UNIVERSITY OF TURKU

<u>**Master´s Thesis**</u>

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MSc. in Chemistry of Drug Development

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BIOPROSPECTING AND MODIFICATION OF MRSA-INHIBITING METABOLITES FROM RARE BOTANICAL SPECIES

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Contents

1-Abstract:

Methicillin-resistant Staphylococcus aureus (MRSA) poses a chronic public threat because it is resistant to conventional medications. In this study, we utilized 90 of the most important plant tissues from a collection of 300 rare plant species from the botanical garden of the University of Turku. The aim was to first investigate how the metabolite composition of the species is linked to the inhibition of MRSA bacterial growth. In the next step the metabolite extracts were treated in three separate ways that were thought to either remove or modify the MRSA-active compounds from the extracts. The changes in metabolite profiles were linked to the changes in MRSA inhibitions.

The methodology involved the collection of two replicate samples per species, enzymatic oxidation of one replicate and freeze-drying all the tissue. The finely ground and weighed tissue was extracted with acetone/water (80/20, v/v) and the aqueous phase freeze-dried. Extracts were prepared in water, filtered and analysed using group-specific MS/MS methods with the UHPLC-DAD-MS/MS system, and for their oxidative activity and protein affinity. Of the treatments originally planned, enzymatic oxidation and nylon-membrane removal of the protein-active compounds were completed; the alkaline (pH 10) oxidation and its two combination treatments were not carried out, for reasons of time and technical constraints. The completed treatments were the ones tested against MRSA bacterial growth.

Across the three completed treatments, the results followed a clear and interpretable trend. For MRSA, the mean inhibition of the 90 control extracts fell after enzymatic oxidation and fell further after nylon filtration, and the number of strong inhibitors dropped from 28 in the control to 16 after oxidation and 10 after filtration. Because nylon filtration removed the most activity, the large protein-binding tannins appear to carry much of the inhibition for most species, while a smaller group of extracts kept their activity through both treatments, marking those as the compounds least dependent on removable or oxidisable polyphenols. One species stood out: the Anacardiaceae member Pleiogynium solandri held strong inhibition of roughly 82 to 84% across control, enzymatic oxidation and nylon filtration alike, which points to a stable, treatment-resistant metabolite and makes it the clearest candidate for isolation and structural identification. It remains to be confirmed whether the metabolites removed by the treatments are the same ones responsible for the activity of the control extracts. The approach developed in this study could significantly help the future bioprospecting of MRSA-active metabolites, since multiple treatment types are likely to reveal the truly active metabolites better than studies using one type of sample only.

2-Introduction:

2-1) The Global Crisis of Antimicrobial Resistance:

Antimicrobial resistance (AMR)is one of the most challenging and serious threats to public health in this current 21st century. In the year 2019, the international body which is World health Organization (WHO) ranked AMR as one of the top ten global health threats. This was based on decades of epidemiological research showing that viruses, fungi, parasites and bacteria around the world are evolving in a way that the drugs could become ineffective which are meant to kill them. *(1)* The implications of this evolutionary pressure are very extreme as infections that are used to treatable can become life-threatening. The hospitals stay can get longer and eventually the healthcare cost and death rates can go up sharply. AMR is not like an epidemic of one pathogen because it is a constant and cumulative process of advancement begin by selective pressure, horizontal gene transfer, and the movement of resistant organisms everywhere in the world across national borders and between human, animals and environmental reservoirs. *(2)*

Murray and his team perform revolutionary research in 2022 for Lancet analysis that fully reasoned the size of the problem. They found that AMR was primarily to blame over 1.27 million deaths worldwide in 2019 and played an essential part in 4.95 million more deaths. *(2)* The subsequent Global Burden of Disease (GBD) 2021 Antimicrobial Resistance Collaborators analysis, released in The Lancet in 2024, expanded this timeline to thirty-one years and 204 countries, encompassing 22 pathogens and 84 pathogen–drug combinations. *(1)* This study determined that AMR directly resulted in a minimum of one million fatalities each year from 1990 to 2021. Projections indicate that, in the absence of additional scientific and policy interventions, drug-resistant bacterial infections will directly result in nearly two million deaths annually by 2050 and be linked to up to 8.22 million excess deaths each year by mid-century. *(1,3)*These numbers put AMR in the same group as heart disease and cancer as one of the main threats to human life in the next few decades.

The economic cost of AMR is just as bad. The World Bank said in 2017 that AMR could lower the world's gross domestic product by 1.1% to 3.8% by 2050. The biggest effects would be felt in nations with low or middle incomes which are unable to pay for longer hospital stays, secondary and third-generation antimicrobial therapies, and better prevention and management of infections infrastructure. *(6)*In medical institutions with wealthy patients, the total yearly expense of AMR is presently in the hundreds of billions of US dollars. Through a pharmaceutical advancement pipeline that has only generated a few utterly new classes of antibiotics in the last 40 years, this socioeconomic and healthcare strain has led to AMR being called a "silent pandemic." This means that it has a long-lasting, deepening effect on worldwide health without the immediate visibility of outbreaks. *(3)*

The World Health Organization 2024 updated Bacterial Priority Pathogens Classification groups the pathogens of greatest significance into levels based on how easy they are to treat, how easy they are to prevent, and how much they affect the world.*(4,5)* Methicillin-resistant Staphylococcus aureus (MRSA) is a "very high priority" pathogen, which is the 2nd highest level. This is because it causes a lot of problems around the world and there are only a few last-resort treatment options available. The 2024 revision is the first full update since 2017, and it includes the much larger death rates from the GBD 2021 analysis. This shows how important it is to find new treatments for MRSA and other resistant organisms.

2-2) Staphylococcus aureus: Biology, Colonisation, and Clinical Significance:

The Staphylococcus aureus is a violet-staining bacteria and spore-free, facultatively oxygen-deprived microbial species belonging to the family Staphylococcaceae. It develops easily on standard culture media such as Columbia blood agar, where it makes distinctive gold-yellow clumps and areas of complete (beta) haemolysis. It forms spores (called "bunches of grapes") on Gram stain and is catalase-positive and coagulase-positive (the latter setting it apart from coagulase-negative staphylococci). *(7,8)* About 30% of people at any given point in time have the organism living in their anterior nares without any symptoms. Another 20–30% have it living there at times, and the rest of the time, they don't have it at all. The nasal cavity is the main place where S. aureus extends to the epidermal layer, perineum, and oropharynx. People who are colonized are three to twelve times more likely to get an invasive infection than people who are not colonized. *(9)* The clinical signs and symptoms of S. aureus bacterial infection exhibit a notable range of anatomical sites and severity. On the less severe finish of this range of severity, the microbe causes generalized skin disorders and soft-tissue infections (SSTIs) like furuncles, carbuncles, impetigo, folliculitis, and non-purulent cellulitis. At the severe end, it causes life-threatening systemic diseases such as bacteraemia and sepsis, infective endocarditis (both native- and prosthetic-valve forms), osteomyelitis, septic arthritis, haematogenous vertebral discitis, pyomyositis, and necrotising pneumonia, the last of which is particularly linked to Panton-Valentine leukocidin-producing community-associated strains. In today's ICUs, the death rate from S. aureus bacteraemia is between 20 and 40%. This number goes up even more in cases of prosthetic a control valve stenosis and vertebral osteomyelitis with epidural abscess. *(8)*

The pathogenic adaptability of S. aureus is due to an exceptionally extensive and systematically regulated array of virulence factors. Microbial surface components that recognize adhesive matrix molecules (MSCRAMMs) are what allow microbes to stick to host extracellular matrix proteins. Some of these are clumping factor A (ClfA), which binds to fibrinogen; clumping factor B (ClfB), which binds to fibrinogen and loricrin; fibronectin-binding proteins A and B (FnBPA and FnBPB); collagen-binding adhesin (CNA); and elastin-binding protein (EbpS). ClfA and ClfB are the main proteins that help platelets stick together and form a network of fibrin in infective endocarditis. FnBPA and FnBPB help non-professional phagocytes invade by bridging fibronectin and integrin, which lets S. aureus stay inside cells and avoid antibiotics. Immune evasion is accomplished via various intricate mechanisms. Protein A, which is made by the spa gene and is used to molecularly type MRSA, binds to the Fc domain of IgG antibodies in a way that does not involve the immune system. This stops neutrophils and macrophages from opsonophagocytosis and at the same time kills B-cells to lower adaptive humoral immunity.*(10)* The staphylococcal complement inhibitor (SCIN) stops the activity of C3 convertase to stop opsonisation by complement. Chemotaxis inhibiting protein of S. aureus

(CHIPS) stops neutrophils from moving to the site of infection by blocking formyl peptide and complement receptors. Extracellular fibrinogen-binding protein (Efb) stops complement convertases and the clumping of platelets.*(11)*

The toxins that S. aureus has make it even better at destroying tissue and causing systemic disease. Alpha-haemolysin (Hla) is a pore-forming toxin that is released as single molecules. These molecules then stick together on the membranes of target cells to form heptameric channels, which kill red blood cells, endothelial cells, and platelets.*(12)*Panton-Valentine leukocidin (PVL) is a two-part leukotoxin that makes holes in the membranes of neutrophils and macrophages. This destroys the body's main defences against bacterial infections that come from outside the body. PVL-positive species are linked to recurrent furunculosis and necrotizing pneumonia in an epidemiological sense. The PVL genes (Luks-PV and lukF-PV) are found on a temperate bacteriophage that is part of the community-associated MRSA chromosome.*(13)* Toxic shock syndrome toxin-1 (TSST-1) is a type of superantigen that attaches to molecules from MHC class II and T-cell receptors at the same time, activating 5–30% of total T cells and starting a cytokine storm.*(12)* Phenol-soluble modulins (PSMs) are small, amphipathic, alpha-helical peptides that break down cell membranes, cause cells that are inflammatory to die, and help biofilms spread.*(14)*

2-3) The genetic foundation, occurrence, and worldwide prevalence of methicillin resistance:

The development of resistance to methicillin in S. aureus indicates the most clinically significant adaptive advancement in the organism's history. Methicillin was initially employed in medical conditions in 1959 for the treatment of bacterial infections triggered by penicillinase-producing S. aureus. This was because the original penicillins were no more effective in clinical conditions because of enzymatic beta-lactam ring hydrolysis. However, methicillin-resistant strains were found in the UK within two years, in 1961.*(15,19)* The rapid emergence of this resistance predicted an ongoing trend whereby newly developed antibiotics had been closely accompanied by novel resistance mechanisms. The hereditary foundation of methicillin resistance has been thoroughly defined. The mecA gene, which encodes penicillin-binding protein 2a (PBP2a), is the main reason for obstruction. PBP2a is a 78-kDa transpeptidase that has a much lower affinity level compared to all commonly administered beta-lactam antibiotics instead of the native penicillin-binding.

proteins.*(15,16,17)* PBP2a takes the place of the native PBPs that are blocked and catalyses the transpeptidation step of peptidoglycan crosslinking. This lets the bacteria make cell walls that are still intact even when beta-lactam levels are high enough to kill sensitive strains. Lim and Strynadka figured out the crystal structure of PBP2a in 2002, and it showed that the active site is hidden behind a narrow allosteric-regulated entrance channel that blocks bigger beta-lactam molecules. This structural feature explains the very wide cross-resistance to almost all beta-lactam groups, such as synthetic penicillins, cephalosporins (except ceftaroline), monobactams, and carbapenems.*(17)*

The gene for mecA is found in a movable genetic strand of 21–67 kb, the staphylococcal cassette chromosome mec (SCCmec), which is attached at a particular attBscc site on the chromosome and could be passed on among staphylococcal strains.*(15,16)* Eleven SCCmec types (I-XI) have been characterised, which vary in dimensions, gene quantity and produced resistance determinants in addition to mecA. Types I, II, and III are substantial parts and are usually found in healthcare-associated MRSA (HA-MRSA) genomes. These kinds frequently contain extra resistance determinants that confer immunity to aminoglycosides, macrolides, tetracyclines and other antibiotics. IV and V are smaller and more mobile elements usually found in community-associated MRSA (CA-MRSA) lineages that typically do not exhibit multi-drug resistance other than to beta-lactams but often harbour virulence genes including PVL. *(15,20)* In 2011 a new mecC gene has been recognised in microbial and human-derived MRSA strains in the UK and Denmark which shared only about 70% nucleotide sequence identity with mecA but generated resistance through a structurally different PBP2a protein, thus broadening the scope of MRSA and challenging the identifying resistance using standard PCR-based methods.*(18)*

From an epidemiological point of view, MRSA infections can be classified into two groups, which have some but not all common features. Healthcare associated MRSA (HA-MRSA) is mainly a disease of hospitalised patients, the immunocompromised, the elderly and patients with invasive treatments such as surgery, dialysis or long-term catheterisation. HA-MRSA strains like in the worldwide clones CC5 (USA100), CC8 (UK EMRSA-15), CC22 (UK EMRSA-15), and CC239, usually harbour large SCCmec elements (types I–III), are Mult resistant, and become less infectious in the community setting given an inactive agr locus. *(19,20)* In the year the 1990s, community-transmitted MRSA (CA-MRSA) was identified to be a physiologically and genetically distinct phenomenon that caused severe SSTIs, necrotising pneumonia and bacterial infections in normally healthy people with no health-related warning signs.*(16)* Worldwide, the USA300 clone (CC8, SCCmec type IVa, PVL+), the EMRSA-15 variant in Europe, and the ST772 in the South Asian region dominate the CA-MRSA strains. These usually contain SCCmec type IV or V and are often PVL-positive, which gives a significantly enhanced leukocyte-killing activity.*(21,22)*

The vancomycin-intermediate S. aureus (VISA) phenotype was originally identified in a therapeutic isolate from Japan in 1996 and occurs by means of several chromosomal alterations that enhance peptidoglycan cell wall thickness, which sequesters vancomycin molecules prior to reaching their D-Ala-D-Ala target and reduces optimal internal concentrations of drugs.*(23–25)* The learning of the vanA operon molecule of bacterial species has been reported in over fifteen clinical isolates collected worldwide, leading to full vancomycin resistance (VRSA, MIC $\geq$16 μg/mL).*(26)* Despite VRSA is still uncommon, its appearance shows that immunity can emerge irrespective of the most effective agents via horizontal gene transfer, highlighting the need for new therapeutic development.

For worldwide death rate, MRSA has shown the highest troubling time-dependent pattern of every single pathogen-pharmacological mixture in the GBD 2021 analysis. The related death toll from MRSA grew from around 57,200 in 1990 to 130,000 in 2021, close to doubling, while the fatality toll from MRSA surged from 261,000 to 550,000 throughout the same period.*(1)*

MRSA is thought to be responsible for roughly 10,000 to 18,000 deaths annually in the United States alone. Sustained infection prevention and control programmes have led to promising decreases in MRSA circulatory system disease rates in several Member States of the EU, but the total burden remains extremely high. The rate of MRSA in clinical S. aureus bloodstream isolates still exceeds 25–40% in Southern and Eastern European countries. *(19,20)*

2-4) The Biofilm Growth: A Microbiological Process of Therapeutic Relevance:

The development of biofilm represents one of the greatest clinically significant characteristics of S. aureus and especially MRSA, and refers to organised, symbiotic, interconnected colonies of bacteria which are embedded in a framework of synthesised extracellular polymeric compounds (EPC) and that adhere to biotic or abiotic surfaces. Biofilm-associated diseases are the cause of an excessive amount of morbidity and fatalities from MRSA, especially in the setting of medical equipment-related infections, resulting chronic wounds, and implant-associated osteomyelitis. *(27,28)* Bacteria within mature biofilms are 10-1000 times more tolerant to antibiotics than planktonic bacteria. Biofilm infections are often hard to destroy using standard antibiotic therapies. *(29)*

2-5) Chemistry of Poylphenol compounds:

Hydrolysable tannins are built on a sugar core, usually glucose, esterified with gallic acid. Add more galloyl groups and you get gallotannins; let two neighbouring galloyls couple oxidatively and you get the hexahydroxydiphenoyl (HHDP) group that defines the ellagitannins, which release ellagic acid on hydrolysis. These are large, oxygen-rich molecules and they are among the strongest protein binders in the plant kingdom, which is precisely why they dominate the nylon-filtration story in the results. In the MRM method they show up as the galloyl and HHDP classes.

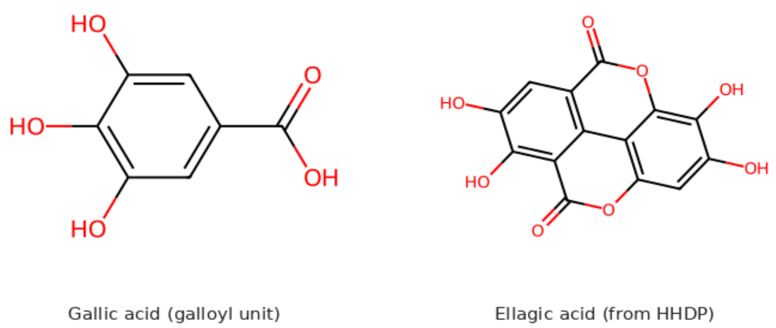


Fig 1: Gallic acid, the galloyl building block of gallotannins, and ellagic acid, released from the HHDP group of ellagitannins.

Condensed tannins, or proanthocyanidins, are chains of flavan-3-ol units. When the units are catechin or epicatechin the chain is a procyanidin (PC) as when they carry the extra hydroxyl of gallocatechin or epigallocatechin it is a prodelphinidin (PD). They range from small oligomers to large polymers, and like the hydrolysable tannins they bind protein strongly, more so as they get bigger. The MRM method resolves them by extension and terminal units across the PC and PD classes, and separates oligomer from polymer, which is what lets the results talk about tannin size rather than just tannin presence.

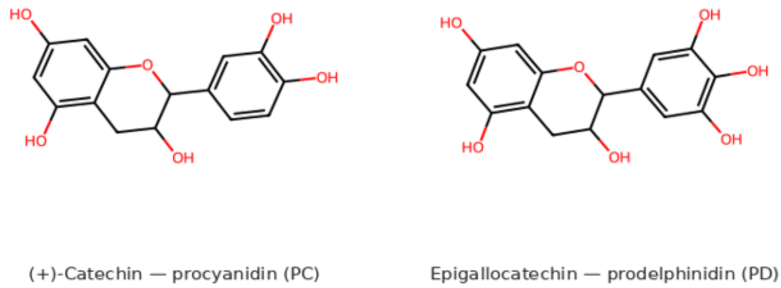


Fig 2: (+)-Catechin, the procyanidin building block, and epigallocatechin, the prodelphinidin building block. The extra B-ring hydroxyl distinguishes PD from PC.

Flavonoids share a three-ring skeleton and mostly travel through the plant as sugar conjugates. The flavanols in this study are kaempferol, quercetin, myricetin and isorhamnetin, which differ only in how many hydroxyls or methyl groups sit on the B-ring; the flavones are apigenin, luteolin and acacetin. They are smaller than the tannins and bind protein far weaklier, so they tend to pass through nylon filtration rather than stick to it. That different behaviour is useful: a species whose activity survives filtration but sits in a flavonoid-rich profile is pointing at a non-tannin active. The MRM method carries a separate class for each of these flavonoid cores.

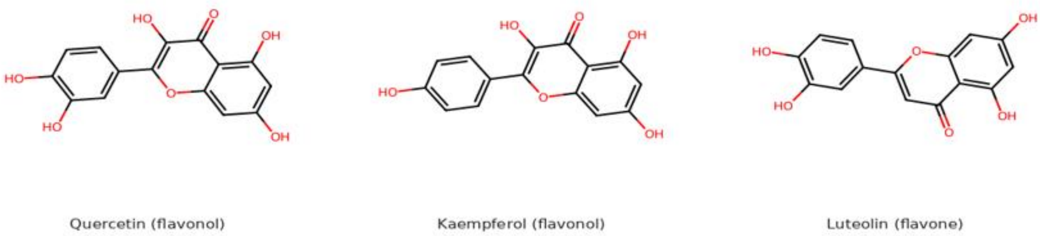


Fig.3: Quercetin and kaempferol (flavanols) and luteolin (flavone). The B-ring hydroxylation pattern is what the MRM method uses to tell the flavanol sub-classes apart.

The simplest polyphenols measured here are the hydroxycinnamic acids, caffeic and ferulic acid, and their esters with quinic acid, of which chlorogenic acid (5-caffeoylquinic acid) is the familiar example. They are small, they oxidise readily because caffeic acid carries a catechol, and they are widespread across plant families. In the results they matter mostly as a marker of the oxidation-sensitive fraction: an extract that loses caffeoyl signal under the enzymatic or alkaline step is showing the catechol chemistry the treatments are designed to catch. The MRM method tracks them as the caffeic-acid, ferulic-acid and quinic-acid derivative classes.

