Gorai Beach and Bhayandar Khadi, both species were identified using standard botanical taxonomic keys.

2. Extraction of Mangrove Leaves

- **Solvents Used:** Methanol and Ethyl Acetate
- **Method:** Cold maceration was employed. Powdered leaves were soaked for 72 hours, filtered, evaporated below 50°C, and stored at 4°C.

3. Pathogen Selection and Revival

- Only two authenticated phytopathogenic bacterial strains were used:
  - *Xanthomonas campestris* (NCIM 5028
  - *Staphylococcus xylosus* (MTCC 7441)
- Both strains were revived and maintained on nutrient agar. No field-infected samples or fungal strains were included in this study.

4. Antimicrobial Activity of Mangrove Extracts

4.1 Positive Control Activity

- **Streptocycline** showed strong antibacterial activity at 50 µg/mL, with inhibition zones of:
  - *X. campestris:* ~22 mm
  - *S. xylosus:* ~20 mm

4.2 Activity of *Avicennia marina* Extracts

Table 5: Antibacterial activity of *Avicennia marina* extracts measured by inhibition zone diameter

Solvent	Pathogen	Zone of Inhibition (mm)
Methanol	*X. campestris*	18.2 ± 0.5
Methanol	*S. xylosus*	17.0 ± 0.6
Ethyl Acetate	*X. campestris*	14.6 ± 0.8
Ethyl Acetate	*S. xylosus*	14.2 ± 0.7

- Methanolic extract of *A. marina* showed the highest activity.

4.3 Activity of *Excoecaria agallocha* Extracts

Table 6: Antibacterial activity of *Excoecaria agallocha* extracts against selected bacterial strains.

Methanol	*Xanthomonas campestris*	16.5 ± 0.4
Methanol	*Staphylococcus xylosus*	16.8 ± 0.5
Ethyl Acetate	*Xanthomonas campestris*	13.5 ± 0.4
Ethyl Acetate	*Staphylococcus xylosus*	13.5 ± 0.5

- Methanolic extract of *Excoecaria agallocha* showed the highest activity.

5. MIC (Minimum Inhibitory Concentration) Analysis

5.1 MIC of Streptocycline (Positive Control)

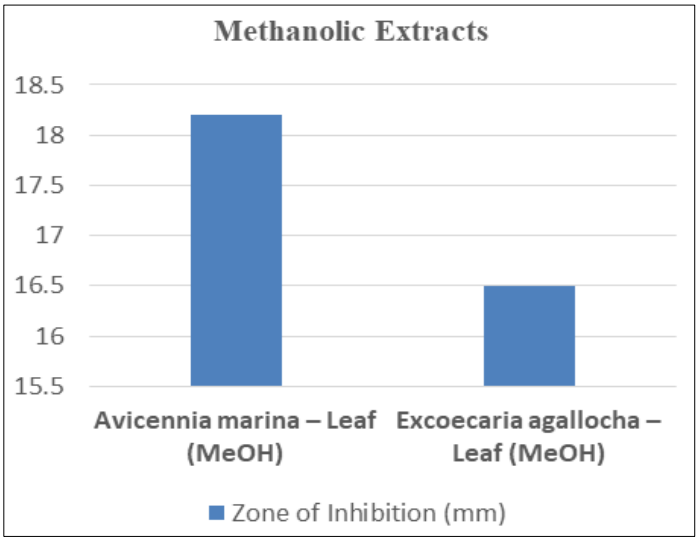
- *X. campestris:* 0.78–1.56 µg/mL
- *S. xylosus:* 1.56–3.12 µg/mL

5.2 MIC of *Avicennia marina* Extracts

Table 7: Minimum Inhibitory Concentration (MIC) of *Avicennia marina* extracts against X. campestris and S. xylosus

Solvent	Pathogen	MIC (mg/mL)
Me thanol	*X. campestris*	6.25
Methanol	*S. xylosus*	3.12
Ethyl Acetate	*X. campestris*	1.56
Ethyl Acetate	*S. xylosus*	3.12

