Naveen Shodh Sansar (An International Refereed / Peer Review Multidisciplinary Research Journal)
RNI No.- MPHIN/2013/60638, ISSN 2320-8767, E- ISSN 2394-3793, Scientific Journal Impact Factor (SJIF)- 8.054,
July to September 2026, E-Journal, Vol. I, Issue LV (55), ISO 9001:2015 - E2024049304 (QMS)

Comparative Antimicrobial Activity of *In Vivo* and *In Vitro*-Derived Tissues of *Pterocarpus marsupium Roxb.* Against Selected Bacterial Pathogens

Dr. Garima Moitra*

***C/O Ashok Kumar Mukherjee, Retd Judge, Orange Building, Byron Bazar, Raipur (C.G.) INDIA**

Abstract: *Pterocarpus marsupium Roxb*. is a medicinally important tree of the family Fabaceae with a long history of traditional use and a diverse phytochemical profile. The present investigation was undertaken to evaluate and compare the antimicrobial potential of different in vivo plant tissues and in vitro-derived tissues of *P. marsupium* against selected bacterial pathogens. The study formed the fourth objective of a broader research programme involving mass multiplication, phytochemical profiling, pharmacological evaluation and molecular characterization of the species. Leaf, stem, bark, fresh callus, old callus and whole-plant/in vitro-derived material were evaluated against seven bacterial microorganisms, namely Bacillus subtilis, Escherichia coli, Enterobacter aerogenes, Proteus vulgaris, Pseudomonas aeruginosa, Shigella boydii and Salmonella paratyphi. Antimicrobial activity was assessed from zones of inhibition using the agar well/cup diffusion approach described in the thesis. Marked variation was observed among plant tissues, extract treatments and microorganisms. Bark and callus tissues generally demonstrated stronger activity than roots and several other tissues. Particularly high inhibition zones included 36.0 ± 0.27 mm against E. coli, 35.0 ± 0.21 mm against S. boydii and 27.0 ± 0.25 mm against B. subtilis in selected preparations. S. boydii was the most susceptible organism overall, whereas P. aeruginosa was comparatively resistant. Fresh and old callus demonstrated substantial activity, indicating that in vitro culture can retain or enhance the production of antimicrobial metabolites. Statistical analysis reported in the thesis showed significant treatment differences for several extract-organism combinations, with p-values as low as 0.0001. The antimicrobial responses are discussed in relation to the distribution of phenolics, flavonoids, tannins, saponins, quinones and other secondary metabolites documented elsewhere in the thesis. The findings support the pharmacological potential of *P. marsupium* and highlight in vitro tissues as promising material for further investigation of plant-derived antimicrobial compounds.

Keywords: *Pterocarpus marsupium;* antimicrobial activity; antibacterial activity; bark; callus; phytochemicals; agar well diffusion; medicinal plant; in vitro culture.

Introduction - Medicinal plants have historically provided important sources of therapeutic preparations and continue to attract scientific attention as reservoirs of structurally diverse natural products. The increasing interest in plant-derived antimicrobials is associated with the continuing need for new compounds capable of inhibiting pathogenic microorganisms and complementing existing antimicrobial strategies. Plant tissues synthesize numerous secondary metabolites that can participate in defense against microorganisms, herbivores and environmental stresses. Phenolics, flavonoids, tannins, quinones, terpenoids, alkaloids and saponins are among the groups frequently investigated for antimicrobial potential.