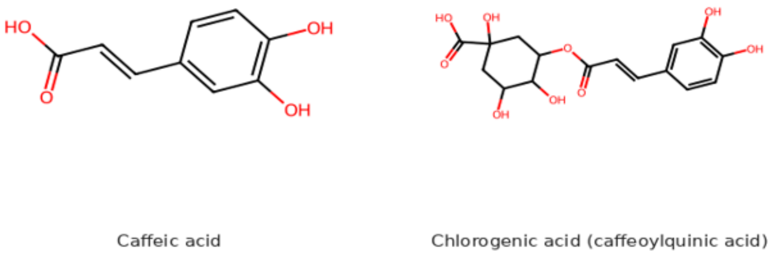


Fig 4: Caffeic acid, a hydroxycinnamic acid, and chlorogenic acid, its ester with quinic acid. The catechol on caffeic acid is what makes this class oxidation-sensitive

2-6) The plants to be Information of Antimicrobial agents Metabolites: Reasons for biological profiling:

Biological activity results from millions of years of selective growth, which makes the natural environment a massive collection of unique chemical structures. Since plants stay in one place and cannot run away from animals or sickness, the natural environment created complex chemical defence systems for survival. Experts claim that these protection methods are extremely complicated in their shape and provide specific healing powers. Because plants are stationary, these defences were developed over an exceedingly long time. The natural environment produces chemical substances that show vast variety because of this evolution. It has been observed that the chemical library of the natural environment contains deep knowledge for medicine. (30,31) Medical history shows that plant derived compounds frequently become the primary sources for medications. Salicylic acid, which is the active part of aspirin, comes from the bark of Salix (willow) species and Salicylic acid has been utilized by humans for more than 3500 years. Quinine was taken from the bark of the Cinchona tree to serve as the primary medicine against malaria for several centuries. Even though new tools exist, morphine and codeine from Papaver somniferum remain particularly important for reducing pain. Medicine known as Taxol from the plant Taxus species brevifolia changed the medical operation for treating regular ovarian, breast and lung cancers. Obtaining Artemisinin from the plant Artemisia annua, which won Tu Youyou the 2015 prestigious Nobel Prize in Science or Medicine, changed the healing of malaria that resists medicines. (32)

Detailed research by Newman and Cragg for the time limit January 1981 up to September 2019 showed that natural compounds and their close metabolites comprise 33.6% of all newly permitted small-molecule medicines. About half of all permitted antibiotics in this period were taken from natural products. *(41)* (30) Experts claim that the substances coming from natural products formed a larger part of the category for killing bacteria. This trend matches the historical importance of microbes and plants in finding antibiotics. There are 374,000 known plant species on Earth, of which only 9% were checked for any medical use. Only small portions were tested by medical professionals against specific pathogens that resist drugs like MRSA.(33,34) Nature's vast chemical library remains unused even though the potential for finding new antimicrobial frameworks is high.

Priority must be given to natural and exotic plant species in bioprospecting programs for finding new antimicrobial compounds. Rare plants possess diverse genetic traits and face heavy pressure from local bacteria, herbivores, and competitors because these pressures force the creation of special secondary metabolic products. These environmental stresses drive the generation of unique chemicals while such production is missing in farm-grown crops. Such crops have gone through genetic narrows when long times of breeding for quality, survival, and commercial viability occurred. (31,35) It has been observed that the survival of these plants depends on these unique chemicals. Special compounds are created because plants must protect themselves from their surroundings. Living beings found in small ecological spaces, like high elevations, dry climates, and salty soils, face very heavy life-related and non-life-related stress. These extra metabolic features might contain strong or completely new defense compounds while difficult conditions exist. Searching through this bioprospecting method uses high-tech UHPLC-MS/MS tools and standard tests to allow a planned study of these rare plant species. Organized study happens at a scale that was not possible before with old ways of testing. Many people believe that the use of advanced tools makes the discovery of medicines faster. Detailed data is gathered through this metabolomics-based bioprospecting method. (36)

Methodical searches for rare plant species are helped by botanical gardens. These places keep living collections of hundreds of rare species from various places that are grown in controlled ways. This system allows a steady number of plants with known history and growth stages to be used. Turku’s botanical garden has a group of about 300 rare species, which acts as the main resource for this thesis. Taking samples of 90 species in one single search was possible because of this collection. A large panel of species was examined to provide the methodological basis of the present study. Experts claim that the analysis of many species together helps in understanding chemical diversity.

2-7) The chemical Identification, Structure-Based Varieties of Plant Polyphenols

A deep examination on Polyphenols has been placed by scientific researchers among the distinct types of secondary metabolites that botanical organisms produce for antimicrobial strength. Polyphenols are understood to be a wide and varied shape of organic items from nature that scientists define through the presence of multiple phenolic hydroxyl groups which are joined to aromatic ring systems. Experts claim that the starting point of the creation of Polyphenols occurs in the phenylpropanoid process. This chemical path begins with the amino acid phenylalanine because PAL-catalysed deamination and condensation create hydroxycinnamic acid derivatives, which are precursors leading to the major Polyphenols categories. (37) Various environmental duties are performed by Polyphenols, such as shielding from UV light and providing coloration like anthocyanins and aurones. It has been observed that Polyphenols act as messengers during the relationships between botanical organisms and microbes, specifically seen with isoflavonoids in legume-rhizobium symbioses. Chemical defences against harmful microorganisms and plant-eating animals are established through Polyphenols using tannins and other astringent compounds. These Polyphenols also serve as the fundamental components for the secondary cell wall when Polyphenols become oxidatively polymerised lignin.

Simple forms of Polyphenols are separated into two primary molecular groups which are hydroxybenzoic acid derivatives and hydroxycinnamic acid derivatives. It has been believe that gallic acid is a significant focus for this academic study because gallic acid is the single unit of gallotannins and the creator of ellagitannins. Because gallic acid contains a pyrogallol ring system, gallic acid is an easily reached target for the enzymatic and alkaline oxidation methods that are shown in Section 3. (38,39) Caffeic acid is recognized as the most frequent hydroxycinnamic acid that holds a catechol moiety. Scientists claim that this catechol moiety is the starting material for enzymes and an important physical sign of antimicrobial power when Polyphenols react with membranes or join with metals.

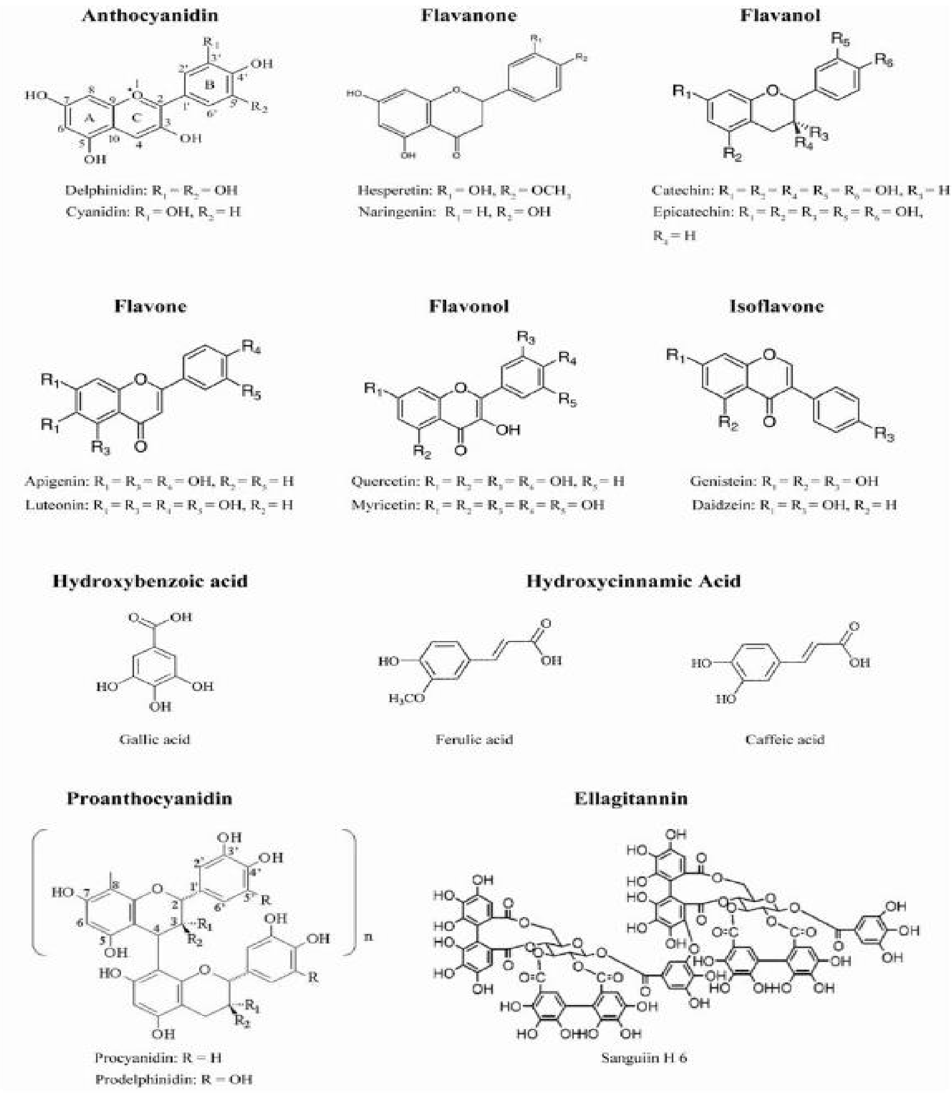


Fig 5: Core structural scaffolds of major plant polyphenol classes

Structural frameworks of Flavonoids possess a C6–C3–C6 carbon skeleton that contains two phenyl rings (A and B) which are joined by a three-carbon unit. This section gives the main pyranone structure or pyrone a conventional C-ring heterocycle properties. (37,38) A variety of individuals think that the classification into flavanols. flavones, flavanones, isoflavones, chalcones and anthocyanidins is defined by the standard saturation, oxidation condition and replacement pattern of this C-ring. Several of highly common natural compounds present in plant species are specific flavanols (quercetin, 3,3',4',5,7-pentahydroxyflavonol; kaempferol, 3,4',5,7-tetrahydroxyflavonol; myricetin, 3,3',4',5,5',7-hexahydroxyflavonol). These substances represent the most examined Flavonoids sub-class for anti-MRSA activity. Learned experts claim that the existence of a catechol group on the B-ring and a 3-hydroxyl on the C-ring of quercetin are structural reasons for the state of weakness of quercetin to enzymatic oxidation and various locations of antimicrobial activity. (40,41)

Certain polyphenols with high molecular weight are called Tannins because these molecules have a protein-precipitating ability. Such quality allows Tannins to work as a natural protection against herbivorous animals and as an origin point of antimicrobial activity and bioassay artifacts. Numerous researchers believe that the two main categories of Tannins are hydrolysable and condensed Tannins (proanthocyanidins, PACs). Generated sugars and phenolic acids are created when hydrolysable Tannins are broken down by weak acid. These Tannins are separated into gallotannins (polyesters of gallic acid and a polyol, usually glucose) and ellagitannins (polyesters of hexahydroxy diphenic acid, HHDP, and a polyol, while the HHDP group is created by oxidative coupling of two adjacent galloyl units). Bonded C4→C8 or C4→C6 carbon-carbon bonds connect condensed Tannins which are oligomers and polymers of flavan-3-ol units and resist acid or base breakdown.(39,42,43) Cloudberry (Rubus chamaemorus) seeds are the source for the ellagitannin sanguiin H-6, which holds significance to this thesis because sanguiin H-6 was identified as a former microbial inhibitor of MRSA biofilm formation in bacterial wound disease models after a study was performed by the research group in which this thesis was conducted. (44)

2-8) Anti-MRSA Mechanisms of Plant Polyphenols:

Because plant polyphenols use various molecular pathways toward MRSA, these substances are studied as an example of new antimicrobial agents. Experts claim that mechanical broadness is one of the most key features that separates plant polyphenols from common medicines, which usually have a single molecular target and are subject to specific gene immunity genetic changes. Figure 6 and table 1 theoretically demonstrate and outline the key anti-MRSA functions of plant polyphenols.

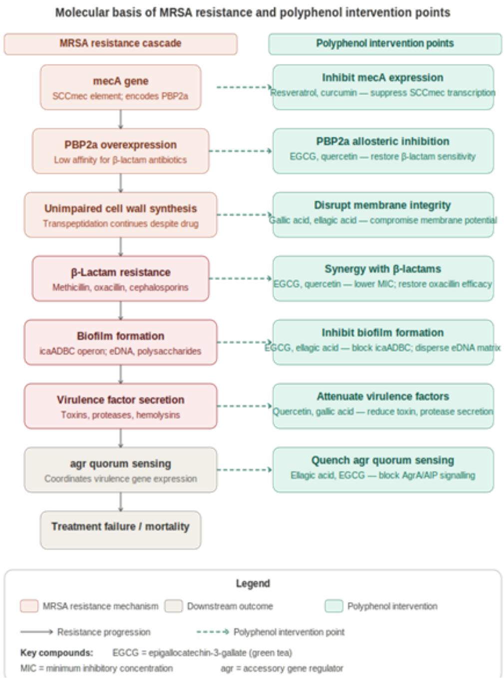


Fig 6: Core structural scaffolds of major plant polyphenol classes

Plant polyphenols are considered diverse by many researchers. Scientists have identified several plant polyphenols as inhibitors of enzymes involved in peptidoglycan biosynthesis through molecular docking and in silico analysis experiments. These enzymes operate at the bacterial cell wall synthesis level, such as MurA (UDP-N-acetylglucosamine enol pyruvyl transferase, the initial active process in peptidoglycan monomeric biosynthesis) and MurB (UDP-N-acetylenolpyruvylglucosamine reductase). (40,41) It has been observed that these inhibitors block the initial active process. Bacterial cell wall synthesis is hindered by these specific plant polyphenols. Many people believe that these objectives are now ahead of the PBP2a-dependent transpeptidation step. Because plant polyphenols induce cellular wall inhibition, scientists predict a preserved normal defence against MRSA strains expressing PBP2a. Plant polyphenols also contain hydroxyl groups that are powerful chelators of divalent metal cations (Ca2+, Mg2+, Zn2+, Fe2+), which are important cofactors for many bacterial enzymes and structural parts of lipopolysaccharide and membrane stability systems. Divalent metal cations are bound by these hydroxyl groups.

Membrane permeability and interruption is a second important mode of antimicrobial effects of plant polyphenols. Many plant polyphenols have an amphipathic arrangement, which integrates polarised phenol-like hydroxyl molecules with nonpolar, aromatic ring systems to help with splitting into microbial lipid bilayers. Plant polyphenols interfere with membrane organization and enhance unspecific ion permeability. This process neutralizes the proton motive force and leads to membrane degradation at a higher level. (45) This effect is well established for epigallocatechin gallate (EGCG), gallic acid and its esters, and condensed tannins through conventional fluorescent dye loss assays, electron microscopy of membrane morphology, and measurements of membrane potential dissipation. (45,46) Membrane interactions are influenced by the catechol group because its planar aromatic structure favours intercalation between phospholipid acyl chains.

One of the most important ways plant polyphenols work against MRSA is by stopping the NorA efflux pump from functioning correctly. This pump is a primary protein belonging to the major facilitator superfamily (MFS) that exists within Staphylococcus aureus. The movement of many items out of the bacterial cell, such as fluoroquinolones, biocides, and coloured substances like cationic dyes, is managed by this transporter. Because this process happens, MRSA develops resistance that is both natural and gained from its environment. (47,48) Research produced by Zhao et al. demonstrated that EGCG at levels of 12.5–25 μg/mL improved how beta-lactam antibiotics like oxacillin and ampicillin worked against clinical MRSA. These medical professionals observed that low fractional inhibitory concentration indices (FICIs) of 0.126–0.375 were present, which indicates that a high level of synergy between these substances exists. (49,50) In a similar way, epicatechin gallate (ECG) and other chemicals like gallate ester catechins stopped strong methicillin resistance from appearing in MRSA USA300. When these were used in combination, the oxacillin MIC dropped from ≥256 μg/mL to ≤2 μg/mL. (51,52) It is claimed by experts that kaempferol and quercetin stop NorA by sticking to the location where the pump recognizes its targets. Because this happens, the number of fluoroquinolones inside the cell stays at a level that kills the bacteria effectively. (47,48) Baicalein and the chemicals made from baicalein have also shown that the power of tetracycline and methicillin can be restored against MRSA. These results offer more evidence that plant polyphenols can fight against the ability of bacteria to resist medicine by stopping the pumps that move substances out. (47)

Other methods of fighting the bacteria include stopping the work done by bacterial DNA gyrase B. This protein is a confirmed target for medicines that is also neutralized by drugs like fluoroquinolones and coumarins. Scientists have seen this effect when EGCG, chebulinic acid, and mixtures of plant polyphenols from green tea are used in experiments. (46) During investigative procedures that look at how molecules connect to each other, quercetin, kaempferol, and myricetin were found as items that might stick to the control site of PBP2a. This location controls how substances enter the active part of the transpeptidase enzyme. This approach is physically different for beating the resistance to beta-lactam that PBP2a causes. Another way that plant polyphenols show their biological activity is by stopping the creation of biofilms and preventing the communication between bacteria at levels below the MIC. Because these levels do not create a pressure that kills the bacteria immediately, the development of common resistance is slowed down. Many people believe that kaempferol at levels of 64–128 μg/mL stops the early sticking stage when S. aureus biofilms are forming. This happens because the chemical interferes with how fibronectin-binding proteins are created by the cell. (40) Sanguiin H-6 stopped the growth of MRSA biofilms in a model that simulates how wounds get infected. This occurred at concentrations that can be reached using standard portions of cloudberry extract. These findings show that plant polyphenols like ellagitannins have a direct use in medical treatment for individuals with health conditions. (44)

Table 1: Plant polyphenol classes: structural features, anti-MRSA mechanisms, and predicted susceptibility to the chemical modification treatments used in this study.