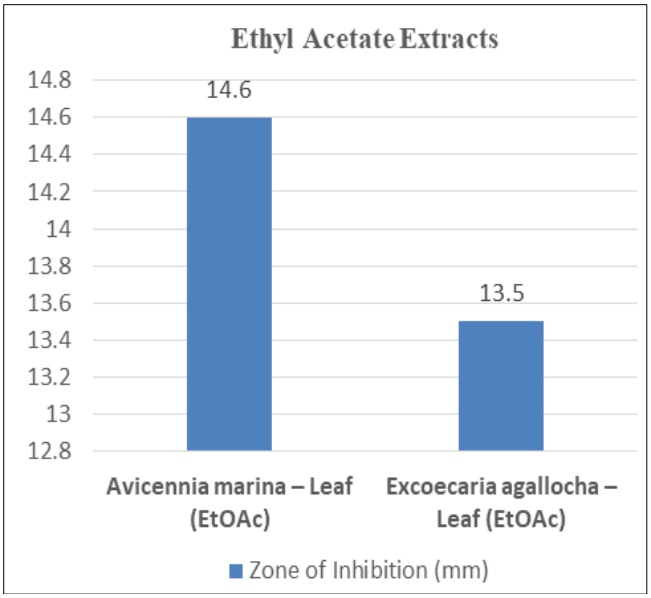
- Lower MIC values indicate better antimicrobial potency. *A. marina* extracts, particularly the ethyl acetate extract against *X. campestris*, performed well relative to other treatments



Graph 1: Activity of Methanolic Extracts against *Xanthomonas campestris*

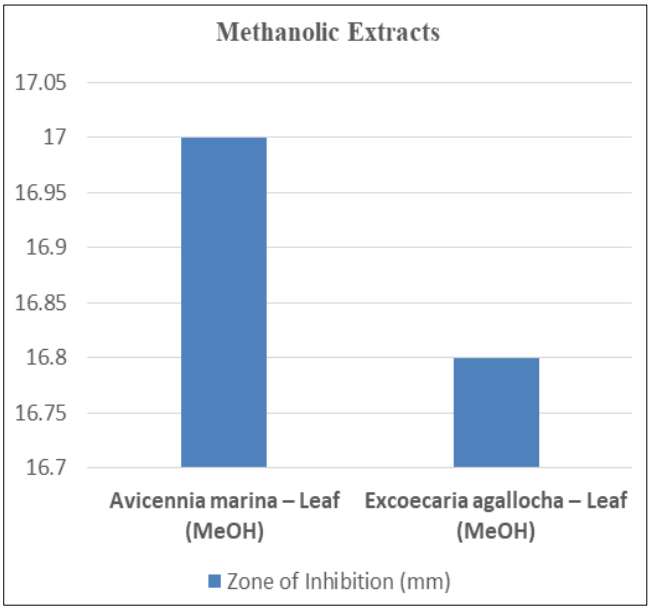
- **X-axis:** Extract type
- **Y-axis:** Zone of inhibition (mm)

Each bar represents the observed inhibition for methanolic extract against *X. campestris* and *A. Marina*



Graph 2: Activity of Ethyl Acetate Extracts against *Xanthomonas campestris*

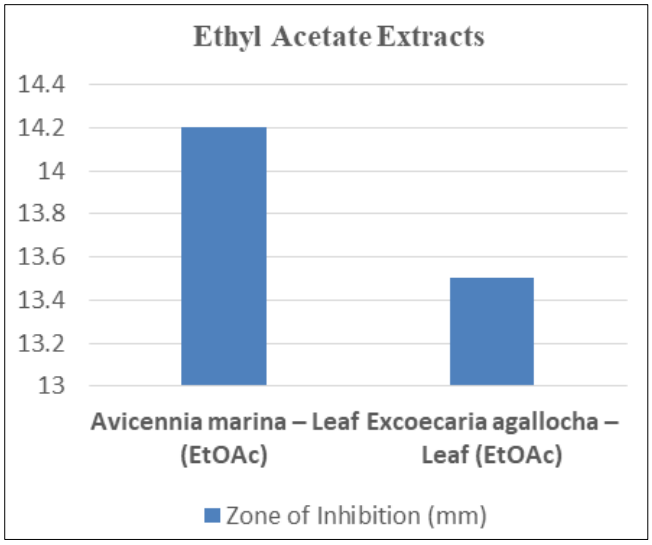
- **X-axis:** Extract type
- **Y-axis:** Zone of inhibition (mm)
- Each bar represents the observed inhibition for Ethyl Acetate Extracts against *Xanthomonas campestris*