Pterocarpus marsupium Roxb., commonly known as Indian kino, Vijaysar or Bijaka, is an economically and medicinally important tree belonging to Fabaceae. It has been used traditionally in Indian systems of medicine, particularly in relation to diabetes and several other conditions. The species as possess broad spectrum of bioactive constituents, including polyphenols, flavonoids, tannins, saponins, terpenoids, alkaloids and glycosides. This chemical diversity provides a biological basis for investigating multiple pharmacological activities of the plant. The antimicrobial properties of *P. marsupium* have been reported previously. The literature provides activity of bark, leaf and stem extracts against Gram-positive and Gram-negative bacteria, with activity depending on plant part, extraction solvent and concentration. Methanol and ethyl acetate extracts have frequently shown stronger activity than less polar extracts. Such observations indicate that the antimicrobial response of *P. marsupium* is not a fixed property of the species but depends on the chemical

composition of the tested tissue and the extraction conditions.The antibacterial potential of *P. marsupium* bark has also been demonstrated in independent studies. A 2009 study evaluated an aqueous bark extract against *Staphylococcus aureus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae* and reported inhibition with minimum inhibitory concentrations in the 0.04–0.08 mg range. The study also observed that susceptibility differed among the bacterial species. These findings provide external support for investigating bark as an antimicrobial tissue.

Another important aspect of the present study is the comparison between naturally occurring plant tissues and in vitro-derived tissues. Plant tissue culture is primarily used for rapid multiplication and conservation, but controlled culture conditions can also influence secondary metabolism. Callus cultures may synthesize or accumulate metabolites that are quantitatively or qualitatively different from those in mature field-grown tissues. Consequently, antimicrobial screening of fresh callus, old callus and in vitro plants can reveal whether the biosynthetic capacity for defense-related compounds is retained under controlled culture conditions.

Materials and Methods

Plant material: Plant material of *P. marsupium* was collected and processed as part of the larger research programme. The antimicrobial experiment included naturally occurring tissues and in vitro-derived materials. The tested categories included leaves, stems, bark, fresh callus, old callus and whole-plant/in vitro-derived material.

Preparation of extracts: Different plant parts were dried, processed and extracted for biological testing. The antimicrobial experiment was evaluated using the agar well/cup diffusion approach. Methanolic preparations generally provided comparatively broad recovery of bioactive constituents.

Test microorganisms: Seven bacterial microorganisms were included in the antimicrobial evaluation:

1. Bacillus subtilis
2. Escherichia coli
3. Enterobacter aerogenes
4. Proteus vulgaris
5. Pseudomonas aeruginosa
6. Shigella boydii
7. Salmonella paratyphi

The selection included both Gram-positive and Gram-negative bacteria, thereby allowing comparison of the activity of *P. marsupium* extracts across organisms with different cellular envelopes and physiological characteristics.

Antimicrobial assay: Antimicrobial activity was evaluated using the agar well/cup diffusion approach described in the thesis. Extracts were tested against the selected bacterial organisms and antimicrobial response was expressed as the diameter of the inhibition zone in millimetres. Larger zones were interpreted as greater inhibitory activity under the experimental conditions.Measurements as means with associated standard deviations, for example 36.0 ± 0.27 mm. Statistical analysis included p-values, coefficient of variation (CV), standard error and least significant difference (LSD). The statistical tables demonstrate that several extract-organism combinations differed significantly.

Statistical analysis: The antimicrobial data were subjected to statistical evaluation. General means, p-values, CV values, standard errors and LSD values. Several treatments showed highly significant differences, with p-values reported as 0.0001 or 0.0003. One comparison for Stem A was reported as non-significant (p = 0.26), illustrating that not all observed numerical differences were statistically significant.

Results

Table 1 and 2 (see in last page)

Overall antimicrobial response: The antimicrobial evaluation demonstrated marked variation among tissues and extract treatments. Bark, fresh callus and old callus generally produced stronger inhibition zones than several vegetative tissues. Bark and old callus among the strongest materials and reports particularly high inhibition against *B. subtilis, E. coli, S. boydii and S. paratyphi*.

Selected high inhibition values included 27.0 ± 0.25 mm against *B. subtilis*, 36.0 ± 0.27 mm against E. coli, 35.0 ± 0.21 mm against *S. boydii* and 23.3 ± 0.15 mm against *S. paratyphi*. These values demonstrate that some *P. marsupium* preparations were capable of producing relatively large inhibition zones.