Class	Key structural feature	Primary anti-MRSA mechanism(s)	Representative compound	Enzymatic oxidation (T1)	Nylon filtration (T3)	Alkaline oxidation (T2)
Gallotannins	Galloyl esters; pyrogallol moiety	Cell wall disruption; DNA gyrase; membrane permeabilization	Pentagalloylglucose, gallic acid	High	Partial	High
Ellagitannins	HHDP ester; hydrolysable	Biofilm inhibition; membrane disruption; anti-quorum sensing	Sanguiin H-6, vescalagin	High	Yes	High
Condensed tannins (PACs)	Flavan-3-ol units; C4-C8 C-C bonds; non-hydrolysable	Protein precipitation; membrane disruption; anti-biofilm	Procyanidin B2, ECG	Moderate	Yes (primary target)	Moderate
Flavanols	3-hydroxyflavone; A-C-B ring; C3-OH	NorA efflux pump inhibition; PBP2a binding; DNA gyrase; anti-biofilm	Quercetin, kaempferol, myricetin	Moderate	Minimal	Moderate
Catechin gallates	Flavan-3-ol; galloyl at C3; pyrogallol B-ring	NorA inhibition; beta-lactam synergy; DNA gyrase B	EGCG, ECG	High	Partial	High
Hydroxycinnamic acids	C6-C3; caffeic acid = catechol	Membrane disruption; cell wall enzyme inhibition	Caffeic acid, chlorogenic acid	High (caffeic)	Minimal	High (caffeic)

2-9) Barriers in the Identification of Essential Metabolites from Complex Plant Extracts:

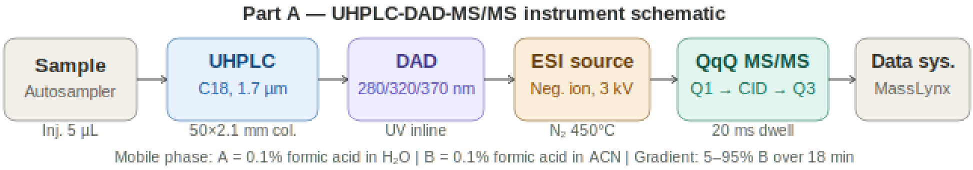
Experts claim that one significant and frequently ignored difficulty regarding plant extracted antimicrobial discovery involves demonstrating that raw mixtures stop a living creature from surviving while also identifying which specific chemical building blocks within the material cause the reported biological action. Raw mixtures of plant materials stay extremely complex chemical blends because raw mixtures usually consist of many unique chemical building blocks from various structural groups and concentration levels spanning multiple orders of magnitude. Many of these molecules might work together for biological action, which makes identifying the power of a single component through systematic bioassay-guided fractionation hard because fractionation separates helpful pairings by dividing chemical building blocks that work together. A decrease in antimicrobial activity during the separation process of an active raw substance is recognized frequently in natural product research. This reduction does not always show that the mixture lacks real medical power; instead, the loss of strength might show the collapse of a helpful interaction between several parts that have little power on their own. (53)

Many researchers believe that another vital issue in the regular testing of plant polyphenol bioassays comes from the possibility of wrong data because tannins possess a characteristic that causes proteins to clump together without selection. Both condensed tannins and large hydrolysable tannins represent materials that can stick to and spread out almost any protein they touch in a liquid, including surface proteins on bacteria and the enzyme systems used in testing. (43, 54-56) Inaccurate growth inhibition can be caused by this random protein clumping in tests that do not show true drug-like antimicrobial effects for making treatments. It has been observed that gut microbes break down tannins quickly while tannins also link strongly with food and saliva substances, so the way tannins move through the body is vastly different from the results seen in glass-dish tests. Therefore, separating true drug-like antimicrobial activity from fake testing results caused by tannins in plant extract screening remains a methodological necessity that requires specific lab tools and comparison groups. (55,57)

Mathematical and computational strategies provide a trustworthy path for addressing these issues through the chemical transformation of botanical extracts during the analysis of metabolite chemistry and biological activity. Experts claim that individuals working in science can link the precise removal or modification of specific metabolites in these extracts to changes in biological results. Due to these connections, the specific chemical groups that are responsible for the antimicrobial impact are discovered by researchers without needing to perform complete separation or isolation. (58) Such a strategy becomes more effective when many different and independent modification techniques are applied at once. When substances disappear or change during several separate treatments and show a consistent loss of biological function, the data provides a stronger proof of cause than a single treatment provides. This interdisciplinary combination of enzymatic oxidation, alkaline oxidation, and nylon filtration, which serves as the primary basis for the research project, utilizes specialized techniques. The study focuses on the specific knowledge that the Salminen group holds regarding polyphenol oxidative chemistry. (58-60)

2-10) UHPLC-DAD-MS Method for Intensive assessing of Common Polyphenol Metabolites:

Modern analytical chemistry methods have completely changed the way scientists find polyphenol metabolites in difficult plant extracts because these methods provide a clarity of separation that was once impossible to reach. Because sub-2 µm particles are used in UHPLC columns, a significantly superior clarity of separation compared to standard HPLC is achieved. The time period for this examination procedure is reduced by three to 10 times because of the Van Deemter correlation among particle size, plate height and optimal movement speed. (61) Working at higher flow rates (0.3–0.6 mL/min) is possible when using sub-2 µm particles while scientists avoid a loss of clarity, even though these particles require machinery designed for functioning at pressures >400 bar. Experts claim that UHPLC systems are necessary because typical HPLC systems are rated only to a maximum of ca. 400 bar. Because the clarity of separation is high and the running times are short, which is typically 15–20 min for a full polyphenol separation, UHPLC is chosen for high-throughput screening of large numbers of plant species.



Part B — Group-specific MRM transition panel

Compound	Polyphenol class	Ion mode	Precursor (m/z)	Product ion (m/z)	CE (eV)
		Hydroxybenzoic acids			
Gallic acid	Phenolic acid	[M−H]□	169.0	125.0 (Q) / 79.0 (q)	18
Ellagic acid	Ellagitannin core	[M−H]□	300.9	145.0 (Q) / 229.0 (q)	25
		Flavonols			
Quercetin	Flavonol	[M−H]□	300.9	151.0 (Q) / 121.0 (q)	22
Kaempferol	Flavonol	[M−H]□	285.0	151.0 (Q) / 93.0 (q)	24
Myricetin	Flavonol	[M−H]□	317.0	179.0 (Q) / 151.0 (q)	20
		Flavanols and condensed tannins (proanthocyanidins)			
Catechin	Flavan-3-ol monomer	[M−H]□	289.1	245.1 (Q) / 205.1 (q)	16
EGCG	Flavan-3-ol gallate	[M−H]□	457.1	169.0 (Q) / 125.0 (q)	28
Procyanidin B2	PA dimer (4β→8)	[M−H]□	577.1	289.1 (Q) / 407.1 (q)	30
Procyanidin B1	PA dimer (4β→8)	[M−H]□	577.1	289.1 (Q) / 245.1 (q)	28
		Hydroxycinnamic acids			
Caffeic acid	Hydroxycinnamic acid	[M−H]□	179.1	135.1 (Q) / 107.0 (q)	16
Ferulic acid	Hydroxycinnamic acid	[M−H]□	193.1	134.1 (Q) / 178.1 (q)	18

Fig 7: UHPLC-DAD-MS/MS instrument schematic and group-specific MRM transition panel

Tandem mass spectrometry (MS/MS) with standard electrospray ionisation (ESI) gives both molecular mass data and physical arrangement data when UHPLC is used. This data comes from the intact deprotonated [M-H] ^- ion in a negative mode and from product ions generated by collision-induced dissociation, CID, of the precursor ion in the collision cell of a triple-quadrupole instrument. (61) It has been observed that negative ion mode ESI is liked better for polyphenols because the deprotonation of phenolic hydroxyl groups happens easily under mildly alkaline spray conditions. These conditions create a gas-phase pH of ~7-9 in the spray origin point, which gives substantial amounts of [M-H] − ions with an important level of detection. The background spectra remain clean compared to the positive mode because the positive mode creates complex adduct patterns for polyphenols. UV-visible absorbance data is provided by the diode-array detector (DAD) located between the UHPLC column and ESI source. Many people believe that this data helps identify substances when flavanols absorb characteristically at 350–380 nm, hydroxycinnamic acid derivatives at 310–330 nm and condensed tannins and gallic acid derivatives at 270–290 nm.

Significant analytical progress for the high-performance bioprospecting technique used in this scientific inquiry resulted from the creation of group-specific multiple reaction monitoring (MRM) methods for extensive polyphenol fingerprinting by Engström, Pälijärvi and Salminen (University of Turku).Experts claim that group-specific MRM methods use the reality that every member of a structural polyphenol class has shared distinct fragmentation transitions, including neutral losses and product ion masses, regardless of the unique substitution pattern of individual substances within the class. Within the MRM mode which relies on a triple-quadrupole (QqQ) technical apparatus, the first quadrupole (Q1) chooses the precursor ion that identifies a specific polyphenol class. The second quadrupole (Q2) serves as the collision cell where specific collision energies (typically 15–35 eV) cause CID fragmentation. The third quadrupole (Q3) selects a final product ion belonging to that same category, and this identification is confirmed when the chromatographic peak space is measured by the technical apparatus. Flavanol glycosides produce a shared aglycone fragment because the sugar portion is lost in Q2. Gallotannins provide a distinct galloyl fragment at m/z 169→125 under CID. Ellagitannins generate an HHDP-derived fragment. (62-64) Compound-specific MRM methods require unique optimized transitions and reference standards for every substance. This specific requirement prevents the simultaneous profiling of hundreds of metabolites across many polyphenol classes during a single analytical run. The group-level approach allows for profiling across a massive collection of biological organisms and treatment environments, which many people believe is a superior strategy.

2-11) Aims and Objectives of this study:

Primary intention of this master's thesis focuses on expanding the systematic discovery of MRSA-resistant metabolites from infrequently found and rare plant species found in the University of Turku botanical garden. Creation and verification of a multi-processing chemical modifying protocol is desired because this master's thesis aims to pinpoint the specific categories of metabolites that trigger the recorded antimicrobial bioactivity. Many people believe that the research study functions as a component of the larger research programme of the Natural Chemistry Research Group at the University of Turku. Global fame was earned by the Natural Chemistry Research Group due to its creative additions to plant polyphenol phytochemistry and UHPLC-MS/MS metabolite profiling methodology.

Specific objectives of the research study involve several distinct targets. Examination of 90 chosen rare plant species from the University of Turku botanical garden occurs to find MRSA growth inhibitory activity and to define the polyphenol metabolite profiles of all 90 species via group-specific UHPLC-DAD-MS/MS. Usage of six various chemical transformation procedures is required, which include enzyme-dependent oxidation of fresh tissue samples, alkaline oxidation of extracts from plants at pH 10, nylon filtration for specialised tannin elimination, and two successive combination methods (enzymatic + alkaline oxidation; nylon filtration + alkaline oxidation) for every extract. It has been observed that the MRSA inhibitory activity and metabolite profiles of every extract must be scrutinized after every treatment is finished. Linking modifications in metabolite synthesis with alterations in MRSA bioactivity happens through multivariate analysing statistical analysis (PCA and PLS regression) while processing all collected species. Verification of whether the multi-processing technique demonstrates the true active metabolites more effectively than any single process type is performed so that a reversible and extensively cultivated suitable standard for antimicrobial bioprospecting from complex plant extract libraries can established.

3- Methodology:

3-1) Background:

The specific procedure in this research helps in building the foundation for what the scientific methods have been invented to achieve the purpose behind every section. This thesis document exists at a meeting point of following three areas.

- Antimicrobial resistance as a medical and community health difficulty,
- Plant polyphenols as a chemically diverse and unstudied origin of antimicrobial leads,
- A specific investigative logic created in the Natural Chemistry Research Group at University of Turku.

This logic was used to distinguish truly functioning metabolites from substances that only appear active because of non-specific tannin protein interactions in the laboratory test. It has been claimed that these three starting points dictate the path of this study. All methodological decisions were formed because these three starting points provided a clear direction.

3.1.1) Solution of the MRSA bacteria problem:

Methicillin-resistant Staphylococcus aureus (MRSA) was named a "high priority" harmful organism in the 2024 update of the WHO Bacterial Priority Pathogen List. The GBD 2021 analysis reported a double growth in MRSA-related deaths between 1990 and 2021, which causes great alarm for medical professionals. It has been observed that the antibacterial development sequence has produced very few truly novel chemical categories during the last forty years. Plant secondary metabolism remains insufficiently utilized as a source for discovery because researchers have focused on other areas. Fewer than ten percent of the approximately 374,000 plant species have been subjected to any medicinal research. The number of species evaluated against specific drug-resistant harmful organisms such as MRSA is even smaller even though many potential cures exist. Practical access to this unknown area is offered by the University of Turku Botanical Garden with its collection of about 300 rare species in controlled cultivation. This collection was selected to provide the large scale necessary for a comparison-based metabolomics study.

3.1.2) Decision for diverse kinds of treatment of Plants Polyphenols:

Rather than being a single type of substance, Polyphenols represent a diverse group of chemical structures including phenolic acids, flavonoids, hydrolysable tannins (gallotannins and ellagitannins), and condensed tannins (proanthocyanidins) which are joined by the existence of phenolic hydroxyls on aromatic rings. Polyphenols are manufactured by flora primarily for protection, and several distinct biological targets are affected by their reported anti-MRSA actions. Researchers suggest that Polyphenols function through stopping the NorA multidrug efflux pump, fixing beta-lactam utility via cell-wall changes, binding DNA gyrase B, making membranes leaky, and stopping biofilm creation at low amounts. Because these actions are so wide, Polyphenols are studied as a group since making resistance is difficult for bacteria when many targets are hit at once. A major difficulty exists because tannins bind to proteins in a way that is not specific. It has been observed that a raw liquid taken from plants might look like it kills germs in an Eppendorf tube simply because tannins cover the surface of the bacteria. Distinguishing the actual germ-killing power from this false experimental result remains the primary problem for this study.

This methodology was first used on 20 samples in Lab Project 1 and has now grown to include 90 biological types where each liquid taken from plants is changed chemically to remove specific parts of the Polyphenols. Each liquid taken from plants is subjected to treatments that reveal which change makes the germ-killing power vanish. It's been believed that if the germ-killing power goes away when tannins are removed, then tannins were the cause of the result. When the germ-killing power remains after tannins are gone but stops after oxygen-based changes, the active chemicals are catechol-type or pyrogallol-type substances. If the germ-killing power continues after all these steps, the active portion of the substance is a different type of chemical and deserves the effort of isolation.

3.1.3) Design of Samples Treatments:

Six treatments are utilized to establish the research investigation for 90 best plant species in this exploration. A single treatment among these remains as the untreated samples labelled as T0, which serves as the baseline standard that maintains the entire polyphenol pattern exactly as the living organism possesses. The other five units previously mentioned represent different alteration methods which includes enzymatic oxidation (T1), alkaline (pH10) oxidation (T2), PPC (Nylon filtration) labelled as (T3) to extract protein-clinging tannins, and the two combinations of these processes which are (Enzymatic oxidation+ pH10 oxidation labelled as (T4) and (PPC + pH 10 oxidation labelled as (T5)). The reason of this approach is to see that which compounds are being lost or changed in this altered treatment against the raw baseline standard of that same species for each of the plant sample. When the variation in MRSA suppression between the two is analysed, the change demonstrates how significant that specific group of natural compounds was for the biological action. The table below labels the six treatments from T0 to T5. Although the science remains identical, the naming system appears more organized within my research.

Table 2: workflow of different treatments

Thesis code	Treatments Types	Procedure	Metabolite target	Goal
T0	A (control)	Untreated extract; 0.22 µm PES filtration only	Full polyphenol profile preserved	Baseline activity for every other treatment to be compared against
T1	B (Enzymatic Oxidation)	Enzymatic oxidation of fresh tissue at ~30 °C, ~2 h; then freeze-dry	Loss of some active compounds are not	Loss of activity means some of the active compounds are lost

Thesis code	Treatments Types	Procedure	Metabolite target	Goal
T2	Alkaline pH10 oxidation	Alkaline oxidation at pH 10, 60 min, then quench with formic acid	Same target class as T1 but driven chemically; broader substrate scope	A chemical complement to T1; together they bracket how oxidation-sensitive the active fraction is
T3	PPC (Nylon filtration)	0.45 µm polyamide (nylon) syringe filtration	Selective retention of protein-binding tannins on the polyamide membrane	If activity drops here, it was tannin-mediated the strongest single piece of evidence for or against tannin artefact
T4	Combination of T1 and T2	(Enzymatic Oxi+pH 10 oxidation)	Catechol/pyrogallol metabolites depleted maximally	Strongest test of whether oxidation-sensitive compounds were responsible for activity
T5	Combination of T3 and T2	(Nylon filtration +pH 10 oxidation)	Tannins removed first, remaining polyphenols then oxidised	Isolates the non-tannin, oxidation-sensitive fraction; useful if T3 alone does not kill activity but T5 does

3.1.4) UPLC-MS/MS analysis of all Treatments:

Every plant species extract and the five other treatments were examined by utilizing a Waters Acquity UPLC-MS/MS instrument linked to a Xevo TQ-S triple-quadrupole weight-measuring device using electrical spray ionization in negative setting. Substance division is performed upon a BEH C18 pillar (1.7 µm, 2.1 × 100 mm) through a two-part flowing mixture of acid-treated water and acetonitrile. This arrangement was created by Engström, Pälijärvi and Salminen for polyphenol identification. It has been observed that light-based verification is provided by light-detecting devices at 280, 310, 320 and 360 nm (acidic phenols at 280 nm, hydroxycinnamic acids at 310–320 nm and flavanols at 360 nm).

The justification for performing MS/MS in class-focused multiple reaction monitoring (MRM) style, instead of individual-molecule MRM, remains essential to the magnitude of this research.

Because MRM for every individual molecule necessitates a comparison material and a specifically adjusted parent-to-child shift, the individual-molecule method is impossible for 90 species and hundreds of natural compounds. Class-focused MRM utilizes the reality that all members of a specific polyphenol category possess an identifying neutral reduction or daughter particle regardless of their side-group arrangement. It has been stated that all gallotannins break apart through a galloyl particle m/z 169 → 125. All ellagitannins discard an HHDP piece (m/z 301 → 239). All flavan-3-ol chains break apart through a 289 → 245 → 137 route. All flavanol sugar-compounds lose their sweet part to yield the core molecule. The scientific tool is instructed to search for these category-identifying shifts throughout the measuring period. One strategy collects the entire polyphenol pattern of a plant species extract in a single 14-minute flow. The final data is presented as a grid of height measurements for every category, every species, and every treatment. This grid provides the specific shape required for the PCA and PLS math evaluations that occur afterward. These math evaluations are conducted to offer a strong explanation of the data while allowing for the recognition of major patterns and ties within the polyphenol patterns. The results are expected to help in gaining a superior grasp of the chemical traits and potential wellness advantages of the plant species extracts.

3-2) Procedural Steps:

This section walks through the actual workflow in the order that the experiments were carried out. The following steps were performed in this research:

- Collection of Samples
- Oxidative pre-treatment of B samples
- Weighing of Samples
- Extraction and Centrifugation
- Removal of Acetone in a concentrator
- UPLC-MS/Ms treatments of all samples

Handling
Grinding
Maceration
Extraction
Freeze drying

3.2.1) Collection of Plant Samples:

The University of Turku Botanical Garden provided all plant samples from its living collection because this educational institution maintains approximately 300 rare species in a managed environment. Two distinct periods were used for the collection of these samples. It has been observed that the pilot work in Lab Project 1 utilized 20 species, which were selected so that leaf, flower, stem, and branch tissue across tropical and temperate groups could be tested as an initial check of the process. Ninety species were then included in the thesis study because these plants met three specific requirements. The selection of the plant material was made based on having sufficient living tissue for two separate tests without harming the garden specimen, wide representation of families with many polyphenols, and extraordinarily little previous research in scientific papers.

I took two tissue samples from every sample during the same day, using the same individual plant when the situation permitted. Labels A and B were attached to these tissues now of cutting; A acted as the control that keeps the natural chemical state, while B was prepared for the process of changing through enzymes. Most samples consisted of leaf tissue, although fruit, flowers were gathered as it is indicated that those parts contained more polyphenols. My tools were cleaned before I cut the plant material, then I placed the pieces into containers that were already marked inside a cold box so that the plant material would stay cool.

3.2.2) Oxidative pre-treatment of the B replicates:

The two separate replicates followed different paths once I arrived at the research facility. All A replicates were placed immediately into the freeze-drier so that no oxidation occurred. I put the B replicates into a Memmert Digital Oven (Model 600) at 30 °C for two hours, after which the plant material was returned to the freezer to halt the change. Many people believe that warming the damaged tissue activates the own enzymes of the plant material like polyphenol oxidase (PPO, EC 1.14.18.1) and laccase-type enzymes (EC 1.10.3.2) because these are freed when the cell walls break. Oxygen in the air is used by these copper enzymes to transform certain polyphenols into ortho-quinones, which then join in chains. This process is the same as when a sliced apple becomes brown. All replicates were freeze-dried and then put into bags with the air removed so that the samples would not gather moisture during the time they were stored in the freezer.

3.2.3) Grinding:

I moved the dried plant material from the storage place into marked 2 mL Eppendorf tubes, which were given codes HZ Bot A for the A replicates and HZ Bot B for the B replicates. Two clean stainless-steel grinding balls were dropped into every tube. It has been observed that the ball mill was operated for approximately 30 minutes until the plant material became a fine powder that flows easily. A magnet was used to remove the metal balls from extracts.

3.2.4) Weighing and Maceration:

Polyphenol extraction was performed using a mixture of acetone and water in an 80:20 ratio. This chemical liquid performs better than methanol or ethanol because of specific chemical reasons. Such chemical liquid pulls out diverse types like gallotannins, ellagitannins, proanthocyanidins, flavonoid glycosides, and phenolic acids. High harvested amounts are achieved because the chemical liquid captures more substances than alcohols. It has been observed that this chemical liquid prevents tannins from bonding with proteins, which ensures high-molecular-weight tannins are not missed.

Substantial amounts of the chemical liquid were prepared from 800 mL reagent-grade acetone and 200 mL UPLC-grade water. Every ground sample weighing approximately 50 mg was put into a fresh 2 mL tube using a weight-measuring instrument. The target weight was 50 mg, while the weight-measuring instrument showed the range of 49.50 to 50.50 mg. Every mass for A and B replicates was written down to two decimal places. Then, 1400 µL of the 80:20 chemical liquid was added to each tube. Tubes were vortexed for five minutes, while followed by passing through the particle-crushing apparatus twice for 30 seconds to crush the particles. For the polyphenols to enter the solution, the tubes were kept in a low-temperature storage area at 4 °C during the night.

3.2.5) Extraction and Centrifugation:

Two extractions on different days were done to complete the maceration. Approximately 90% of the extractable polyphenols were pulled out during the first stage. The second extraction with fresh chemical liquid on the remaining solid residue captures the final 10%. The second chemical liquid was mixed with the first chemical liquid after the process finished.