Graph 3: Activity of Methanolic Extracts against *Staphylococcus xylosus*

- **X-axis:** Extract type
- **Y-axis:** Zone of inhibition (mm)

Each bar represents the observed inhibition for methanolic extract against *Staphylococcus xylosus* and *A. Marina*



Graph 4: Activity of Ethyl Acetate Extracts against *Staphylococcus xylosus*

- **X-axis:** Extract type
- **Y-axis:** Zone of inhibition (mm)
- Each bar represents the observed inhibition for Ethyl Acetate Extracts against *Staphylococcus xylosus*

Discussion

This study revealed significant antibacterial potential of *Avicennia marina*, particularly in its methanolic and ethyl acetate leaf extracts. Both *X. campestris* and *S. xylosus* were inhibited, with clear zones of inhibition and MIC values supporting extract efficacy.

Though Streptocycline was active at lower µg/mL concentrations, the activity of crude plant extracts at higher mg/mL levels remains noteworthy and relevant for early-phase bioprospecting.

Conclusion

- *A. marina* and *E. agallocha* were successfully collected, identified, and extracted using cold maceration.
- Methanol and ethyl acetate extracts exhibited antibacterial activity against *X. campestris* and *S. xylosus*.
- MIC testing showed *A. marina* ethyl acetate extract was particularly potent.
- These findings support the use of mangrove plants as sources of natural antibacterial agents for crop protection and biomedical exploration.

Future Prospects

- Further fractionation and compound isolation from *A. marina* is recommended.
- Broader screening of mangrove plants against multiple phytopathogens can be pursued.
- *In vivo* testing and formulation development are needed to translate lab findings into field application.

- The integration of mangrove-based antimicrobials into sustainable agriculture and biopesticide development is a promising direction.