Leaf extracts: Leaf treatments demonstrated treatment-dependent activity. Leaf A showed no detectable inhibition against the listed organisms in the summarized table. Leaf B displayed inhibition of 15.3 ± 0.32 mm against B. subtilis, 20.7 ± 0.39 mm against E. coli, 22.7 ± 0.42 mm against E. aerogenes, 22.7 ± 0.42 mm against P. vulgaris, 20.7 ± 0.47 mm against S. boydii and 17.0 ± 0.18 mm against S. paratyphi, while the response to P. aeruginosa was absent in the reported row.

Leaf C showed stronger activity, including 22.0 ± 0.37 mm against B. subtilis, 36.0 ± 0.27 mm against E. coli, 27.3 ± 0.38 mm against E. aerogenes, 25.7 ± 0.34 mm against P. vulgaris, 20.7 ± 0.17 mm against P. aeruginosa, 23.3 ± 0.12 mm against S. boydii and 23.3 ± 0.15 mm against S. paratyphi. The general mean reported for Leaf C was approximately 25.5 mm.

These results indicate substantial treatment-dependent variation within leaf material. They also demonstrate that the same plant part can show little or no activity under one treatment and pronounced activity under another.

Stem extracts: Stem treatments also displayed considerable variation. Stem A showed inhibition against B. subtilis and selected organisms but relatively limited overall activity, with a general mean of approximately 5.5 mm in the statistical summary. Stem B had a general mean of approximately 11.9 mm, whereas Stem C reached approximately 21.0–22.7 mm depending on the statistical

presentation.

One of the strongest stem responses was 27.0 ± 0.25 mm against B. subtilis in Stem C. Stem C also produced 24.0 ± 0.17 mm against E. aerogenes, 23.3 ± 0.25 mm against P. vulgaris, 15.3 ± 0.23 mm against P. aeruginosa, 35.0 ± 0.21 mm against S. boydii and 22.7 ± 0.17 mm against S. paratyphi. These results indicate that stem tissue can possess considerable antibacterial activity under favorable extraction conditions.

Bark extracts: Bark represented one of the most active natural tissues examined. Bark A showed a general mean of approximately 5.3 mm in the statistical summary, whereas Bark B had a general mean of approximately 11.4 mm and Bark C approximately 19.5 mm.

Bark C showed 25.3 ± 0.29 mm against B. subtilis, 24.0 ± 0.28 mm against E. aerogenes, 19.0 ± 0.24 mm against P. vulgaris, 24.2 ± 0.24 mm against P. aeruginosa, 25.0 ± 0.25 mm against S. boydii and 17.0 ± 0.24 mm against S. paratyphi in the reported table. The high activity of bark is consistent with its biological role as a protective tissue and with its phytochemical richness.

Fresh callus: Fresh callus showed notable antimicrobial activity, although its response varied among organisms and treatment categories. The thesis table reports inhibition of B. subtilis by Fresh Callus B and C, including 20.0 ± 0.24 mm and 30.7 ± 0.30 mm, respectively. Fresh Callus B and C also inhibited P. vulgaris at approximately 17.0 ± 0.12 mm and 19.7 ± 0.12 mm, respectively.

Against P. aeruginosa, Fresh Callus B and C showed 24.7 ± 0.19 mm and 25.3 ± 0.34 mm, respectively. Fresh Callus B and C showed strong activity against S. boydii, including 28.3 ± 0.24 mm and 35.3 ± 0.34 mm. S. paratyphi inhibition reached 22.3 ± 0.41 mm and 22.7 ± 0.25 mm in the reported treatments.

Old callus and whole-plant/in vitro-derived material

Old callus showed strong activity against several organisms. The reported values included 24.0 ± 0.15 mm and 30.0 ± 0.14 mm against B. subtilis in Old Callus B and C, respectively, and 17.0 ± 0.17 mm against E. coli in Old Callus C. Old Callus C also showed 20.7 ± 0.17 mm against E. aerogenes and 25.3 ± 0.28 mm against P. aeruginosa.