Shaking of the tubes happened for three hours at 300 rpm after the long soak. Centrifugation followed at 14,000 rpm for 10 minutes so that the plant material stayed at the bottom. Such a process ensures the remaining top liquid stays clear. The remaining top liquid was moved into new 2 mL tubes used for concentrating. Commonly, it has been observed that re-extracting the solid residue with 80:20 chemical liquid for the second maceration improves the results. Shaking and spinning happened again, and the second remaining top liquid joined the first remaining top liquid in the same tube.

3.2.6) Concentration and Acetone-removal:

Concentration of the combined chemical liquids took place on evaporation equipment for 1.5 to 2 hours. Air-allowing covers with small holes were used so the acetone gas could leave the tube. Only a portion of the chemical liquid was removed because dryness is not the goal. Keeping space in the tube is necessary because the second extraction needs room. After the second maceration was added, the aqueous extract was processed for another 2 to 2.5 hours. Deepening color served as a sign that the acetone was leaving. A concentrate that is entirely water looks darker than a concentrate that still has acetone.

3.2.7) Freeze-Drying of the extracts:

Freeze-drying changed the concentrated aqueous extracts until they became a dry polyphenol powder overnight. Each tube was checked for small pieces stuck to the lid after the process. The dry powder residue was put back into the tube, even though some deviations were recorded against the sample numbers. Solid sealing caps replaced the air-allowing covers for protection. The storing the dried extracts at −20 °C keeps them safe until UPLC-MS/MS analysis.

3.2.8) Sample preparation for UPLC-MS/MS analysis of the control extracts:

Untreated A replicates fulfilled two specific working positions when the study started on the UPLC-MS/MS. These control extracts provided the baseline polyphenol profile that every treated variant would later be measured against, while these same extracts indicated which species were suitable for chemical treatments. It has been observed that the baseline must be established before the experiment proceeds because the baseline defines the starting point for the polyphenol content.

The preparation procedure used freeze-dried extract powder as the starting material. Every dried A extract was placed back into a liquid mixture using 500 µL of Milli-Q water, then the mixture was vortexed for five minutes until the powder achieved full rehydration. A PTFE syringe filter was used to strain the liquid into a clean 1.5 mL tube so that any solid debris would not obstruct the column or the autosampler line. To create the injection sample, 50 µL of that stock was combined with 700 µL of Milli-Q water and vortexed for 15 minutes. This thinning process is significant because the thinning process places every extract on the same concentration scale. The equalizing concentrations allows for the comparison of peak areas between species without causing the detector to become overloaded by the strongest extracts. For the inner-tube format, 100 µL of the diluted sample was pipetted into a glass insert and the insert was tapped on the bench to remove any air bubble before the loading process began.

The analytical run was conducted on a Waters Acquity UPLC joined with a Xevo TQ-S triple-quadrupole mass spectrometer using negative-mode electrospray and a BEH C18 column. The diode-array detector performed readings in parallel while the machine operated. A group-specific MRM method was employed for the analysis, which identifies the fragment transitions that each polyphenol class shares instead of identifying one compound at a time. A single injection is sufficient to return the full polyphenol fingerprint of the extract when this method is used.

The selection of samples happened within the control run stage. Peak areas across the polyphenol classes were read for each species, and a working cut-off was established at 10 000 area units. An extract clearly contained enough polyphenol to produce a solid signal when the value remained above that number. It has been observed that a treatment which strips or oxidizes part of the polyphenol will still result in a measurable change if the initial signal is strong. These samples provided the data that the whole multi-treatment comparison required to function properly even though the process was complex.

A different situation existed for samples measuring below 10 000 area units. Because truly little polyphenol existed at the start, any shift occurring after enzymatic oxidation or nylon filtration would disappear into the background noise. Experts claim that processing these samples through the full treatment series would waste time and material for numbers that lack reliability. The species with good control areas above 10 000 were the only ones carried into the enzymatic oxidation and nylon filtration treatments while the others were excluded.

4-Experimental Section:

The entire scientific study utilized the 300 plant species collection of the University of Turku Botanical Garden. Each biological species was gathered as two identical replicates. One replicate remained untreated as group A and the other replicate was enzymatically oxidised as group B. These botanical materials were dehydrated and crushed and prepared in 80:20 acetone/water. The extracts were concentrated and freeze-dried. Replicates of group A were then reconstituted for profiling through UPLC-MS/MS. The profiling stage narrows the experimental focus because only species whose control extract possessed enough polyphenol for meaningful observation proceeded to the chemical procedures and the MRSA assays.

4.1) UPLC-MS/MS profiling of the control extracts and sample selection:

Preparation for the control evaluation followed the steps for sample preparation. Each freeze-dried A extract was reconstituted in 500 µL of Milli-Q water. The liquid was shaken for 15 minutes and moved into a 96-well plate for filtration. After the filtration happened, the extract was placed in a collection plate. The samples were diluted for injection while 50 µL of the filtered extract was mixed with 450 µL of Milli-Q water. A second 15-minute shaking process occurred. It has been observed that 200 µL of each diluted sample must go into the analysis plate for UPLC-MS/MS on the Xevo instrument. The group-specific MRM method reads each polyphenol class by observing the specific fragment transitions.

4.1.1) Interpretation of data in the masslynx software:

All biological samples were processed in MassLynx up to the 300A point. The peak areas for every polyphenol category were copied into an Excel document. Every sample that possessed a sufficient area was marked. The cut-off of 10 000 area units serves as a good working rule. A class peak above that number was seen as a real and readable signal even though some signals were low. A species with one or more classes above this limit was selected for further work. Any species where every class stayed near the baseline was recorded as "practically nothing" and discarded. The specific categories that the scientific investigation followed were galloyl derivatives, HHDP (ellagitannin) derivatives, catechin, gallocatechin, PC (procyanidin) oligomers and polymers, and PD (prodelphinidin) oligomers and polymers.

Among the 300 A extracts, 101 extracts showed good areas in at least one polyphenol category while 199 extracts showed almost no results. Table 3 explains the way the successful hits are divided among the categories. It can be showed that the strongest samples are the ones that showed results in several categories simultaneously. These strong biological samples represent the core of the treatment study.

Table 3. Summary of the 300-sample control screen. "Good area" means at least one polyphenol class above the working cut-off; a sample can appear under more than one class.

Screen outcome / class	Count	Notes
Samples with good area (any class)	101	carried forward to selection
Samples with nothing	199	set aside
— galloyl derivatives	25	hydrolysable, gallotannin-type
— HHDP / ellagitannin derivatives	32	hydrolysable, ellagitannin-type
— catechin (in oligomer/polymer context)	42	flavan-3-ol building block
— PC oligomers / polymers	96	condensed tannins (procyanidin)
— PD oligomers / polymers	49	condensed tannins (prodelphinidin)

4.2) Weight of samples:

I had done the weight of approximately 50mg for all the samples requires for further studies. The plant mass could range between 49.50 to 50.50 mg. The below sample table no. 4 represents the sample number and plant species which were interesting after interpretation of data.

Table 4: weight of samples

No.	Sample No.	A mass (mg)	B mass (mg)	No.	Sample no.	A mass (mg)	B mass (mg)

1	**01**	**50.18**	**50.34**	46	**188**	**50.41**	**49.90**
2	**04**	**50.31**	**50.00**	47	**190**	**50.38**	**50.21**
3	**19**	**50.40**	**50.21**	48	**191**	**50.20**	**49.93**
4	**25**	**50.40**	**50.11**	49	**193**	**50.03**	**50.14**
5	**34**	**50.14**	**50.27**	50	**194**	**50.29**	**49.96**
6	**44**	**50.10**	**49.97**	51	**195**	**49.99**	**50.20**
7	**45**	**50.14**	**50.03**	52	**197**	**49.98**	**50.13**
8	**48**	**50.09**	**50.18**	53	**198**	**50.11**	**50.06**
9	**53**	**50.34**	**50.03**	54	**199**	**50.03**	**50.04**
10	**55**	**49.97**	**50.14**	55	**203**	**50.12**	**50.27**
11	**56**	**50.31**	**50.33**	56	**206**	**50.15**	**50.16**
12	**58**	**50.36**	**50.18**	57	**208**	**50.28**	**50.37**
13	**62**	**50.45**	**50.01**	58	**210**	**50.42**	**50.01**
14	**63**	**50.20**	**50.19**	59	**211**	**50.17**	**50.23**
15	**70**	**50.15**	**49.98**	60	**212**	**49.99**	**50.04**
16	**74**	**50.10**	**49.89**	61	**213**	**50.23**	**50.14**
17	**80**	**50.11**	**49.86**	62	**214**	**50.16**	**50.10**
18	**84**	**50.36**	**50.20**	63	**225**	**50.06**	**50.20**
19	**86**	**50.19**	**49.87**	64	**228**	**50.25**	**50.38**
20	**88**	**50.43**	**49.97**	65	**229**	**50.30**	**50.27**
21	**89**	**50.18**	**50.01**	66	**238**	**50.35**	**50.07**
22	**92**	**49.99**	**49.72**	67	**242**	**50.36**	**50.19**
23	**96**	**50.02**	**50.07**	68	**243**	**50.22**	**50.10**
24	**100**	**50.26**	**50.13**	69	**245**	**50.16**	**50.10**
25	**106**	**50.37**	**50.26**	70	**249**	**49.98**	**50.26**
26	**110**	**50.10**	**50.14**	71	**253**	**50.11**	**49.92**
27	**111**	**50.36**	**50.05**	72	**254**	**50.17**	**50.44**
28	**112**	**50.25**	**50.15**	73	**257**	**50.23**	**49.90**
29	**113**	**50.18**	**50.29**	74	**258**	**50.36**	**50.17**
30	**120**	**50.26**	**50.35**	75	**264**	**50.07**	**50.25**
31	**125**	**50.40**	**50.10**	76	**269**	**49.85**	**50.02**
32	**126**	**50.00**	**50.15**	77	**270**	**49.92**	**50.24**
33	**129**	**50.02**	**50.12**	78	**271**	**50.31**	**49.72**
34	**133**	**50.28**	**50.01**	79	**273**	**50.03**	**50.29**
35	**137**	**50.22**	**50.10**	80	**275**	**50.28**	**50.35**
36	**148**	**50.19**	**50.14**	81	**276**	**50.26**	**50.22**
37	**159**	**50.18**	**50.33**	82	**279**	**50.31**	**50.03**
38	**161**	**50.31**	**50.35**	83	**281**	**50.06**	**50.00**
39	**163**	**50.23**	**50.01**	84	**284**	**50.19**	**50.18**
40	**164**	**50.42**	**50.05**	85	**285**	**50.21**	**49.87**
41	**165**	**50.45**	**49.94**	86	**288**	**50.10**	**49.98**
42	**166**	**50.23**	**50.37**	87	**289**	**50.06**	**50.20**
43	**169**	**50.19**	**50.03**	88	**290**	**50.17**	**50.27**
44	**172**	**49.97**	**50.11**	89	**291**	**50.33**	**50.35**
45	**176**	**50.01**	**50.11**	90	**293**	**50.13**	**50.20**

4.3) Precipitation testing before the preparation of samples for bacteria analysis:

Before any selected sample went to MRSA testing it went through a precipitation screen, run from the protocol Martta Airola wrote for the Natural Chemistry Research Group in 2024. The screen exists because tannin-rich extracts can drop out of solution the moment they hit bacterial growth medium, and an extract that has precipitated is no longer available to act on the bacteria. Without this check a blank result in the antibacterial assay would be uninterpretable: it could mean no activity, or it could just mean no compound left in solution. Turbidity from precipitation also looks the same as bacterial growth to a plate reader, which is the other reason to measure it directly first. The medium was Tryptic Soy Broth (TSB), taken from either the biology lab or the biomedical department. TSB must be used within six hours of opening; past that I could not assume it was sterile and opened a fresh bottle.

4.3.1) Initial Screening:

Samples were thawed and shaken for an hour before pipetting. I put 50 µL of each extract into a single well of a 96-well flat-bottom plate, no duplicates at this stage, and filled H12 with 50 µL of the sample solvent (water) as the plate background. With the reader set to 595 nm, I added 150 µL of TSB to every well and read absorbance straight away and again three hours later. Subtracting the H12 background from each well gives the precipitation signal on its own.

4.3.2) Selection of dilution series:

The most strongly precipitating samples from the initial screen went on to a dilution series. I put 100 µL of each into row A and pre-loaded 50 µL of water into rows B to G and down the whole of column 12. With a multichannel set to 11 tips, I moved 50 µL from row A to row B, mixed by pipetting three times, and carried on down, B to C, C to D, and so on to H. That builds a series of 1:1, 1:2, 1:4, 1:8, 1:16, 1:32, 1:64 and 1:128 across the plate. Then 150 µL of TSB into every well, and absorbance at 595 nm immediately and again after three hours of shaking. The point of the series is to find the dilution at which each extract stops coming out of solution, so the MRSA assay can be set safely above it.

4.4) Preparing the bacterial measurement assays solutions:

With the precipitation behaviour mapped, I picked a 15-fold dilution as the working concentration for the MRSA assay. The reasoning, close to how I wrote it at the time, is that the assay has to hold enough extract for the bacteria to respond if there is anything to respond to, but not so much that the extract has already precipitated before the bacteria can meet it. The 15× point sits on the soluble side of the precipitation curve while keeping a meaningful polyphenol concentration. A higher dilution would clear the solution entirely but would also dilute the compounds away to nothing.

Working dilutions were made by combining 40 µL of each sample with 560 µL of water, giving 600 µL of 15× diluted extract per sample. The arithmetic is one part sample to fourteen parts solvent, fifteen parts in total. I vortexed the diluted samples for an hour to homogenise them, then split each one, half to the MRSA plate and half back to the freezer as backup.

4.5) MRSA growth inhibition assay:

Activity against MRSA was measured by broth microdilution in 96-well flat-bottom plates, following the EUCAST and CLSI M07 guidance. Reservoir plates held 500 µL of each 15× diluted extract per well, with the first six wells of row A kept for the reference set (Argentina Answering at 1 mg/mL in 10% ethanol at a 2× working dilution, water, 5% or 10% ethanol depending on the run, medium plus bacteria as the growth control, medium alone, and an empty well). Forty-two extracts filled rows B to H of each reservoir plate, and three reservoir plates covered the 90-sample set plus the 13 extra B samples added later in the time-course. The Bella platform robot transferred the samples into the assay plates in duplicate. Three plates ran for MRSA, plus three each for E. coli and S. aureus as reference organisms, nine plates per run. Plates were incubated at 37 °C for 18 to 20 hours and read at OD 600 nm, and the full set was run in biological duplicate across two independent days.

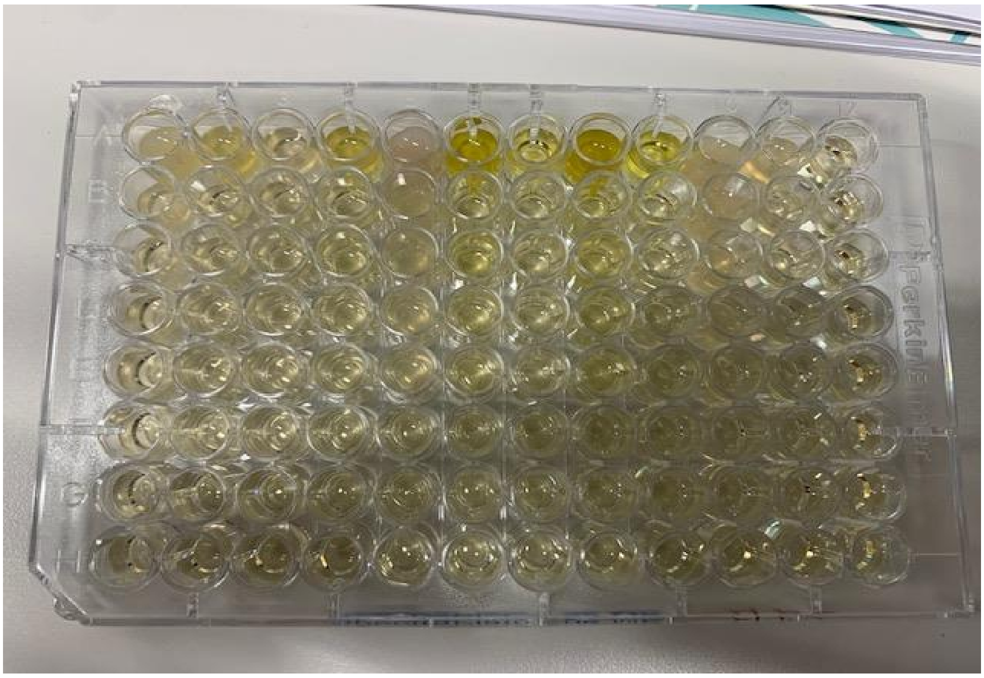


Fig 8: Preparation of control samples for bacterial strains (S. aureus, E. coli and MRSA)

Fig 9: Preparation of Enzymatic oxidation samples for bacterial strains (S. aureus, E. coli and MRSA)

Fig 10: Preparation of nylon filtration samples for bacterial strains (S. aureus, E. coli and MRSA)

4.6) Different treatments:

The study was designed around six conditions: the untreated control, enzymatic oxidation, alkaline oxidation at pH 10, nylon filtration, and two combinations built on the alkaline step (enzymatic plus alkaline, and nylon plus alkaline). In the end three of these were not carried out: the pH 10 alkaline oxidation and both combination treatments that depend on it. I stopped at control, enzymatic oxidation and nylon filtration for reasons of time and for practical and technical constraints in running and reading the extra oxidation steps within the period available for the study. The three completed conditions still span the two most informative axes, tannin removal by nylon and oxidation of the catechol and pyrogallol phenolics by the enzymatic step, which is enough to sort the library into treatment-robust and treatment-sensitive hits. The pH 10 and combination treatments remain a natural extension for future work.

4-7) Data handling:

UPLC-MS/MS areas were processed in MassLynx, exported to Excel, and normalised to extract dry mass. MRSA inhibition for each sample and treatment was calculated from the 24-hour endpoint OD against the solvent-control reference on each plate, then averaged across the two replicates with the standard deviation recorded.

5-Results and Discussions:

5-1) Results:

5.1.1) Control Samples (A) Results:

5.1.1.1) Calculation of Activity:

This work state that a sample which keeps a low final density compared with MRSA bacteria growing freely in solvent is blocking growth. If the density rises higher, the sample is feeding the MRSA bacteria. Measurements were taken with one endpoint number for each well:

$$\text{Inhibition } (\%) = [(\text{ODref} - \text{ODsample}) \div \text{ODref}] \times 100$$

ODsample is the final density of the extract-treated well. Solvent-control wells (A3 and A4 MRSA bacteria, growth medium and 5% ethanol, but no extract) provided the average final density known as ODref. Replicate inhibition values from duplicate runs were averaged because the spread had to be represented as a standard deviation. Rows which contained two replicates that disagreed by more than >10% were marked as high variability by the research group. Strong inhibition is identified for results higher than 50%, while moderate inhibition is understood as 20–50%, and weak or no inhibition was recorded as 0–20%. Growth promotion was demonstrated when results showed negative values in the final data because these figures show a different direction of effect.

Table 5: Activity of bacterial strains

Strain	Avg Inh %	Strong ≥50%	Moderate 20–50%	Weak 0–20%	Promoter <0%
MRSA (DSM 21979) — primary target	**28.8%**	**28 (31%)**	**12 (13%)**	**28 (31%)**	**22 (24%)**
S. aureus (DSM 1104)	-13.6%	20 (22%)	5 (6%)	15 (17%)	50 (56%)
E. coli (DSM 682)	12.1%	1 (1%)	27 (30%)	46 (51%)	16 (18%)

5.1.1.2) MRSA Results:

MRSA was strongly inhibited by 28 samples which is (31%) of the total 90 plant extracts, while 12 showed moderate results, 28 were weak samples, and 22 samples showed promoted growth. It has been observed that the central, encouraging result of this work is that many plant extracts can hold back a strain defined by its resistance to conventional medical substances. The results suggests that these plant extracts carry compounds because their mode of action sidesteps the mechanisms that make MRSA clinically stubborn. These plant extracts are valuable because the compounds within these plant extracts work differently than standard medical substances. Table 6 shows all the 90 samples calculation and table no 7 below shows the 5 outstanding samples from MRSA measurement of control samples.