Photogallery

Antipathogenic activity of Plant extracts

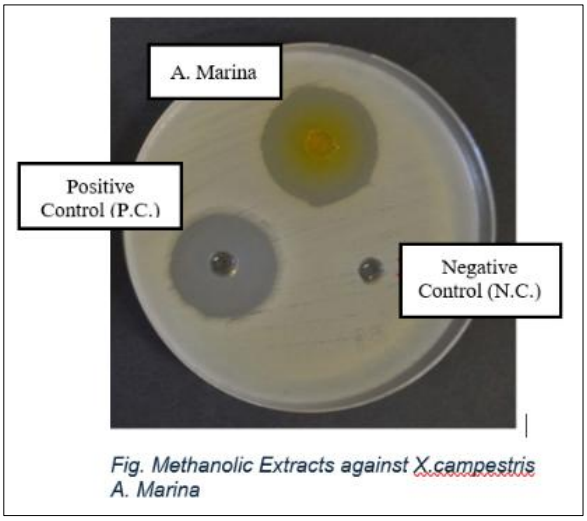


Fig. Methanolic Extracts against X.campestris A. Marina

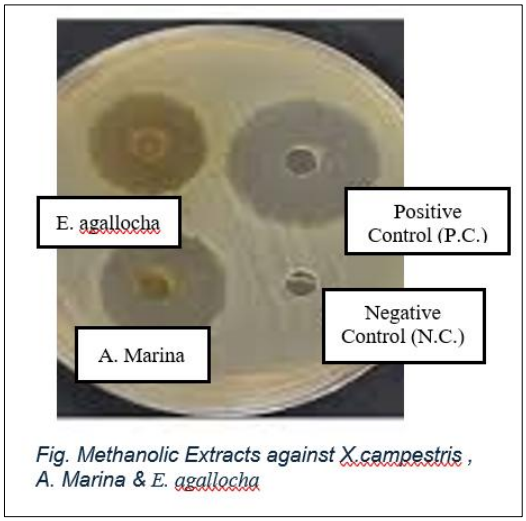


Fig. Methanolic Extracts against X.campestris , A. Marina & E. agallocha

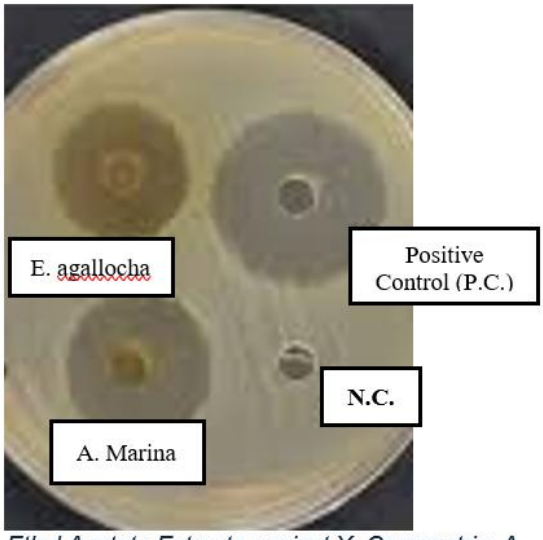


Fig . Ethyl Acetate Extracts against X. Campestris, A. Marina & E. agallocha

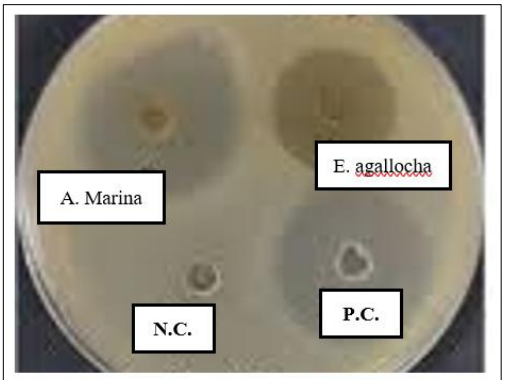


Fig . Methanolic Extracts against S. xylosus , A. Marina & Excoecaria agallocha

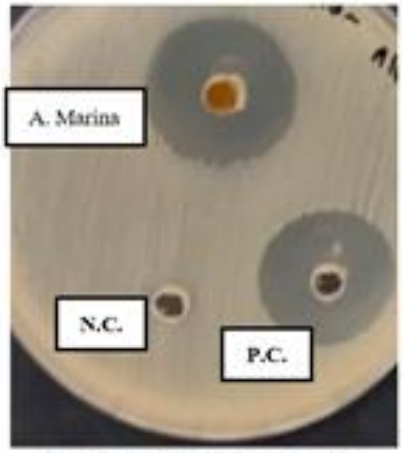


Fig., Ethyl Acetate Extracts of A. Marina against Staphylococcus xylosus