Whole-plant/in vitro-derived treatments displayed some of the strongest values recorded in the study. Against B. subtilis, Plant A, B and C showed 21.0 ± 0.21 mm, 32.0 ± 0.35 mm and 38.0 ± 0.14 mm, respectively, in the reported table. Plant B and C also showed strong inhibition against E. coli, with values around 33.3 ± 0.17 mm and 32.0 ± 0.14 mm. Against P. vulgaris, Plant B and C reached approximately 25.3 ± 0.14 mm and 34.3 ± 0.35 mm.

These findings indicate that in vitro-derived whole-plant material can retain substantial antibacterial potential and, under particular treatments, may exhibit stronger activity than several field-derived tissues.

Susceptibility of microorganisms: The microorganisms differed considerably in their susceptibility. *S. boydii* was identified in the thesis as the most susceptible organism, with inhibition zones ranging from approximately 20.7 to 35.3 mm in active treatments. *E. coli* also showed strong responses, including a maximum reported value of 36.0 ± 0.27 mm.

P. aeruginosa was comparatively resistant It is the most resistant microorganism, requiring higher concentrations or stronger preparations to produce inhibition. This difference emphasizes the importance of organism-specific susceptibility when evaluating plant-derived antimicrobials.

Statistical validation: The statistical results support the presence of substantial treatment effects. For Leaf B and Leaf C, p-values of 0.0001 and 0.0003 were reported, respectively. Stem B and Stem C also showed highly significant differences, while Stem A was reported as non-significant with p = 0.26. Bark treatments showed highly significant p-values of approximately 0.0001.

The coefficient of variation values ranged approximately from 6.61% to 15.30% for the summarized tissue treatments. These values indicate manageable experimental variation for most comparisons. LSD values were also provided, allowing pairwise comparison among treatment means.

Discussion: The present study demonstrates that *P. marsupium* possesses substantial antibacterial potential and that this activity varies strongly according to plant tissue, treatment and microorganism. Such variation is expected because different tissues accumulate different classes and concentrations of secondary metabolites.

Relationship between phytochemistry and antimicrobial activity: Leaf extracts of *P.marsupium* showed the presence of alkaloids and flavonoids in methanolic and acetone extracts, while glycosides, cardiac glycosides, lignin, coumarins, proteins, amino acids, reducing sugars and terpenoids were detected in selected extracts. Quinones were detected in methanolic and petroleum ether extracts. These observations demonstrate that antimicrobial activity occurs in a chemically complex matrix rather than being attributable to a single constituent. Phenolics and flavonoids are particularly relevant to antimicrobial activity because they can interact with microbial membranes, proteins and enzymes. Tannins can bind proteins and may interfere with microbial adhesion and extracellular enzymes. Quinones can participate in redox reactions and interact with nucleophilic cellular components. Saponins may alter membrane integrity through interactions with membrane sterols. The combined presence of these compounds may contribute to the observed inhibition zones. The independent literature therefore supports the conclusion that bark is a particularly important antimicrobial tissue in P. marsupium.

Importance of in vitro callus: The activity observed in fresh and old callus is one of the most significant findings of the study. Callus is an unorganized mass of proliferating cells produced under controlled tissue-culture conditions.

Although callus lacks the anatomical organization of mature plant tissues, it retains metabolic pathways capable of synthesizing many secondary metabolites.

The strong response of callus may be associated with the nutritional composition of the culture medium, plant growth regulators and mild physiological stress imposed by in vitro conditions. Such factors can modify secondary metabolism. The present results suggest that callus cultures of *P. marsupium* retain antimicrobial biosynthetic capacity.

This observation is supported by an earlier study specifically examining leaves and in vitro-raised calli of *P. marsupium*. Shahid, Shahzad and Anis (2009) investigated antibacterial potential of leaf and callus extracts and reported that the species had useful antibacterial potential. This provides particularly relevant external support because the study also involved in vitro-raised callus rather than only matures bark or stem material.