Inhibition % Calculations — MRSA (Control samples, 24 h endpoint)

Inhibition (%) Formula = [(ODreference − ODsample) / ODreference] × 100 | ODreference = AVERAGE (A3, A4) | SD% = (Std Dev / |Mean Inh%|) × 100 | CV% flag >10% = ⚠ HIGH VAR

>50% Strong inhibitor = Sage Green

20–50% Moderate inhibitor= yellow green

0–20% Weak/negligible inhibitor= sand

<0% Growth promoter=clay

Table 6: Coloured representation of MRSA inhibition for all 90 control samples

Sample #	Rep 1 Well	Rep 2 Well	ODref (avg A3+A4)	Rep 1 Abs (24h)	Rep 2 Abs (24h)	Inh % Rep 1	Inh % Rep 2	Ave Inh %	Std Dev (±)	SD % (Std Dev/Mean×100)	CV% / Flag
▸ Set A3 (Samples 1–42) — MRSA | ODreference (A3+A4 avg) = 1.100 (A3=1.103, A4=1.097)											
1	B1	B2	1,100	0,134	0,137	87,82	87,55	**88**	0,19	0 %	OK
4	B3	B4	1,100	0,118	0,137	89,27	87,55	**88**	1,22	1 %	OK
19	B5	B6	1,100	0,787	0,815	28,45	25,91	**27**	1,80	7 %	OK
25	B7	B8	1,100	0,642	0,599	41,64	45,55	**44**	2,76	6 %	OK
34	B9	B10	1,100	1,113	1,311	-1,18	-19,18	**-10**	12,73	-125 %	⚠ HIGH VAR
44	B11	B12	1,100	1,158	1,179	-5,27	-7,18	**-6**	1,35	-22 %	OK
45	C1	C2	1,100	0,243	0,158	77,91	85,64	**82**	5,46	7 %	OK
48	C3	C4	1,100	0,986	0,986	10,36	10,36	**10**	0,00	0 %	OK
53	C5	C6	1,100	1,031	1,030	6,27	6,36	**6**	0,06	1 %	OK
55	C7	C8	1,100	1,019	0,971	7,36	11,73	**10**	3,09	32 %	OK
58	C9	C10	1,100	0,120	0,667	89,09	39,36	**64**	35,16	55 %	⚠ HIGH VAR
56	C11	C12	1,100	0,703	0,172	36,09	84,36	**60**	34,13	57 %	⚠ HIGH VAR
62	D1	D2	1,100	1,056	1,060	4,00	3,64	**4**	0,26	7 %	OK
63	D3	D4	1,100	0,432	0,467	60,73	57,55	**59**	2,25	4 %	OK
70	D5	D6	1,100	1,047	1,059	4,82	3,73	**4**	0,77	18 %	OK
74	D7	D8	1,100	0,885	0,979	19,55	11,00	**15**	6,04	40 %	OK
80	D9	D10	1,100	1,126	0,961	-2,36	12,64	**5**	10,61	207 %	⚠ HIGH VAR
84	D11	D12	1,100	0,139	0,156	87,36	85,82	**87**	1,09	1 %	OK
86	E1	E2	1,100	0,235	0,250	78,64	77,27	**78**	0,96	1 %	OK
88	E3	E4	1,100	1,145	1,163	-4,09	-5,73	**-5**	1,16	-24 %	OK
89	E5	E6	1,100	0,984	0,951	10,55	13,55	**12**	2,12	18 %	OK
92	E7	E8	1,100	0,536	1,120	51,27	-1,82	**25**	37,54	152 %	⚠ HIGH VAR
96	E9	E10	1,100	0,844	0,829	23,27	24,64	**24**	0,96	4 %	OK
100	E11	E12	1,100	0,101	0,695	90,82	36,82	**64**	38,18	60 %	⚠ HIGH VAR
106	F1	F2	1,100	0,615	0,580	44,09	47,27	**46**	2,25	5 %	OK
110	F3	F4	1,100	0,099	0,100	91,00	90,91	**91**	0,06	0 %	OK
111	F5	F6	1,100	0,872	0,917	20,73	16,64	**19**	2,89	15 %	OK
112	F7	F8	1,100	0,692	0,800	37,09	27,27	**32**	6,94	22 %	OK

113	F9	F10	1,100	0,279	0,256	74,64	76,73	**76**	1,48	2 %	OK
120	F11	F12	1,100	0,240	0,419	78,18	61,91	**70**	11,51	16 %	⚠ HIGH VAR
125	G1	G2	1,100	1,039	0,995	5,55	9,55	**8**	2,83	37 %	OK
126	G3	G4	1,100	0,308	0,281	72,00	74,45	**73**	1,74	2 %	OK
129	G5	G6	1,100	1,010	1,472	8,18	-33,82	**-13**	29,70	-232 %	⚠ HIGH VAR
133	G7	G8	1,100	1,009	1,043	8,27	5,18	**7**	2,19	32 %	OK
148	G9	G10	1,100	0,219	1,079	80,09	1,91	**41**	55,28	135 %	⚠ HIGH VAR
137	G11	G12	1,100	1,050	1,160	4,55	-5,45	**0**	7,07	-1556 %	OK
159	H1	H2	1,100	0,829	0,857	24,64	22,09	**23**	1,80	8 %	OK
161	H3	H4	1,100	1,159	1,144	-5,36	-4,00	**-5**	0,96	-21 %	OK
163	H5	H6	1,100	1,353	0,687	-23,00	37,55	**7**	42,81	589 %	⚠ HIGH VAR
164	H7	H8	1,100	1,179	1,183	-7,18	-7,55	**-7**	0,26	-3 %	OK
165	H9	H10	1,100	0,970	1,050	11,82	4,55	**8**	5,14	63 %	OK
166	H11	H12	1,100	1,185	1,180	-7,73	-7,27	**-7**	0,32	-4 %	OK
▸ Set B3 (Samples 43–84) — MRSA | ODreference (A3+A4 avg) = 1.097 (A3=1.092, A4=1.103)											
169	B1	B2	1,098	1,172	1,197	-6,79	-9,07	**-8**	1,61	-20 %	OK
172	B3	B4	1,098	1,166	1,157	-6,24	-5,42	**-6**	0,58	-10 %	OK
176	B5	B6	1,098	0,712	0,639	35,13	41,78	**38**	4,70	12 %	OK
188	B7	B8	1,098	1,105	1,128	-0,68	-2,78	**-2**	1,48	-86 %	OK
190	B9	B10	1,098	0,997	1,044	9,16	4,87	**7**	3,03	43 %	OK
191	B11	B12	1,098	1,081	1,099	1,50	-0,14	**1**	1,16	170 %	OK
193	C1	C2	1,098	0,129	0,124	88,25	88,70	**88**	0,32	0 %	OK
194	C3	C4	1,098	0,983	0,977	10,43	10,98	**11**	0,39	4 %	OK
195	C5	C6	1,098	1,127	1,167	-2,69	-6,33	**-5**	2,58	-57 %	OK
197	C7	C8	1,098	1,127	1,167	-2,69	-6,33	**-5**	2,58	-57 %	OK
198	C9	C10	1,098	1,016	1,054	7,43	3,96	**6**	2,45	43 %	OK
199	C11	C12	1,098	1,039	1,023	5,33	6,79	**6**	1,03	17 %	OK
203	D1	D2	1,098	0,158	0,165	85,60	84,97	**85**	0,45	1 %	OK
206	D3	D4	1,098	0,314	0,362	71,39	67,02	**69**	3,09	4 %	OK
208	D5	D6	1,098	0,175	0,177	84,05	83,87	**84**	0,13	0 %	OK
210	D7	D8	1,098	0,119	0,638	89,16	41,87	**66**	33,44	51 %	⚠ HIGH VAR
211	D9	D10	1,098	0,157	0,161	85,69	85,33	**86**	0,26	0 %	OK
212	D11	D12	1,098	0,984	0,982	10,34	10,52	**10**	0,13	1 %	OK
213	E1	E2	1,098	0,190	0,182	82,69	83,42	**83**	0,52	1 %	OK
214	E3	E4	1,098	0,133	0,140	87,88	87,24	**88**	0,45	1 %	OK
225	E5	E6	1,098	1,478	0,961	-34,67	12,44	**-11**	33,31	-300 %	⚠ HIGH VAR
228	E7	E8	1,098	1,468	1,222	-33,76	-11,34	**-23**	15,85	-70 %	⚠ HIGH VAR
229	E9	E10	1,098	0,853	0,828	22,28	24,56	**23**	1,61	7 %	OK
238	E11	E12	1,098	0,983	0,967	10,43	11,89	**11**	1,03	9 %	OK
242	F1	F2	1,098	0,149	0,151	86,42	86,24	**86**	0,13	0 %	OK
243	F3	F4	1,098	0,153	0,154	86,06	85,97	**86**	0,06	0 %	OK
245	F5	F6	1,098	0,602	0,481	45,15	56,17	**51**	7,80	15 %	⚠ HIGH VAR
249	F7	F8	1,098	1,050	1,127	4,33	-2,69	**1**	4,96	605 %	OK

253	F9	F10	1,098	1,110	1,114	-1,14	-1,50	**-1**	0,26	-20 %	OK
254	F11	F12	1,098	1,223	1,224	-11,44	-11,53	**-11**	0,06	-1 %	OK
257	G1	G2	1,098	0,219	0,222	80,05	79,77	**80**	0,19	0 %	OK
258	G3	G4	1,098	1,207	1,162	-9,98	-5,88	**-8**	2,90	-37 %	OK
264	G5	G6	1,098	1,019	1,026	7,15	6,51	**7**	0,45	7 %	OK
269	G7	G8	1,098	1,069	1,045	2,60	4,78	**4**	1,55	42 %	OK
270	G9	G10	1,098	0,152	0,149	86,15	86,42	**86**	0,19	0 %	OK
271	G11	G12	1,098	1,101	1,083	-0,32	1,32	**1**	1,16	231 %	OK
273	H1	H2	1,098	0,840	0,892	23,46	18,72	**21**	3,35	16 %	OK
275	H3	H4	1,098	0,807	0,827	26,47	24,65	**26**	1,29	5 %	OK
276	H5	H6	1,098	1,163	1,175	-5,97	-7,06	**-7**	0,77	-12 %	OK
279	H7	H8	1,098	1,018	1,015	7,24	7,52	**7**	0,19	3 %	OK
281	H9	H10	1,098	1,084	1,087	1,23	0,96	**1**	0,19	18 %	OK
284	H11	H12	1,098	0,148	0,133	86,51	87,88	**87**	0,97	1 %	OK
▸ Set C3 (Samples 85–90) — MRSA | ODreference (A3+A4 avg) = 1.122 (A3=1.115, A4=1.129)											
285	B1	B2	1,122	0,357	0,462	68,18	58,82	**64**	6,62	10 %	OK
288	B3	B4	1,122	0,944	0,946	15,86	15,69	**16**	0,13	1 %	OK
289	B5	B6	1,122	1,153	1,154	-2,76	-2,85	**-3**	0,06	-2 %	OK
290	B7	B8	1,122	1,218	1,212	-8,56	-8,02	**-8**	0,38	-5 %	OK
291	B9	B10	1,122	1,179	1,169	-5,08	-4,19	**-5**	0,63	-14 %	OK
293	B11	B12	1,122	1,032	1,079	8,02	3,83	**6**	2,96	50 %	OK

So, the growth of MRSA was inhibited by 91.0% by the most impressive sample number 110 (Plumbago auriculata), where MRSA growth was completely stopped. The results shows that a tight cluster followed just behind, with all results above 85%: for sample number 193 (Leea rubra) at 88.5%, sample number 04 (Camellia tsaii) at 88.4%, sample number 01 (Sequaiadendron giganterum) at 87.7%, sample number 214 (Myrsine africana) at 87.6%. This outcome shows comfortably with the long-standing reputation of myrrh-bearing resins as antibacterial agents.

Table 7: 5 best control samples for MRSA

#	Plant species	Avg Inh %	SD	Reliability
110	*Plumbago auriculata*	**91.0**	0.06	OK
193	*Leea rubra*	**88.5**	0.32	OK
4	*Camellia tsaii*	**88.4**	1.22	OK
1	*Sequaiadendron giganterum*	**87.7**	0.19	OK
214	*Myrsine africana*	**87.6**	0.45	OK

5.1.1.2) S. aureus Results:

A very separated result was seen when looking at the methicillin-sensitive strain. The average reduction of the S. aures strain showed a negative value of -13.6% because many extracts (50 of 90, 56%) caused the bacteria to increase in number instead of stopping them. Certain samples like 279 (Pilea pegeromioides), sample number 197 (Garcinia xanthochymus) and sample number 275 (Etlingera elatior) pushed the expansion to 100–128% more than the control group.

It shows that several of these samples carried high-variability markers. These results are believed to be caused by either very inconsistent replicates or a growth response that depends on the specific composition of the extract. While several extracts assisted the microscopic organisms (S. aureus) to grow, 20 extracts (22%) were observed to possess a heavy power for stopping the methicillin-sensitive strain. It has been confirmed from the measurement that Plumbago auriculata (sample no.110) kept the highest place at 76.6%. Following the first one, Eugenia uniflora (#270) stayed at 74.7%, while Pistacia atlantica (#45) reached 74.4% and Myrsine africana (#214) reached 70.8%.

Inhibition % Calculations — S. aureus (Control samples, 24 h endpoint)

Inhibition (%) Formula = [(ODreference − ODsample) / ODreference] × 100 | ODreference = AVERAGE (A3, A4) | SD% = (Std Dev / |Mean Inh%|) × 100 | CV% flag >10% = ⚠ HIGH VAR

>50% Strong inhibitor = Sage Green

20–50% Moderate inhibitor= yellow green

0–20% Weak/negligible inhibitor= sand

<0% Growth promoter=clay

Table 8: S. aureus (DSM 1104) inhibition for all 90 control samples

Sample #	Rep 1 Well	Rep 2 Well	OD ref (avg A3+ A4)	Rep 1 Abs (24h)	Rep 2 Abs (24h)	Inh % Rep 1	Inh % Rep 2	Ave Inh %	Std Dev (±)	SD % (Std Dev/Mean×100)	CV% / Flag
▸ Set A1 (Samples 1–42) — S. aureus | ODreference (A3+A4 avg) = 0.457 (A3=0.451, A4=0.463)											
1	B1	B2	0,457	0,127	0,178	72,21	61,05	**67**	7,89	12 %	⚠ HIGH VAR
4	B3	B4	0,457	0,162	0,158	64,55	65,43	**65**	0,62	1 %	OK
19	B5	B6	0,457	0,702	0,682	-53,61	-49,23	**-51**	3,09	-6 %	OK
25	B7	B8	0,457	0,690	0,092	-50,98	79,87	**14**	92,53	641 %	⚠ HIGH VAR
34	B9	B10	0,457	0,104	0,822	77,24	-79,87	**-1**	111,09	-8462 %	⚠ HIGH VAR
44	B11	B12	0,457	0,405	0,426	11,38	6,78	**9**	3,25	36 %	OK
45	C1	C2	0,457	0,121	0,113	73,52	75,27	**74**	1,24	2 %	OK
48	C3	C4	0,457	0,857	0,863	-87,53	-88,84	**-88**	0,93	-1 %	OK
53	C5	C6	0,457	0,536	0,599	-17,29	-31,07	**-24**	9,75	-40 %	⚠ HIGH VAR
55	C7	C8	0,457	0,716	0,659	-56,67	-44,20	**-50**	8,82	-17 %	⚠ HIGH VAR
58	C9	C10	0,457	0,132	0,141	71,12	69,15	**70**	1,39	2 %	OK
56	C11	C12	0,457	0,160	0,132	64,99	71,12	**68**	4,33	6 %	OK
62	D1	D2	0,457	0,424	0,651	7,22	-42,45	**-18**	35,12	-199 %	⚠ HIGH VAR
63	D3	D4	0,457	0,941	0,941	-105,91	-105,91	**-106**	0,00	0 %	OK
70	D5	D6	0,457	0,782	0,829	-71,12	-81,40	**-76**	7,27	-10 %	⚠ HIGH VAR
74	D7	D8	0,457	0,730	0,831	-59,74	-81,84	**-71**	15,63	-22 %	⚠ HIGH VAR
80	D9	D10	0,457	0,135	0,747	70,46	-63,46	**4**	94,69	2705 %	⚠ HIGH VAR
84	D11	D12	0,457	0,150	0,142	67,18	68,93	**68**	1,24	2 %	OK

86	E1	E2	0,457	0,822	0,868	-79,87	-89,93	**-85**	7,12	-8 %	⚠ HIGH VAR
88	E3	E4	0,457	0,451	0,596	1,31	-30,42	**-15**	22,44	-154 %	⚠ HIGH VAR
89	E5	E6	0,457	0,842	0,833	-84,25	-82,28	**-83**	1,39	-2 %	OK
92	E7	E8	0,457	0,463	0,454	-1,31	0,66	**0**	1,39	-424 %	OK
96	E9	E10	0,457	0,532	0,724	-16,41	-58,42	**-37**	29,71	-79 %	⚠ HIGH VAR
100	E11	E12	0,457	0,660	0,237	-44,42	48,14	**2**	65,45	3519 %	⚠ HIGH VAR
106	F1	F2	0,457	0,157	0,172	65,65	62,36	**64**	2,32	4 %	OK
110	F3	F4	0,457	0,107	0,107	76,59	76,59	**77**	0,00	0 %	OK
111	F5	F6	0,457	0,764	0,640	-67,18	-40,04	**-54**	19,19	-36 %	⚠ HIGH VAR
112	F7	F8	0,457	0,176	0,176	61,49	61,49	**61**	0,00	0 %	OK
113	F9	F10	0,457	0,287	0,300	37,20	34,35	**36**	2,01	6 %	OK
120	F11	F12	0,457	0,816	0,829	-78,56	-81,40	**-80**	2,01	-3 %	OK
125	G1	G2	0,457	0,899	0,945	-96,72	-106,78	**-102**	7,12	-7 %	⚠ HIGH VAR
126	G3	G4	0,457	0,774	0,120	-69,37	73,74	**2**	101,19	4624 %	⚠ HIGH VAR
129	G5	G6	0,457	0,393	0,396	14,00	13,35	**14**	0,46	3 %	OK
133	G7	G8	0,457	0,691	0,746	-51,20	-63,24	**-57**	8,51	-15 %	⚠ HIGH VAR
148	G9	G10	0,457	0,429	0,400	6,13	12,47	**9**	4,49	48 %	OK
137	G11	G12	0,457	0,462	0,952	-1,09	-108,32	**-55**	75,82	-139 %	⚠ HIGH VAR
159	H1	H2	0,457	0,502	0,139	-9,85	69,58	**30**	56,17	188 %	⚠ HIGH VAR
161	H3	H4	0,457	0,414	0,431	9,41	5,69	**8**	2,63	35 %	OK
163	H5	H6	0,457	0,836	0,841	-82,93	-84,03	**-83**	0,77	-1 %	OK
164	H7	H8	0,457	0,560	0,536	-22,54	-17,29	**-20**	3,71	-19 %	OK
165	H9	H10	0,457	0,726	0,759	-58,86	-66,08	**-62**	5,11	-8 %	OK
166	H11	H12	0,457	0,394	0,408	13,79	10,72	**12**	2,17	18 %	OK

▸ Set B1 (Samples 43–84) — S. aureus | ODreference (A3+A4 avg) = 0.416 (A3=0.414, A4=0.419)

169	B1	B2	0,417	0,517	0,530	-24,13	-27,25	**-26**	2,21	-9 %	OK
172	B3	B4	0,417	0,501	0,492	-20,29	-18,13	**-19**	1,53	-8 %	OK
176	B5	B6	0,417	0,817	0,820	-96,16	-96,88	**-97**	0,51	-1 %	OK
188	B7	B8	0,417	0,423	0,241	-1,56	42,14	**20**	30,90	152 %	⚠ HIGH VAR
190	B9	B10	0,417	0,127	0,879	69,51	-111,04	**-21**	127,67	-615 %	⚠ HIGH VAR
191	B11	B12	0,417	0,847	0,848	-103,36	-103,60	**-103**	0,17	0 %	OK
193	C1	C2	0,417	0,128	0,122	69,27	70,71	**70**	1,02	1 %	OK
194	C3	C4	0,417	0,837	0,845	-100,96	-102,88	**-102**	1,36	-1 %	OK
195	C5	C6	0,417	0,420	0,515	-0,84	-23,65	**-12**	16,13	-132 %	⚠ HIGH VAR
197	C7	C8	0,417	0,913	0,956	-119,21	-129,53	**-124**	7,30	-6 %	⚠ HIGH VAR
198	C9	C10	0,417	0,801	0,924	-92,32	-121,85	**-107**	20,88	-20 %	⚠ HIGH VAR
199	C11	C12	0,417	0,628	0,624	-50,78	-49,82	**-50**	0,68	-1 %	OK
203	D1	D2	0,417	0,167	0,167	59,90	59,90	**60**	0,00	0 %	OK
206	D3	D4	0,417	0,115	0,260	72,39	37,58	**55**	24,62	45 %	⚠ HIGH VAR
208	D5	D6	0,417	0,174	0,197	58,22	52,70	**55**	3,90	7 %	OK