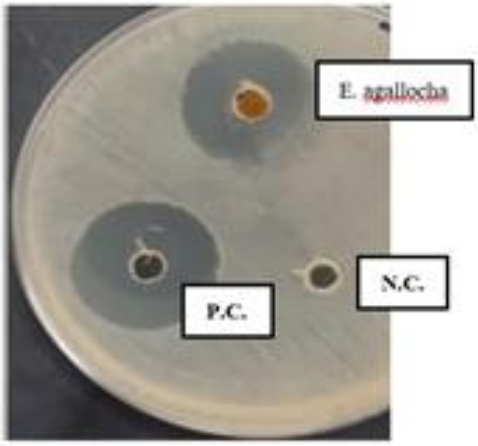
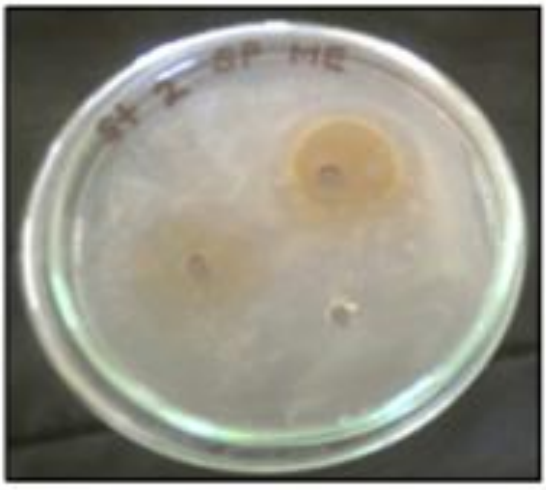
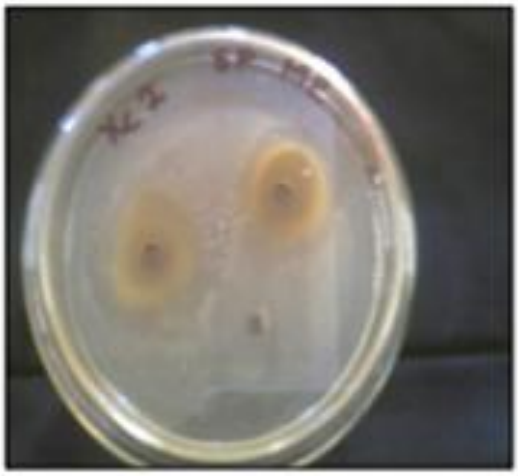
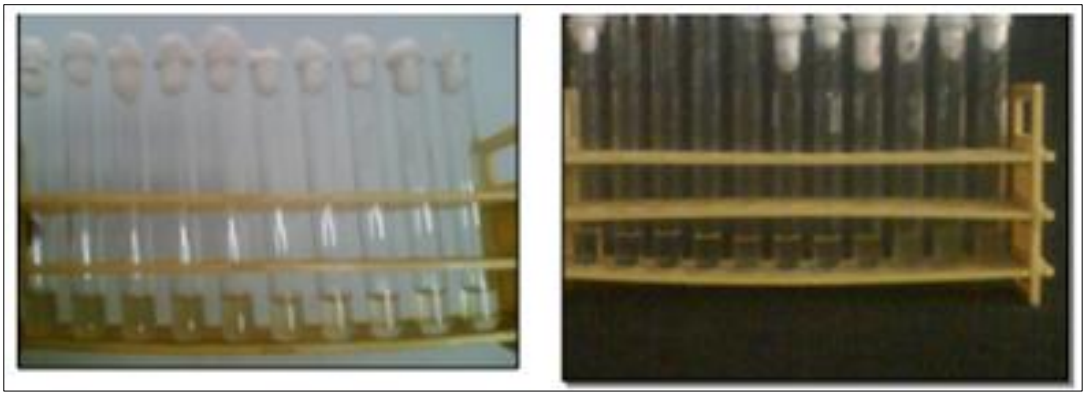


Fig., Ethyl Acetate Extracts of Excoecaria agallocha against Staphylococcus xylosus





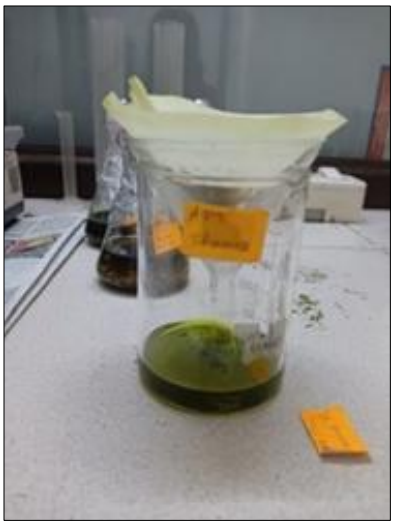


Phytochemical Extraction Process

10g sample

Sample in solvent

Samples with Ethanol

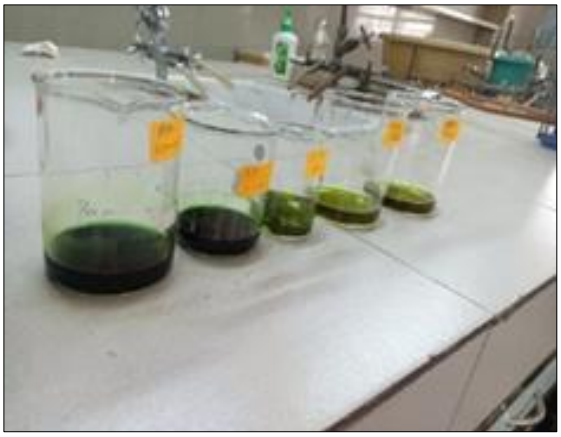


Filteration

17.72+Hrs On mild Rotatory shaker

Sample in Ethyl Alc.

Solvent Evaporation at 50°C or below Monitored



Phytochemical Extraction

Extraction Storage at 4°C until use

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