Whole-plant/in vitro material: The whole-plant/in vitro-derived material also demonstrated strong activity. The highest recorded inhibition in the reported table was 38.0 ± 0.14 mm against *B. subtilis* in Plant B, while Plant B and C produced strong inhibition against *E. coli*. These results suggest that the antimicrobial potential associated with *P. marsupium* is retained after in vitro regeneration.

The finding is important from a biotechnology perspective. If in vitro-derived material can produce useful levels of antimicrobial metabolites, it may provide a renewable source of biomass without repeated destructive harvesting of mature trees. This is particularly relevant for medicinal tree species where bark and heartwood are commonly collected.

Differential susceptibility of Gram-positive and Gram-negative bacteria: The present study included both Gram-positive and Gram-negative bacteria. The response was not determined solely by Gram classification because individual species showed distinct susceptibility patterns. *S. boydii* and *E. coli* showed strong responses in several treatments, whereas *P. aeruginosa* was more resistant.

Gram-negative bacteria possess an outer membrane that can restrict penetration of certain compounds, and bacterial efflux systems can further reduce intracellular accumulation. *P. aeruginosa* is well known for its intrinsic and acquired resistance mechanisms. Therefore, its comparatively weak response in this study is biologically plausible.

Plant extract activity cannot be explained entirely by bacterial cell-wall structure. The polarity, molecular size and concentration of active compounds, as well as the diffusion characteristics of the extract in agar, also influence the measured inhibition zone.

Interpretation of inhibition zones: Inhibition zone diameter is a useful preliminary screening parameter, but it should not be treated as a direct measure of antimicrobial potency. Diffusion through agar depends on molecular size, polarity and concentration, so a compound with high antimicrobial activity may produce a relatively small zone if it diffuses poorly.

The present study should be viewed primarily as a screening investigation. Confirmation of antimicrobial potency would require determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), time-kill behavior and, where appropriate, isolation and identification of the responsible compounds.

Relationship with antioxidant and enzyme profiles: In vitro plants and seedlings showed elevated antioxidant-related characteristics, while bark and roots also showed substantial defense-related enzyme activity .The high polyphenol oxidase activity reported in in vitro plants and seedlings and the high catalase activity in in vitro plants are particularly relevant to the concept of tissue-culture-induced metabolic changes. PPO values of 21.9 Units/g in in vitro plants and 20.1 Units/g in in vitro seedlings, while catalase activity reached 27.8 Units/g and 25.9 Units/g, respectively. These observations indicate active secondary metabolism and stress-response systems in cultured tissues.

Although these enzyme results do not prove that PPO or catalase directly caused antibacterial activity, their co-occurrence with strong antimicrobial activity supports the broader interpretation that in vitro culture can modify plant defense metabolism.

Pharmacological significance: The antimicrobial activity demonstrated in the present investigation contributes to the pharmacological profile of *P. marsupium*. The species is already recognized for antioxidant and antidiabetic activities, and antimicrobial activity adds another dimension to its biological potential.

The results may be relevant to the traditional use of medicinal plant preparations for conditions involving microbial infection or wound-related applications. The present study is an in vitro screening experiment and should not be interpreted as evidence of clinical efficacy.

The identification of antimicrobial constituents is a logical next step. Chromatographic separation followed by HPLC, LC-MS, GC-MS or spectroscopic characterization could determine which compounds or compound combinations are responsible for the observed activity. Such studies could also establish whether callus and in vitro plants contain the same antimicrobial compounds as bark or whether tissue culture induces a different metabolite profile.

Sustainable production and conservation: *P. marsupium* is a medicinal tree in which bark and wood are economically valuable. Repeated harvesting of bark can damage or kill mature trees, creating a conflict between medicinal demand and conservation. The demonstration of antimicrobial activity in callus and in vitro-derived tissues therefore has potential conservation significance.