210	D7	D8	0,417	0,685	0,701	-64,47	-68,31	**-66**	2,72	-4 %	OK
211	D9	D10	0,417	0,137	0,162	67,11	61,10	**64**	4,24	7 %	OK
212	D11	D12	0,417	0,876	0,774	- 110,32	-85,83	**-98**	17,32	-18 %	⚠ HIGH VAR
213	E1	E2	0,417	0,155	0,206	62,79	50,54	**57**	8,66	15 %	⚠ HIGH VAR
214	E3	E4	0,417	0,120	0,123	71,19	70,47	**71**	0,51	1 %	OK
225	E5	E6	0,417	0,569	0,131	-36,61	68,55	**16**	74,36	466 %	⚠ HIGH VAR
228	E7	E8	0,417	0,496	0,506	-19,09	-21,49	**-20**	1,70	-8 %	OK
229	E9	E10	0,417	0,836	0,825	- 100,72	-98,08	**-99**	1,87	-2 %	OK
238	E11	E12	0,417	0,763	0,807	-83,19	-93,76	**-88**	7,47	-8 %	⚠ HIGH VAR
242	F1	F2	0,417	0,154	0,159	63,03	61,82	**62**	0,85	1 %	OK
243	F3	F4	0,417	0,158	0,158	62,06	62,06	**62**	0,00	0 %	OK
245	F5	F6	0,417	0,117	0,380	71,91	8,76	**40**	44,65	111 %	⚠ HIGH VAR
249	F7	F8	0,417	0,459	0,422	-10,20	-1,32	**-6**	6,28	-109 %	OK
253	F9	F10	0,417	0,491	0,546	-17,89	-31,09	**-24**	9,34	-38 %	⚠ HIGH VAR
254	F11	F12	0,417	0,531	0,509	-27,49	-22,21	**-25**	3,74	-15 %	OK
257	G1	G2	0,417	0,208	0,241	50,06	42,14	**46**	5,60	12 %	OK
258	G3	G4	0,417	0,558	0,650	-33,97	-56,06	**-45**	15,62	-35 %	⚠ HIGH VAR
264	G5	G6	0,417	0,335	0,380	19,57	8,76	**14**	7,64	54 %	⚠ HIGH VAR
269	G7	G8	0,417	0,456	0,837	-9,48	- 100,96	**-55**	64,68	-117 %	⚠ HIGH VAR
270	G9	G10	0,417	0,099	0,112	76,23	73,11	**75**	2,21	3 %	OK
271	G11	G12	0,417	0,447	0,345	-7,32	17,17	**5**	17,32	352 %	⚠ HIGH VAR
273	H1	H2	0,417	0,774	0,737	-85,83	-76,95	**-81**	6,28	-8 %	OK
275	H3	H4	0,417	0,752	1,066	-80,55	- 155,94	**-118**	53,31	-45 %	⚠ HIGH VAR
276	H5	H6	0,417	0,402	0,421	3,48	-1,08	**1**	3,23	269 %	OK
279	H7	H8	0,417	0,853	1,050	- 104,80	- 152,10	**-128**	33,45	-26 %	⚠ HIGH VAR
281	H9	H10	0,417	0,559	0,442	-34,21	-6,12	**-20**	19,86	-98 %	⚠ HIGH VAR
284	H11	H12	0,417	0,148	0,204	64,47	51,02	**58**	9,51	16 %	⚠ HIGH VAR
▸ Set C1 (Samples 85–90) — S. aureus | ODreference (A3+A4 avg) = 0.465 (A3=0.468, A4=0.462)											
285	B1	B2	0,465	0,479	0,536	-3,01	-15,27	**-9**	8,67	-95 %	⚠ HIGH VAR
288	B3	B4	0,465	0,385	0,541	17,20	-16,34	**0**	23,72	5515 %	⚠ HIGH VAR
289	B5	B6	0,465	0,500	0,503	-7,53	-8,17	**-8**	0,46	-6 %	OK
290	B7	B8	0,465	0,551	0,553	-18,49	-18,92	**-19**	0,30	-2 %	OK
291	B9	B10	0,465	0,580	0,591	-24,73	-27,10	**-26**	1,67	-6 %	OK
293	B11	B12	0,465	0,403	0,422	13,33	9,25	**11**	2,89	26 %	OK

Table 9: 5 best control samples S. aureus

#	Plant species	Avg Inh %	SD	Reliability
110	*Plumbago auriculata*	**76.6**	0.00	OK
270	*Eugenia uniflora*	**74.7**	2.21	OK
45	*Pistacia atlantica*	**74.4**	1.24	OK
214	*Myrsine africana*	**70.8**	0.51	OK
58	*aristea*	**70.1**	1.39	OK

5.1.1.3) E. Coli Results:

Testing against E. coli presented a harder challenge for the samples because E. coli is a Gram-negative organism. The mean reduction of 12.1% for E. coli was higher than others, yet this number creates confusion since the general reaction remained very flat. A single sample no. 110 (Plumbago auriculata), reached a strong level of 70.3%, while 27 items stayed in the middle range and 46 samples were very weak. The results shows that that Cissus antarctica (sample no.206) at 47.5%, Pistacia atlantica (sample no.45) at 40.7% and Podocarpus neriifolius (sample no.56) at 38.3% occupied the subsequent ranks. This specific arrangement of reactions is to be created by the outer-membrane barrier belonging to Gram-negative bacteria. The outer-membrane barrier restricts many plant samples which usually destroy Gram-positive bacteria because the outer-membrane barrier performs the work of a filter.

Inhibition % Calculations — S. aureus (Control samples, 24 h endpoint)

Inhibition (%) Formula = [(ODreference − ODsample) / ODreference] × 100 | ODreference = AVERAGE (A3, A4) | SD% = (Std Dev / |Mean Inh%|) × 100 | CV% flag >10% = ⚠ HIGH VAR

>50% Strong inhibitor = Sage Green
20–50% Moderate inhibitor= yellow green
0–20% Weak/negligible inhibitor= sand
<0% Growth promoter=clay

Table 10: E. coli (DSM 682) inhibition for all 90 control samples

Sample #	Rep 1 Well	Rep 2 Well	ODref (avg A3+A4)	Rep 1 Abs (24h)	Rep 2 Abs (24h)	Inh % Rep 1	Inh % Rep 2	Ave Inh %	Std Dev (±)	SD % (Std Dev/Mean×100)	CV% / Flag
▸ **Set A2 (Samples 1–42) — E. coli | ODreference (A3+A4 avg) = 0.740 (A3=0.734, A4=0.745)**											
1	B1	B2	0,740	0,501	0,487	32,252	34,145	**33**	1,34	4 %	OK
4	B3	B4	0,740	0,759	0,812	-2,637	-9,804	**-6**	5,07	-81 %	OK

19	B5	B6	0,740	0,622	0,566	15,889	23,462	**20**	5,35	27 %	OK
25	B7	B8	0,740	0,608	0,568	17,782	23,191	**20**	3,82	19 %	OK
34	B9	B10	0,740	0,611	0,514	17,377	30,494	**24**	9,28	39 %	⚠ HIGH VAR
44	B11	B12	0,740	0,608	0,595	17,782	19,540	**19**	1,24	7 %	OK
45	C1	C2	0,740	0,404	0,473	45,368	36,038	**41**	6,60	16 %	OK
48	C3	C4	0,740	0,549	0,545	25,761	26,302	**26**	0,38	1 %	OK
53	C5	C6	0,740	0,684	0,679	7,505	8,181	**8**	0,48	6 %	OK
55	C7	C8	0,740	0,603	1,011	18,458	-36,714	**-9**	39,01	-427 %	⚠ HIGH VAR
58	C9	C10	0,740	1,002	0,742	-35,497	-0,338	**-18**	24,86	-139 %	⚠ HIGH VAR
56	C11	C12	0,740	0,413	0,500	44,151	32,387	**38**	8,32	22 %	⚠ HIGH VAR
62	D1	D2	0,740	0,622	0,616	15,889	16,700	**16**	0,57	4 %	OK
63	D3	D4	0,740	0,657	0,582	11,156	21,298	**16**	7,17	44 %	⚠ HIGH VAR
70	D5	D6	0,740	0,609	0,643	17,647	13,049	**15**	3,25	21 %	OK
74	D7	D8	0,740	0,607	0,564	17,918	23,732	**21**	4,11	20 %	OK
80	D9	D10	0,740	0,620	1,005	16,160	-35,903	**-10**	36,81	-373 %	⚠ HIGH VAR
84	D11	D12	0,740	0,608	0,664	17,782	10,210	**14**	5,35	38 %	OK
86	E1	E2	0,740	0,545	0,551	26,302	25,490	**26**	0,57	2 %	OK
88	E3	E4	0,740	0,679	0,754	8,181	-1,961	**3**	7,17	231 %	⚠ HIGH VAR
89	E5	E6	0,740	0,670	0,562	9,398	24,003	**17**	10,33	62 %	⚠ HIGH VAR
92	E7	E8	0,740	0,784	0,740	-6,018	-0,068	**-3**	4,21	-138 %	OK
96	E9	E10	0,740	0,616	0,589	16,700	20,352	**19**	2,58	14 %	OK
100	E11	E12	0,740	0,536	0,535	27,519	27,654	**28**	0,10	0 %	OK
106	F1	F2	0,740	0,598	0,580	19,135	21,569	**20**	1,72	8 %	OK
110	F3	F4	0,740	0,221	0,218	70,115	70,521	**70**	0,29	0 %	OK
111	F5	F6	0,740	0,715	0,720	3,313	2,637	**3**	0,48	16 %	OK
112	F7	F8	0,740	0,618	0,591	16,430	20,081	**18**	2,58	14 %	OK
113	F9	F10	0,740	0,703	0,683	4,936	7,640	**6**	1,91	30 %	OK
120	F11	F12	0,740	0,662	0,680	10,480	8,046	**9**	1,72	19 %	OK
125	G1	G2	0,740	0,553	0,557	25,220	24,679	**25**	0,38	2 %	OK
126	G3	G4	0,740	0,676	0,322	8,587	56,457	**33**	33,85	104 %	⚠ HIGH VAR
129	G5	G6	0,740	0,707	0,691	4,395	6,558	**5**	1,53	28 %	OK
133	G7	G8	0,740	0,529	0,595	28,465	19,540	**24**	6,31	26 %	OK
148	G9	G10	0,740	0,701	0,680	5,206	8,046	**7**	2,01	30 %	OK
137	G11	G12	0,740	0,657	0,702	11,156	5,071	**8**	4,30	53 %	OK
159	H1	H2	0,740	0,620	0,611	16,160	17,377	**17**	0,86	5 %	OK
161	H3	H4	0,740	0,673	0,638	8,993	13,725	**11**	3,35	29 %	OK
163	H5	H6	0,740	0,576	0,544	22,110	26,437	**24**	3,06	13 %	OK
164	H7	H8	0,740	0,710	0,764	3,989	-3,313	**0**	5,16	1527 %	OK
165	H9	H10	0,740	0,657	0,680	11,156	8,046	**10**	2,20	23 %	OK
166	H11	H12	0,740	0,730	0,821	1,285	-11,021	**-5**	8,70	-179 %	⚠ HIGH VAR
▸ Set B2 (Samples 43–84) — E. coli | ODreference (A3+A4 avg) = 0.697 (A3=0.69, A4=0.703)											
169	B1	B2	0,697	0,668	0,682	4,092	2,082	**3**	1,42	46 %	OK
172	B3	B4	0,697	0,849	0,639	-21,895	8,256	**-7**	21,32	-313 %	⚠ HIGH VAR
176	B5	B6	0,697	0,635	0,680	8,830	2,369	**6**	4,57	82 %	OK

188	B7	B8	0,697	0,775	0,746	-11,271	-7,107	-9	2,94	-32 %	OK
190	B9	B10	0,697	0,655	0,512	5,958	26,490	16	14,52	89 %	⚠ HIGH VAR
191	B11	B12	0,697	0,605	0,544	13,137	21,895	18	6,19	35 %	OK
193	C1	C2	0,697	0,452	0,528	35,104	24,192	30	7,72	26 %	⚠ HIGH VAR
194	C3	C4	0,697	0,523	0,639	24,910	8,256	17	11,78	71 %	⚠ HIGH VAR
195	C5	C6	0,697	0,666	0,704	4,379	-1,077	2	3,86	234 %	OK
197	C7	C8	0,697	0,778	0,701	-11,701	-0,646	-6	7,82	-127 %	⚠ HIGH VAR
198	C9	C10	0,697	0,798	0,689	-14,573	1,077	-7	11,07	-164 %	⚠ HIGH VAR
199	C11	C12	0,697	0,697	0,684	-0,072	1,795	1	1,32	153 %	OK
203	D1	D2	0,697	0,712	0,655	-2,225	5,958	2	5,79	310 %	OK
206	D3	D4	0,697	0,391	0,341	43,862	51,041	47	5,08	11 %	OK
208	D5	D6	0,697	0,683	0,606	1,938	12,994	7	7,82	105 %	⚠ HIGH VAR
210	D7	D8	0,697	0,596	0,495	14,429	28,930	22	10,25	47 %	⚠ HIGH VAR
211	D9	D10	0,697	0,685	0,699	1,651	-0,359	1	1,42	220 %	OK
212	D11	D12	0,697	0,489	0,564	29,792	19,024	24	7,61	31 %	⚠ HIGH VAR
213	E1	E2	0,697	0,576	0,343	17,301	50,754	34	23,65	70 %	⚠ HIGH VAR
214	E3	E4	0,697	0,612	0,273	12,132	60,804	36	34,42	94 %	⚠ HIGH VAR
225	E5	E6	0,697	0,688	0,974	1,220	-39,842	-19	29,04	-150 %	⚠ HIGH VAR
228	E7	E8	0,697	0,683	0,814	1,938	-16,870	-7	13,30	-178 %	⚠ HIGH VAR
229	E9	E10	0,697	0,521	0,467	25,197	32,950	29	5,48	19 %	OK
238	E11	E12	0,697	0,553	0,625	20,603	10,266	15	7,31	47 %	⚠ HIGH VAR
242	F1	F2	0,697	0,698	0,672	-0,215	3,518	2	2,64	160 %	OK
243	F3	F4	0,697	1,071	1,054	-53,769	-51,328	-53	1,73	-3 %	OK
245	F5	F6	0,697	0,739	0,285	-6,102	59,081	26	46,09	174 %	⚠ HIGH VAR
249	F7	F8	0,697	0,564	0,702	19,024	-0,790	9	14,01	154 %	⚠ HIGH VAR
253	F9	F10	0,697	0,661	0,645	5,097	7,394	6	1,62	26 %	OK
254	F11	F12	0,697	0,717	0,705	-2,943	-1,220	-2	1,22	-59 %	OK
257	G1	G2	0,697	0,516	0,567	25,915	18,593	22	5,18	23 %	OK
258	G3	G4	0,697	0,613	0,617	11,989	11,414	12	0,41	3 %	OK
264	G5	G6	0,697	0,562	0,574	19,311	17,588	18	1,22	7 %	OK
269	G7	G8	0,697	0,656	0,635	5,815	8,830	7	2,13	29 %	OK
270	G9	G10	0,697	0,680	0,650	2,369	6,676	5	3,05	67 %	OK
271	G11	G12	0,697	0,687	0,671	1,364	3,661	3	1,62	65 %	OK
273	H1	H2	0,697	0,528	0,524	24,192	24,767	24	0,41	2 %	OK
275	H3	H4	0,697	0,552	0,537	20,747	22,900	22	1,52	7 %	OK
276	H5	H6	0,697	0,659	0,644	5,384	7,538	6	1,52	24 %	OK
279	H7	H8	0,697	0,577	0,540	17,157	22,469	20	3,76	19 %	OK
281	H9	H10	0,697	0,691	0,678	0,790	2,656	2	1,32	77 %	OK
284	H11	H12	0,697	0,501	0,581	28,069	16,583	22	8,12	36 %	⚠ HIGH VAR
▸ **Set C2 (Samples 85–90) — E. coli | ODreference (A3+A4 avg) = 0.754 (3=0.751, A4=0.757)**											
285	B1	B2	0,754	0,548	0,512	27,321	32,095	30	3,38	11 %	OK
288	B3	B4	0,754	0,781	0,731	-3,581	3,050	0	4,69	-1768 %	OK
289	B5	B6	0,754	0,780	0,751	-3,448	0,398	-2	2,72	-178 %	OK

290	B7	B8	0,754	0,760	0,742	-0,796	1,592	**0**	1,69	424 %	OK
291	B9	B10	0,754	0,737	0,755	2,255	-0,133	**1**	1,69	159 %	OK
293	B11	B12	0,754	0,726	0,689	3,714	8,621	**6**	3,47	56 %	OK

Table 11: 5 best control samples for E. Coli

#	Plant species	Avg Inh %	SD	Reliability
110	*Plumbago auriculata*	**70.3**	0.29	OK
206	*Cissus antarctica*	47.5	5.08	OK
45	*Pistacia atlantica*	40.7	6.60	OK
56	*Podocarpus neriifolius*	38.3	8.32	⚠High var
214	*Myrsine africana*	36.5	34.42	⚠High var
213	*Pleiogynium solandri*	34.0	23.65	⚠High var

5.1.1.4) Broad Spectrum of the Strains Results:

When each bacterial strain is interpreted, the following figure divides the data into three smaller radar charts that show the eight most potent plant extracts for each bacterium in a sequence. It is observed that the variation in the resistance challenge is made noticeably clear through these charts. Even though the MRSA and S. aureus panels reach far toward the outer perimeters, the most successful E. coli extracts stay remarkably close to the centre point. This visual representation highlights the contrast because E. coli presents a greater level of protection against these extracts. Information is separated into these charts so that a better comparison is permitted.

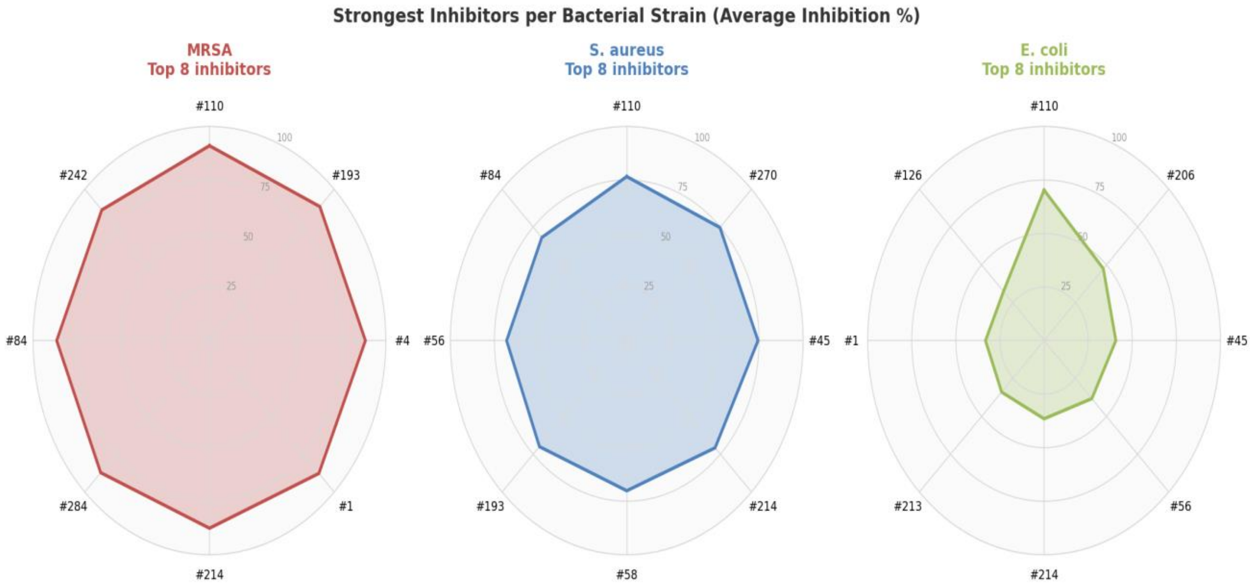


Fig 11: Strongest inhibitors for each strain shown separately (top 8 per strain). The smaller reach of the E. coli panel reflects this strain's broad resistance to the library.

Also, a small number of plant extracts deserve to be highlighted because these extracts perform effectively across multiple bacteria. It shows that eighteen plant extracts stopped at least two out of the three tiny organisms with strong power, while the specific sample #110 (Plumbago auriculata) was observed to restrain all three at once (S. aureus 76.6%, E. coli 70.3%, MRSA 91.0%), which makes Plumbago auriculata the most important extract in the whole group. Because Plumbago auriculata contains a chemical nature full of plumbagin, this wide reach of strength is anticipated by researchers before. Plumbagin is a naphthoquinone that operates against Gram-negative bacteria using biological paths that do not rely on the common locations where regular medicine works. Success was demonstrated by Plumbago auriculata throughout the laboratory evaluation. Those grouped bars below place the leading broad-spectrum extracts side by side, while the dashed line marks the 50% strong-inhibition point. This layout provides a systematic approach to see which extracts carry their inhibitory potency across strains and which extracts rely mostly on a single bacterium.

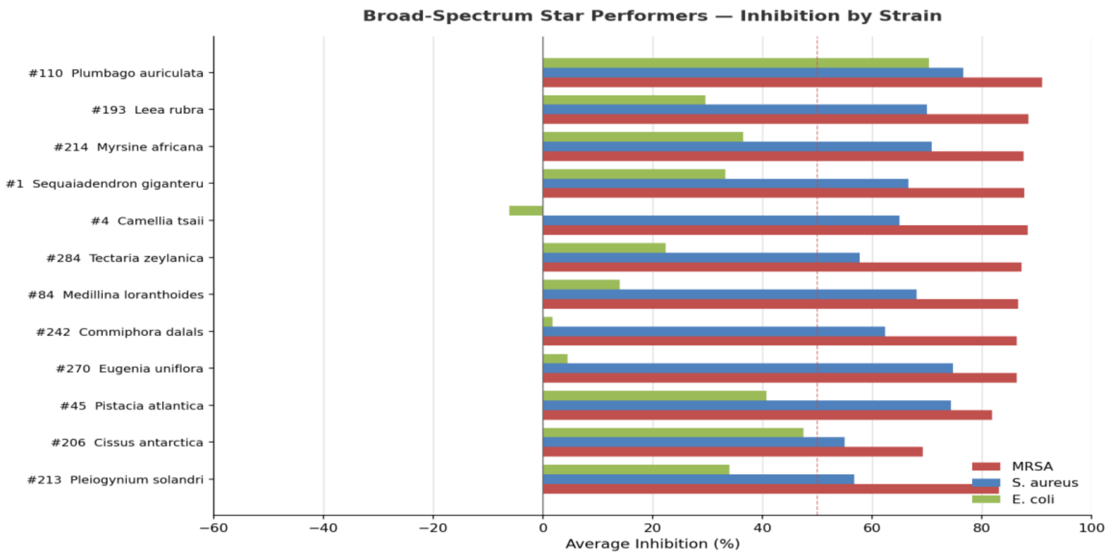


Figure 12: Broad-spectrum candidates, inhibition by strain. The dashed line marks the 50% strong-inhibition threshold; bars to the left of zero indicate growth promotion.

Table 12: Star performers — strong MRSA activity paired with notable breadth (ranked by MRSA inhibition).

#	Plant species	S. aureus	E. coli	MRSA	Notes
110	*Plumbago auriculata*	76.6	70.3	**91.0**	Broad-spectrum ⭑— strong on all three
193	*Leea rubra*	70.0	29.6	**88.5**	Strong SA + MRSA
214	*Myrsine africana*	70.8	36.5	**87.6**	Strong SA + MRSA
1	*Sequaiadendron giganterum*	66.6	33.2	**87.7**	Strong SA + MRSA, moderate EC
4	*Camellia tsaii*	65.0	-6.2	**88.4**	Strong SA + MRSA
45	*Pistacia atlantica*	74.4	40.7	**81.8**	Strong all three, moderate EC
206	*Cissus antarctica*	55.0	47.5	**69.2**	Broadest spectrum after #110
213	*Pleiogynium solandri*	56.7	34.0	**83.1**	Even strength across all three
84	*Medillina loranthoides*	68.1	14.0	**86.6**	Strong SA + MRSA
270	*Eugenia uniflora*	74.7	4.5	**86.3**	Strong SA + MRSA
242	*Commiphora dalals*	62.4	1.7	**86.3**	Strong MRSA (Commiphora resin)
243	*Commiphora gileadensis*	62.1	-52.5	**86.0**	Strong MRSA (Commiphora resin)

5.1.2) Enzymatic Oxidation (B) Samples Results:

5.1.2.1) Calculation of Activity:

I used the same method as the control screen, so the two sets of numbers line up directly. Each oxidized extract grew with bacteria for 24 hours while I tracked optical density at 600 nm. I read inhibition off the final 24-hour point:

$$\text{Inhibition (\%)} = [(\text{ODref} - \text{ODsample}) \div \text{ODref}] \times 100$$

ODref is the average final density of the solvent-control wells, A3 and A4. ODsample is the final density of the treated well. Every sample ran in duplicate. I averaged the two, reported the spread as a standard deviation, and flagged any pair that disagreed by more than 10 points. Above 50% counts as strong, 20 to 50% moderate, 0 to 20% weak, and anything below zero means the extract helped the bacteria grow rather than holding them back.

Oxidation changed the library. Against MRSA, 16 of the 90 extracts still held strong at 50% or better, but the average dropped to 4.1% and 56 samples slipped into promotion. That is a clear step down from the untreated control, which is what I expected oxidation to do. The reference strains came out heavily negative on average (-139.2% for S. aureus, -20.4% for E. coli), but that is the baseline problem talking, not real biology. For those two, the count of strong inhibitors (6 and 1) says more than the average does.

Table 13. Activity across all 90 oxidized samples, by strain. Reference-strain averages are affected by the low baseline.

Strain	Avg inh %	Strong ≥50%	Moderate 20–50%	Weak 0–20%	Promoter <0%
MRSA (DSM 21979), primary target	**4.1%**	**16 (18%)**	**9 (10%)**	**9 (10%)**	**56 (62%)**
S. aureus (DSM 1104) †	-139.2%	6 (7%)	14 (16%)	1 (1%)	69 (77%)
E. coli (DSM 682) †	-20.4%	1 (1%)	7 (8%)	11 (12%)	71 (79%)

5.1.2.2) MRSA Results:

This is the part that matters, and it held up better than I feared. 16 extracts still inhibited MRSA strongly after oxidation. The front-runners were #84 (Medillina loranthoides) at 85.4%, #214 (Myrsine africana) at 83.1%, #211 (Eucalyptus Antipolitenis) at 82.7%, #242 (Commiphora dalals) at 82.7%, #4 (Camellia tsaii) at 82.6%, and #243 (Commiphora gileadensis) at 82.5%. Both Commiphora species landed near the top again, which I did not want to read too much into until I saw them survive the treatment. A run of others, #213 (Pleiogynium solandri), #203 (Melaleuca decora), #270 (Eugenia uniflora) and #45 (Pistacia atlantica), stayed above 80%. Their active compounds clearly shrug off oxidation.

Inhibition % Calculations — MRSA (Control samples, 24 h endpoint)

Inhibition (%) Formula = [(ODreference − ODsample) / ODreference] × 100 | ODreference = AVERAGE (A3, A4) | SD% = (Std Dev / |Mean Inh%|) × 100 | CV% flag >10% = ⚠ HIGH VAR

>50% Strong inhibitor = Sage Green
20–50% Moderate inhibitor= yellow green
0–20% Weak/negligible inhibitor= sand
<0% Growth promoter=clay

Table 14: MRSA inhibition for all 90 oxidized samples

Sample #	Rep 1 Well	Rep 2 Well	ODref (avg A3+A4)	Rep 1 Abs (24h)	Rep 2 Abs (24h)	Inh % Rep 1	Inh % Rep 2	Ave Inh %	Std Dev (±)	SD % (StdDev/Mean)	CV% / Flag
▸ Set 1 (Samples 1–42) — MRSA | ODref (A3+A4 avg) = 0.885 (A3=0.883, A4=0.887)											
1	B1	B2	0,885	0,195	0,193	78,0	78,2	78	0,2	0 %	OK
4	B3	B4	0,885	0,147	0,161	83,4	81,8	83	1,1	1 %	OK
19	B5	B6	0,885	0,819	0,810	7,5	8,5	8	0,7	9 %	OK
25	B7	B8	0,885	1,075	0,915	-21,5	-3,4	-12	12,8	-103 %	⚠ HIGH VAR
34	B9	B10	0,885	0,966	1,349	-9,2	-52,4	-31	30,6	-99 %	⚠ HIGH VAR
44	B11	B12	0,885	1,172	1,171	-32,4	-32,3	-32	0,1	0 %	OK
45	C1	C2	0,885	0,161	0,186	81,8	79,0	80	2,0	2 %	OK
48	C3	C4	0,885	1,071	1,040	-21,0	-17,5	-19	2,5	-13 %	OK
53	C5	C6	0,885	1,024	1,044	-15,7	-18,0	-17	1,6	-9 %	OK
55	C7	C8	0,885	0,938	1,514	-6,0	-71,1	-39	46,0	-119 %	⚠ HIGH VAR
56	C9	C10	0,885	0,585	0,603	33,9	31,9	33	1,4	4 %	OK
58	C11	C12	0,885	1,006	0,969	-13,7	-9,5	-12	3,0	-26 %	OK
62	D1	D2	0,885	1,164	1,111	-31,5	-25,5	-29	4,2	-15 %	OK
63	D3	D4	0,885	0,933	0,649	-5,4	26,7	11	22,7	214 %	⚠ HIGH VAR
70	D5	D6	0,885	1,097	1,110	-24,0	-25,4	-25	1,0	-4 %	OK
74	D7	D8	0,885	1,095	1,083	-23,7	-22,4	-23	1,0	-4 %	OK
80	D9	D10	0,885	0,765	0,786	13,6	11,2	12	1,7	14 %	OK
84	D11	D12	0,885	0,132	0,127	85,1	85,6	85	0,4	0 %	OK
86	E1	E2	0,885	0,734	0,638	17,1	27,9	22	7,7	34 %	⚠ HIGH VAR
88	E3	E4	0,885	1,248	1,194	-41,0	-34,9	-38	4,3	-11 %	OK
89	E5	E6	0,885	0,966	0,999	-9,2	-12,9	-11	2,6	-24 %	OK
92	E7	E8	0,885	1,033	1,031	-16,7	-16,5	-17	0,2	-1 %	OK
96	E9	E10	0,885	0,995	0,990	-12,4	-11,9	-12	0,4	-3 %	OK
100	E11	E12	0,885	0,949	1,033	-7,2	-16,7	-12	6,7	-56 %	OK
106	F1	F2	0,885	0,672	0,564	24,1	36,3	30	8,6	29 %	⚠ HIGH VAR

110	F3	F4	0,885	0,702	0,607	20,7	31,4	26	7,6	29 %	⚠ HIGH VAR
111	F5	F6	0,885	1,038	1,012	-17,3	-14,4	-16	2,1	-13 %	OK
112	F7	F8	0,885	1,077	1,090	-21,7	-23,2	-22	1,0	-5 %	OK
113	F9	F10	0,885	0,257	0,252	71,0	71,5	71	0,4	1 %	OK
120	F11	F12	0,885	0,680	0,777	23,2	12,2	18	7,8	44 %	⚠ HIGH VAR
126	G1	G2	0,885	0,390	0,373	55,9	57,9	57	1,4	2 %	OK
125	G3	G4	0,885	0,992	1,407	-12,1	-59,0	-36	33,2	-93 %	⚠ HIGH VAR
129	G5	G6	0,885	1,069	1,083	-20,8	-22,4	-22	1,1	-5 %	OK
133	G7	G8	0,885	0,767	0,750	13,3	15,3	14	1,4	10 %	OK
137	G9	G10	0,885	0,863	0,817	2,5	7,7	5	3,7	72 %	OK
148	G11	G12	0,885	1,192	1,198	-34,7	-35,4	-35	0,5	-1 %	OK
159	H1	H2	0,885	0,780	0,818	11,9	7,6	10	3,0	31 %	OK
161	H3	H4	0,885	1,051	0,944	-18,8	-6,7	-13	8,5	-67 %	⚠ HIGH VAR
163	H5	H6	0,885	0,888	0,904	-0,3	-2,1	-1	1,3	-103 %	OK
164	H7	H8	0,885	1,214	1,219	-37,2	-37,7	-37	0,4	-1 %	OK
165	H9	H10	0,885	1,184	1,181	-33,8	-33,4	-34	0,2	-1 %	OK
166	H11	H12	0,885	1,144	1,156	-29,3	-30,6	-30	1,0	-3 %	OK
▸ **Set 2 (Samples 43–84) — MRSA | ODref (A3+A4 avg) = 0.922 (A3=0.925, A4=0.92)**											
169	B1	B2	0,923	1,193	1,210	-29,3	-31,2	-30	1,3	-4 %	OK
172	B3	B4	0,923	1,119	1,110	-21,3	-20,3	-21	0,7	-3 %	OK
176	B5	B6	0,923	0,975	0,927	-5,7	-0,5	-3	3,7	-119 %	OK
188	B7	B8	0,923	1,164	1,167	-26,2	-26,5	-26	0,2	-1 %	OK
190	B9	B10	0,923	1,114	1,075	-20,8	-16,5	-19	3,0	-16 %	OK
191	B11	B12	0,923	1,116	1,112	-21,0	-20,5	-21	0,3	-1 %	OK
193	C1	C2	0,923	0,588	0,128	36,3	86,1	61	35,3	58 %	⚠ HIGH VAR
194	C3	C4	0,923	1,056	1,055	-14,5	-14,4	-14	0,1	-1 %	OK
195	C5	C6	0,923	1,042	1,044	-13,0	-13,2	-13	0,2	-1 %	OK
197	C7	C8	0,923	1,173	1,169	-27,2	-26,7	-27	0,3	-1 %	OK
198	C9	C10	0,923	1,098	1,102	-19,0	-19,5	-19	0,3	-2 %	OK
199	C11	C12	0,923	1,053	1,058	-14,1	-14,7	-14	0,4	-3 %	OK
203	D1	D2	0,923	0,170	0,164	81,6	82,2	82	0,5	1 %	OK
206	D3	D4	0,923	0,683	0,551	26,0	40,3	33	10,1	31 %	⚠ HIGH VAR
208	D5	D6	0,923	0,180	0,186	80,5	79,8	80	0,5	1 %	OK
210	D7	D8	0,923	0,682	0,413	26,1	55,2	41	20,6	51 %	⚠ HIGH VAR
211	D9	D10	0,923	0,158	0,161	82,9	82,5	83	0,2	0 %	OK
212	D11	D12	0,923	0,857	0,731	7,1	20,8	14	9,7	69 %	⚠ HIGH VAR
213	E1	E2	0,923	0,166	0,161	82,0	82,5	82	0,4	0 %	OK
214	E3	E4	0,923	0,120	0,192	87,0	79,2	83	5,5	7 %	OK
225	E5	E6	0,923	1,026	1,010	-11,2	-9,5	-10	1,2	-12 %	OK

228	E7	E8	0,923	1,252	1,246	-35,7	-35,1	-35	0,5	-1 %	OK
229	E9	E10	0,923	0,592	0,403	35,8	56,3	46	14,5	31 %	⚠ HIGH VAR
238	E11	E12	0,923	1,016	1,013	-10,1	-9,8	-10	0,2	-2 %	OK
242	F1	F2	0,923	0,156	0,164	83,1	82,2	83	0,6	1 %	OK
245	F3	F4	0,923	0,561	0,476	39,2	48,4	44	6,5	15 %	OK
243	F5	F6	0,923	0,161	0,162	82,5	82,4	82	0,1	0 %	OK
249	F7	F8	0,923	1,140	1,139	-23,6	-23,5	-24	0,1	0 %	OK
253	F9	F10	0,923	1,117	1,116	-21,1	-21,0	-21	0,1	0 %	OK
254	F11	F12	0,923	1,157	1,165	-25,4	-26,3	-26	0,6	-2 %	OK
257	G1	G2	0,923	1,548	1,178	-67,8	-27,7	-48	28,4	-59 %	⚠ HIGH VAR
258	G3	G4	0,923	1,164	1,168	-26,2	-26,6	-26	0,3	-1 %	OK
264	G5	G6	0,923	1,045	1,053	-13,3	-14,1	-14	0,6	-4 %	OK
269	G7	G8	0,923	1,043	1,033	-13,1	-12,0	-13	0,8	-6 %	OK
270	G9	G10	0,923	0,179	0,167	80,6	81,9	81	0,9	1 %	OK
271	G11	G12	0,923	1,169	1,179	-26,7	-27,8	-27	0,8	-3 %	OK
273	H1	H2	0,923	0,862	0,930	6,6	-0,8	3	5,2	181 %	OK
275	H3	H4	0,923	0,946	1,540	-2,5	-66,9	-35	45,5	-131 %	⚠ HIGH VAR
276	H5	H6	0,923	1,175	1,194	-27,4	-29,4	-28	1,5	-5 %	OK
279	H7	H8	0,923	1,261	1,263	-36,7	-36,9	-37	0,2	0 %	OK
281	H9	H10	0,923	1,238	1,243	-34,2	-34,7	-34	0,4	-1 %	OK
284	H11	H12	0,923	0,648	0,638	29,8	30,8	30	0,8	3 %	OK
▸ Set 3 (Samples 85–90) — MRSA | ODref (A3+A4 avg) = 0.896 (A3=0.895, A4=0.898)											
285	B1	B2	0,897	0,457	0,363	49,0	59,5	54	7,4	14 %	⚠ HIGH VAR
288	B3	B4	0,897	1,156	1,152	-28,9	-28,5	-29	0,3	-1 %	OK
289	B5	B6	0,897	0,970	0,985	-8,2	-9,9	-9	1,2	-13 %	OK
290	B7	B8	0,897	1,103	1,061	-23,0	-18,3	-21	3,3	-16 %	OK
291	B9	B10	0,897	1,128	1,150	-25,8	-28,3	-27	1,7	-6 %	OK
293	B11	B12	0,897	0,936	0,870	-4,4	3,0	-1	5,2	-718 %	OK

5.1.2.3) S. aureus Results:

The clearest way to see the effect is to put each strong untreated inhibitor next to its oxidized self. Most of the bars barely shift, which tells me those extracts keep working after oxidation. Two break the pattern hard. #110 (*Plumbago auriculata*), the best inhibitor in the whole control set, falls from 91% down to 26%. #284 (*Tectaria zeylanica*) drops from 87% to around 30%. Whatever does the work in those two gets destroyed by oxidation, and that is a useful thing to know rather than a disappointment.