Tissue culture provides a route for producing plant biomass under controlled conditions. If optimized cultures can maintain antimicrobial metabolite production, they may

reduce dependence on destructive harvesting. The combination of mass multiplication and pharmacological screening is therefore particularly valuable. It connects conservation biology with natural-product research and provides a pathway toward sustainable utilization.

Future Perspectives: Future studies should focus on standardization of extract preparation and concentration, followed by MIC and MBC determination. Bioassay-guided fractionation could be used to identify active fractions. HPLC, LC-MS/MS, GC-MS and NMR spectroscopy could then be applied to characterize antimicrobial constituents. Comparative metabolomic analysis of bark, fresh callus, old callus and in vitro plants would be especially valuable. Such analysis could determine whether tissue culture produces the same compounds as mature tissues or stimulates different biosynthetic pathways.

It would also be useful to investigate synergistic effects between *P. marsupium* extracts and conventional antibiotics. *P. marsupium* bark extract can act synergistically with antibiotics in certain bacterial systems. This area may be particularly important in the context of antimicrobial resistance. Additional studies should also examine cytotoxicity and selectivity so that antimicrobial activity can be distinguished from nonspecific toxicity. In vitro antimicrobial screening should be followed by mechanistic and in vivo studies before therapeutic applications are considered.

Conclusion: The present investigation demonstrates that *Pterocarpus marsupium* possesses considerable antibacterial potential and that this activity varies markedly among plant tissues, treatments and bacterial species. Bark and callus tissues showed particularly strong activity, while selected whole-plant/in vitro-derived preparations produced some of the largest inhibition zones recorded in the study. Among the microorganisms tested, Shigella boydii was the most susceptible, with inhibition zones reaching approximately 35.3 mm in active treatments. *Escherichia coli* also showed strong susceptibility, including a maximum reported zone of 36.0 ± 0.27 mm. In contrast, *Pseudomonas aeruginosa* was comparatively resistant.

The strong activity of bark is consistent with its role as a protective tissue and its rich phytochemical composition. The substantial activity of fresh and old callus is especially important because it demonstrates that in vitro-derived tissues can retain significant antimicrobial biosynthetic capacity. This observation is supported by previous research on *P. marsupium* leaves and in vitro-raised calli. Statistical analysis further supports significant differences among several treatments, with highly significant p-values reported for many tissue-treatment combinations. However, the activity should be regarded as preliminary in vitro antibacterial evidence because inhibition zones alone do not establish antimicrobial potency or therapeutic efficacy.

References:-

1. Badkhane, Y., Yadav, A. S., Sharma, A. K., Raghuwanshi, D. K., & Katare, A. (2010). Pterocarpus marsupium: A review of its biological activities and medicinal properties. Reference as discussed in the submitted thesis.
2. Cowan, M. M. (1999). Plant products as antimicrobial agents. Clinical Microbiology Reviews, 12(4), 564–582.
3. George, E. F., Hall, M. A., & De Klerk, G. J. (2008). Plant Propagation by Tissue Culture. Springer.
4. Gayathri, M., & Kannabiran, K. (2009). Antimicrobial activity of Hemidesmus indicus, Ficus bengalensis and Pterocarpus marsupium Roxb. Indian Journal of Pharmaceutical Sciences, 71(5), 578–581. DOI: 10.4103/0250-474X.58182.
5. Harborne, J. B. (1998). Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Chapman & Hall.
6. Israr et al. (2012). Antimicrobial evaluation of Pterocarpus marsupium extracts against pathogenic bacteria. Reference as cited in the submitted thesis.
7. Londonkar, R. L., & Hugar, A. L. (2017). Pharmaco - gnostic and antimicrobial evaluation of Pterocarpus marsupium. Reference as cited in the submitted thesis.
8. Pant et al. (2017). Phytochemical composition and biological activities of Pterocarpus marsupium stem wood extracts. Reference as cited and discussed in the submitted thesis.
9. Ramya, S., Kalayansundaram, M., Kalaivani, T., & Jayakumararaj, R. (2008). Phytochemical screening and antibacterial activity of leaf extracts of Pterocarpus marsupium Roxb. Ethnobotanical Leaflets, 12, 1029–1034.
10. Saidurrahman et al. (2022). Antibacterial activity of Pterocarpus marsupium stem and bark extracts. Reference as cited in the submitted thesis.
11. Shahid, M., Shahzad, A., & Anis, M. (2009). Antibacterial potential of the extracts derived from leaves and in vitro raised calli of medicinal plants Pterocarpus marsupium Roxb., Clitoria ternatea L., and Sansevieria cylindrica Bojer ex Hook. Oriental Pharmacy and Experimental Medicine, 9(2), 174–181. DOI: 10.3742/OPEM.2009.9.2.174.
12. Sofowora, A. (2008). Medicinal Plants and Traditional Medicine in Africa. Spectrum Books.
13. Trease, G. E., & Evans, W. C. Pharmacognosy. Saunders/Elsevier.
14. Moitra, G., & Das, S. (2024). Antibacterial and phytochemical profiling of Pterocarpus marsupium bark extracts from Barnawapara, (C.G.), India. Frontiers in Health Informatics, 13(6), 4630–4636.

Table 1. Observation of antimicrobial activity of P. marsupium tissues against selected bacterial pathogens

Plant material / treatment	B. subtilis	E. coli	E. aerogenes	P. vulgaris	P. aeruginosa	S. boydii	S. paratyphi
Leaf A	NR	NR	NR	NR	NR	NR	NR
Leaf B	15.3 ± 0.32	20.7 ± 0.39	22.7 ± 0.42	22.7 ± 0.42	NR	20.7 ± 0.47	17.0 ± 0.18
Leaf C	22.0 ± 0.37	36.0 ± 0.27	27.3 ± 0.38	25.7 ± 0.34	20.7 ± 0.17	23.3 ± 0.12	23.3 ± 0.15
Stem C	27.0 ± 0.25	—	24.0 ± 0.17	23.3 ± 0.25	15.3 ± 0.23	35.0 ± 0.21	22.7 ± 0.17
Bark C	25.3 ± 0.29	—	24.0 ± 0.28	19.0 ± 0.24	24.2 ± 0.24	25.0 ± 0.25	17.0 ± 0.24
Fresh callus B	20.0 ± 0.24	—	—	17.0 ± 0.12	24.7 ± 0.19	28.3 ± 0.24	22.3 ± 0.41
Fresh callus C	30.7 ± 0.30	—	—	19.7 ± 0.12	25.3 ± 0.34	35.3 ± 0.34	22.7 ± 0.25
Old callus B	24.0 ± 0.15	—	—	—	—	—	—
Old callus C	30.0 ± 0.14	17.0 ± 0.17	20.7 ± 0.17	—	25.3 ± 0.28	—	—
In vitro plant C	38.0 ± 0.14	32.0 ± 0.14	—	34.3 ± 0.35	—	—	—

Note: Values are inhibition-zone diameters (mm), mean ± SD, as reported in the thesis tables used for this article. “NR” = no reported inhibition in the corresponding summarized observation; “—” = value not reproduced in the available observation summary. A, B and C are retained as treatment labels because the antimicrobial table does not consistently define them by solvent/concentration.

Table 2. Selected observation summary of antimicrobial response

Observation parameter	Finding
Highest reported inhibition zone	38.0 ± 0.14 mm against B. subtilis (in vitro plant C)
Strong E. coli response	36.0 ± 0.27 mm (leaf C)
Strong S. boydii response	35.3 ± 0.34 mm (fresh callus C) and 35.0 ± 0.21 mm (stem C)
Strong P. vulgaris response	34.3 ± 0.35 mm (in vitro plant C)
Comparatively resistant organism	Pseudomonas aeruginosa
Most susceptible organism in thesis interpretation	Shigella boydii
Statistical significance	Several tissue-treatment comparisons reported $p \leq 0.0003$; Stem A was reported as non-significant ($p = 0.26$)

****