Inhibition % Calculations — S. aureus (Control samples, 24 h endpoint)

Inhibition (%) Formula = [(ODreference − ODsample) / ODreference] × 100 | ODreference = AVERAGE (A3, A4) | SD% = (Std Dev / |Mean Inh%|) × 100 | CV% flag >10% = ⚠ HIGH VAR

>50% Strong inhibitor = Sage Green

20–50% Moderate inhibitor= yellow green

0–20% Weak/negligible inhibitor= sand

<0% Growth promoter=clay

Table 15. S. aureus (DSM 1104) inhibition for all 90 oxidized samples

Sample #	Rep 1 Well	Rep 2 Well	ODref (avg A3+A4)	Rep 1 Abs (24h)	Rep 2 Abs (24h)	Inh % Rep 1	Inh % Rep 2	Ave Inh %	Std Dev (±)	SD % (StdDev/Mean)	CV% / Flag
▸ Set 1 (Samples 1–42) — S. aureus | ODref (A3+A4 avg) = 0.218 (A3=0.218, A4=0.218)											
1	B1	B2	0,218	0,166	0,214	23,9	1,8	13	15,6	121 %	⚠ HIGH VAR
4	B3	B4	0,218	0,138	0,138	36,7	36,7	37	0,0	0 %	OK
19	B5	B6	0,218	0,747	0,758	-242,7	-247,7	-245	3,6	-1 %	OK
25	B7	B8	0,218	0,671	0,713	-207,8	-227,1	-217	13,6	-6 %	⚠ HIGH VAR
34	B9	B10	0,218	0,845	0,850	-287,6	-289,9	-289	1,6	-1 %	OK
44	B11	B12	0,218	0,884	0,890	-305,5	-308,3	-307	1,9	-1 %	OK
45	C1	C2	0,218	0,099	0,100	54,6	54,1	54	0,3	1 %	OK
48	C3	C4	0,218	0,834	0,828	-282,6	-279,8	-281	1,9	-1 %	OK
53	C5	C6	0,218	0,534	0,534	-145,0	-145,0	-145	0,0	0 %	OK
55	C7	C8	0,218	0,785	0,788	-260,1	-261,5	-261	1,0	0 %	OK
56	C9	C10	0,218	0,106	0,101	51,4	53,7	53	1,6	3 %	OK
58	C11	C12	0,218	0,662	0,713	-203,7	-227,1	-215	16,5	-8 %	⚠ HIGH VAR
62	D1	D2	0,218	0,773	0,700	-254,6	-221,1	-238	23,7	-10 %	⚠ HIGH VAR
63	D3	D4	0,218	0,885	0,879	-306,0	-303,2	-305	1,9	-1 %	OK
70	D5	D6	0,218	0,825	0,846	-278,4	-288,1	-283	6,8	-2 %	OK
74	D7	D8	0,218	0,868	0,831	-298,2	-281,2	-290	12,0	-4 %	⚠ HIGH VAR
80	D9	D10	0,218	0,121	0,123	44,5	43,6	44	0,6	1 %	OK
84	D11	D12	0,218	0,133	0,146	39,0	33,0	36	4,2	12 %	OK
86	E1	E2	0,218	0,742	0,767	-240,4	-251,8	-246	8,1	-3 %	⚠ HIGH VAR
88	E3	E4	0,218	0,860	0,691	-294,5	-217,0	-256	54,8	-21 %	⚠ HIGH VAR
89	E5	E6	0,218	0,788	0,774	-261,5	-255,0	-258	4,5	-2 %	OK
92	E7	E8	0,218	0,394	0,392	-80,7	-79,8	-80	0,6	-1 %	OK
96	E9	E10	0,218	0,630	0,487	-189,0	-123,4	-156	46,4	-30 %	⚠ HIGH VAR
100	E11	E12	0,218	0,610	0,584	-179,8	-167,9	-174	8,4	-5 %	⚠ HIGH VAR
106	F1	F2	0,218	0,241	0,648	-10,6	-197,2	-104	132,0	-127 %	⚠ HIGH VAR
110	F3	F4	0,218	0,099	0,102	54,6	53,2	54	1,0	2 %	OK

111	F5	F6	0,218	0,742	0,757	-240,4	-247,2	-244	4,9	-2 %	OK
112	F7	F8	0,218	0,825	0,853	-278,4	-291,3	-285	9,1	-3 %	⚠ HIGH VAR
113	F9	F10	0,218	0,278	0,269	-27,5	-23,4	-25	2,9	-11 %	OK
120	F11	F12	0,218	0,634	0,681	-190,8	-212,4	-202	15,2	-8 %	⚠ HIGH VAR
126	G1	G2	0,218	0,106	0,101	51,4	53,7	53	1,6	3 %	OK
125	G3	G4	0,218	0,846	0,840	-288,1	-285,3	-287	1,9	-1 %	OK
129	G5	G6	0,218	0,550	0,534	-152,3	-145,0	-149	5,2	-3 %	OK
133	G7	G8	0,218	0,671	0,630	-207,8	-189,0	-198	13,3	-7 %	⚠ HIGH VAR
137	G9	G10	0,218	0,536	0,502	-145,9	-130,3	-138	11,0	-8 %	⚠ HIGH VAR
148	G11	G12	0,218	0,404	0,424	-85,3	-94,5	-90	6,5	-7 %	OK
159	H1	H2	0,218	0,777	0,764	-256,4	-250,5	-253	4,2	-2 %	OK
161	H3	H4	0,218	0,587	0,577	-169,3	-164,7	-167	3,2	-2 %	OK
163	H5	H6	0,218	0,756	0,762	-246,8	-249,5	-248	1,9	-1 %	OK
164	H7	H8	0,218	0,466	0,478	-113,8	-119,3	-117	3,9	-3 %	OK
165	H9	H10	0,218	0,394	0,406	-80,7	-86,2	-83	3,9	-5 %	OK
166	H11	H12	0,218	0,421	0,424	-93,1	-94,5	-94	1,0	-1 %	OK
▸ **Set 2 (Samples 43–84) — S. aureus | ODref (A3+A4 avg) = 0.212 (A3=0.211, A4=0.214)**											
169	B1	B2	0,213	0,481	0,465	-126,4	-118,8	-123	5,3	-4 %	OK
172	B3	B4	0,213	0,343	0,398	-61,4	-87,3	-74	18,3	-25 %	⚠ HIGH VAR
176	B5	B6	0,213	0,810	0,834	-281,2	-292,5	-287	8,0	-3 %	⚠ HIGH VAR
188	B7	B8	0,213	0,417	0,455	-96,2	-114,1	-105	12,6	-12 %	⚠ HIGH VAR
190	B9	B10	0,213	0,860	0,867	-304,7	-308,0	-306	2,3	-1 %	OK
191	B11	B12	0,213	0,762	0,707	-258,6	-232,7	-246	18,3	-7 %	⚠ HIGH VAR
193	C1	C2	0,213	0,117	0,121	44,9	43,1	44	1,3	3 %	OK
194	C3	C4	0,213	0,780	0,778	-267,1	-266,1	-267	0,7	0 %	OK
195	C5	C6	0,213	0,607	0,574	-185,6	-170,1	-178	11,0	-6 %	⚠ HIGH VAR
197	C7	C8	0,213	0,806	0,843	-279,3	-296,7	-288	12,3	-4 %	⚠ HIGH VAR
198	C9	C10	0,213	0,901	0,910	-324,0	-328,2	-326	3,0	-1 %	OK
199	C11	C12	0,213	0,491	0,431	-131,1	-102,8	-117	20,0	-17 %	⚠ HIGH VAR
203	D1	D2	0,213	0,153	0,158	28,0	25,6	27	1,7	6 %	OK
206	D3	D4	0,213	0,109	0,111	48,7	47,8	48	0,7	1 %	OK
208	D5	D6	0,213	0,160	0,157	24,7	26,1	25	1,0	4 %	OK
210	D7	D8	0,213	0,147	0,316	30,8	-48,7	-9	56,2	-629 %	⚠ HIGH VAR
211	D9	D10	0,213	0,147	0,150	30,8	29,4	30	1,0	3 %	OK
212	D11	D12	0,213	0,882	0,865	-315,1	-307,1	-311	5,7	-2 %	OK
213	E1	E2	0,213	0,160	0,163	24,7	23,3	24	1,0	4 %	OK
214	E3	E4	0,213	0,124	0,112	41,6	47,3	44	4,0	9 %	OK
225	E5	E6	0,213	0,743	0,763	-249,6	-259,1	-254	6,7	-3 %	OK

228	E7	E8	0,213	0,687	0,862	-223,3	-305,6	-264	58,2	-22 %	⚠ HIGH VAR
229	E9	E10	0,213	0,652	0,632	-206,8	-197,4	-202	6,7	-3 %	OK
238	E11	E12	0,213	0,898	0,855	-322,6	-302,4	-312	14,3	-5 %	⚠ HIGH VAR
242	F1	F2	0,213	0,153	0,153	28,0	28,0	28	0,0	0 %	OK
245	F3	F4	0,213	0,101	0,104	52,5	51,1	52	1,0	2 %	OK
243	F5	F6	0,213	0,151	0,154	28,9	27,5	28	1,0	4 %	OK
249	F7	F8	0,213	0,359	0,389	-68,9	-83,1	-76	10,0	-13 %	⚠ HIGH VAR
253	F9	F10	0,213	0,659	0,613	-210,1	-188,5	-199	15,3	-8 %	⚠ HIGH VAR
254	F11	F12	0,213	0,471	0,472	-121,6	-122,1	-122	0,3	0 %	OK
257	G1	G2	0,213	0,371	0,385	-74,6	-81,2	-78	4,7	-6 %	OK
258	G3	G4	0,213	0,642	0,661	-202,1	-211,1	-207	6,3	-3 %	OK
264	G5	G6	0,213	0,818	0,820	-284,9	-285,9	-285	0,7	0 %	OK
269	G7	G8	0,213	0,195	0,303	8,2	-42,6	-17	35,9	-209 %	⚠ HIGH VAR
270	G9	G10	0,213	0,136	0,135	36,0	36,5	36	0,3	1 %	OK
271	G11	G12	0,213	0,508	0,489	-139,1	-130,1	-135	6,3	-5 %	OK
273	H1	H2	0,213	0,855	0,902	-302,4	-324,5	-313	15,6	-5 %	⚠ HIGH VAR
275	H3	H4	0,213	0,109	0,169	48,7	20,5	35	20,0	58 %	⚠ HIGH VAR
276	H5	H6	0,213	0,386	0,386	-81,6	-81,6	-82	0,0	0 %	OK
279	H7	H8	0,213	0,505	0,531	-137,6	-149,9	-144	8,7	-6 %	⚠ HIGH VAR
281	H9	H10	0,213	0,528	0,543	-148,5	-155,5	-152	5,0	-3 %	OK
284	H11	H12	0,213	0,658	0,555	-209,6	-161,2	-185	34,3	-18 %	⚠ HIGH VAR
▸ Set 3 (Samples 85–90) — S. aureus | ODref (A3+A4 avg) = 0.221 (A3=0.222, A4=0.219)											
285	B1	B2	0,221	0,105	0,104	52,4	52,8	53	0,3	1 %	OK
288	B3	B4	0,221	0,344	0,337	-56,0	-52,8	-54	2,2	-4 %	OK
289	B5	B6	0,221	0,704	0,797	-219,3	-261,5	-240	29,8	-12 %	⚠ HIGH VAR
290	B7	B8	0,221	0,380	0,383	-72,3	-73,7	-73	1,0	-1 %	OK
291	B9	B10	0,221	0,494	0,488	-124,0	-121,3	-123	1,9	-2 %	OK
293	B11	B12	0,221	0,818	0,767	-271,0	-247,8	-259	16,4	-6 %	⚠ HIGH VAR

5.1.2.4) E. Coli Results:

Inhibition % Calculations — E. Coli (Control samples, 24 h endpoint)

Inhibition (%) Formula = [(ODreference − ODsample) / ODreference] × 100 | ODreference = AVERAGE (A3, A4) | SD% = (Std Dev / |Mean Inh%|) × 100 | CV% flag >10% = ⚠ HIGH VAR

>50% Strong inhibitor = Sage Green

20–50% Moderate inhibitor= yellow green

0–20% Weak/negligible inhibitor= sand

<0% Growth promoter=clay

Table 16: E. coli (DSM 682) inhibition for all 90 oxidized samples

Sample #	Rep 1 Well	Rep 2 Well	ODref (avg A3+A4)	Rep 1 Abs (24h)	Rep 2 Abs (24h)	Inh % Rep 1	Inh % Rep 2	Ave Inh %	Std Dev (±)	SD % (StdDev/Mean)	CV% / Flag
▸ Set 1 (Samples 1–42) — E. coli | ODref (A3+A4 avg) = 0.430 (A3=0.417, A4=0.444)											
1	B1	B2	0,431	0,465	0,468	-8,0	-8,7	-8	0,5	-6 %	OK
4	B3	B4	0,431	0,557	0,551	-29,4	-28,0	-29	1,0	-3 %	OK
19	B5	B6	0,431	0,535	0,666	-24,3	-54,7	-39	21,5	-54 %	⚠ HIGH VAR
25	B7	B8	0,431	0,524	0,529	-21,7	-22,9	-22	0,8	-4 %	OK
34	B9	B10	0,431	0,502	0,558	-16,6	-29,6	-23	9,2	-40 %	⚠ HIGH VAR
44	B11	B12	0,431	0,488	0,657	-13,4	-52,6	-33	27,8	-84 %	⚠ HIGH VAR
45	C1	C2	0,431	0,363	0,369	15,7	14,3	15	1,0	7 %	OK
48	C3	C4	0,431	0,514	0,513	-19,4	-19,2	-19	0,2	-1 %	OK
53	C5	C6	0,431	0,654	0,679	-51,9	-57,7	-55	4,1	-7 %	OK
55	C7	C8	0,431	0,476	0,404	-10,6	6,2	-2	11,8	-536 %	⚠ HIGH VAR
56	C9	C10	0,431	0,389	0,418	9,6	2,9	6	4,8	76 %	OK
58	C11	C12	0,431	0,584	0,649	-35,7	-50,8	-43	10,7	-25 %	⚠ HIGH VAR
62	D1	D2	0,431	0,606	0,607	-40,8	-41,0	-41	0,2	0 %	OK
63	D3	D4	0,431	0,475	0,517	-10,3	-20,1	-15	6,9	-45 %	OK
70	D5	D6	0,431	0,438	0,553	-1,7	-28,5	-15	18,9	-125 %	⚠ HIGH VAR
74	D7	D8	0,431	0,495	0,580	-15,0	-34,7	-25	14,0	-56 %	⚠ HIGH VAR
80	D9	D10	0,431	0,522	0,494	-21,3	-14,8	-18	4,6	-26 %	OK
84	D11	D12	0,431	0,447	0,456	-3,8	-5,9	-5	1,5	-30 %	OK
86	E1	E2	0,431	0,510	0,572	-18,5	-32,9	-26	10,2	-40 %	⚠ HIGH VAR
88	E3	E4	0,431	0,524	0,536	-21,7	-24,5	-23	2,0	-9 %	OK
89	E5	E6	0,431	0,551	0,616	-28,0	-43,1	-36	10,7	-30 %	⚠ HIGH VAR
92	E7	E8	0,431	0,439	0,459	-2,0	-6,6	-4	3,3	-76 %	OK
96	E9	E10	0,431	0,480	0,464	-11,5	-7,8	-10	2,6	-27 %	OK
100	E11	E12	0,431	0,600	0,586	-39,4	-36,1	-38	2,3	-6 %	OK

106	F1	F2	0,431	0,434	0,434	-0,8	-0,8	-1	0,0	0 %	OK
110	F3	F4	0,431	0,266	0,251	38,2	41,7	40	2,5	6 %	OK
111	F5	F6	0,431	0,649	0,680	-50,8	-58,0	-54	5,1	-9 %	OK
112	F7	F8	0,431	0,515	0,564	-19,6	-31,0	-25	8,0	-32 %	⚠ HIGH VAR
113	F9	F10	0,431	0,699	0,635	-62,4	-47,5	-55	10,5	-19 %	⚠ HIGH VAR
120	F11	F12	0,431	0,477	0,552	-10,8	-28,2	-20	12,3	-63 %	⚠ HIGH VAR
126	G1	G2	0,431	0,296	0,282	31,2	34,5	33	2,3	7 %	OK
125	G3	G4	0,431	0,549	0,559	-27,5	-29,8	-29	1,6	-6 %	OK
129	G5	G6	0,431	0,583	0,601	-35,4	-39,6	-38	3,0	-8 %	OK
133	G7	G8	0,431	0,470	0,432	-9,2	-0,3	-5	6,2	-131 %	OK
137	G9	G10	0,431	0,958	0,515	-122,5	-19,6	-71	72,8	-102 %	⚠ HIGH VAR
148	G11	G12	0,431	0,615	0,611	-42,9	-41,9	-42	0,7	-2 %	OK
159	H1	H2	0,431	0,508	0,618	-18,0	-43,6	-31	18,1	-59 %	⚠ HIGH VAR
161	H3	H4	0,431	0,485	0,518	-12,7	-20,3	-16	5,4	-33 %	OK
163	H5	H6	0,431	0,579	0,500	-34,5	-16,1	-25	13,0	-51 %	⚠ HIGH VAR
164	H7	H8	0,431	0,583	0,587	-35,4	-36,4	-36	0,7	-2 %	OK
165	H9	H10	0,431	0,572	0,643	-32,9	-49,4	-41	11,7	-28 %	⚠ HIGH VAR
166	H11	H12	0,431	0,761	0,474	-76,8	-10,1	-43	47,1	-109 %	⚠ HIGH VAR
▸ **Set 2 (Samples 43–84) — E. coli | ODref (A3+A4 avg) = 0.477 (A3=0.479, A4=0.475)**											
169	B1	B2	0,477	0,762	0,799	-59,7	-67,5	-64	5,5	-9 %	OK
172	B3	B4	0,477	0,781	0,741	-63,7	-55,3	-60	5,9	-10 %	OK
176	B5	B6	0,477	0,449	0,466	5,9	2,3	4	2,5	62 %	OK
188	B7	B8	0,477	0,735	0,769	-54,1	-61,2	-58	5,0	-9 %	OK
190	B9	B10	0,477	0,589	0,601	-23,5	-26,0	-25	1,8	-7 %	OK
191	B11	B12	0,477	0,575	0,602	-20,5	-26,2	-23	4,0	-17 %	OK
193	C1	C2	0,477	0,426	0,430	10,7	9,9	10	0,6	6 %	OK
194	C3	C4	0,477	0,642	0,635	-34,6	-33,1	-34	1,0	-3 %	OK
195	C5	C6	0,477	0,517	0,542	-8,4	-13,6	-11	3,7	-34 %	OK
197	C7	C8	0,477	0,711	0,724	-49,1	-51,8	-50	1,9	-4 %	OK
198	C9	C10	0,477	0,444	0,428	6,9	10,3	9	2,4	28 %	OK
199	C11	C12	0,477	0,633	0,696	-32,7	-45,9	-39	9,3	-24 %	⚠ HIGH VAR
203	D1	D2	0,477	0,589	0,593	-23,5	-24,3	-24	0,6	-2 %	OK
206	D3	D4	0,477	0,370	0,341	22,4	28,5	25	4,3	17 %	OK
208	D5	D6	0,477	0,564	0,661	-18,2	-38,6	-28	14,4	-51 %	⚠ HIGH VAR
210	D7	D8	0,477	0,467	0,412	2,1	13,6	8	8,2	104 %	⚠ HIGH VAR
211	D9	D10	0,477	0,601	0,588	-26,0	-23,3	-25	1,9	-8 %	OK
212	D11	D12	0,477	0,427	0,413	10,5	13,4	12	2,1	17 %	OK
213	E1	E2	0,477	0,322	0,229	32,5	52,0	42	13,8	33 %	⚠ HIGH VAR

214	E3	E4	0,477	0,246	0,226	48,4	52,6	51	3,0	6 %	OK
225	E5	E6	0,477	0,593	0,653	-24,3	-36,9	-31	8,9	-29 %	⚠ HIGH VAR
228	E7	E8	0,477	0,791	0,838	-65,8	-75,7	-71	7,0	-10 %	OK
229	E9	E10	0,477	0,318	0,362	33,3	24,1	29	6,5	23 %	OK
238	E11	E12	0,477	0,499	0,590	-4,6	-23,7	-14	13,5	-95 %	⚠ HIGH VAR
242	F1	F2	0,477	0,708	0,727	-48,4	-52,4	-50	2,8	-6 %	OK
245	F3	F4	0,477	0,337	0,291	29,4	39,0	34	6,8	20 %	OK
243	F5	F6	0,477	0,693	0,681	-45,3	-42,8	-44	1,8	-4 %	OK
249	F7	F8	0,477	0,722	0,742	-51,4	-55,6	-53	3,0	-6 %	OK
253	F9	F10	0,477	0,593	0,608	-24,3	-27,5	-26	2,2	-9 %	OK
254	F11	F12	0,477	0,494	0,461	-3,6	3,4	0	4,9	-4667 %	OK
257	G1	G2	0,477	0,618	0,591	-29,6	-23,9	-27	4,0	-15 %	OK
258	G3	G4	0,477	0,631	0,616	-32,3	-29,1	-31	2,2	-7 %	OK
264	G5	G6	0,477	0,558	0,665	-17,0	-39,4	-28	15,9	-56 %	⚠ HIGH VAR
269	G7	G8	0,477	0,642	0,709	-34,6	-48,6	-42	9,9	-24 %	⚠ HIGH VAR
270	G9	G10	0,477	0,606	0,547	-27,0	-14,7	-21	8,7	-42 %	⚠ HIGH VAR
271	G11	G12	0,477	0,703	0,681	-47,4	-42,8	-45	3,3	-7 %	OK
273	H1	H2	0,477	0,422	0,441	11,5	7,5	10	2,8	30 %	OK
275	H3	H4	0,477	0,402	0,511	15,7	-7,1	4	16,2	376 %	⚠ HIGH VAR
276	H5	H6	0,477	0,662	0,653	-38,8	-36,9	-38	1,3	-4 %	OK
279	H7	H8	0,477	0,752	0,700	-57,7	-46,8	-52	7,7	-15 %	⚠ HIGH VAR
281	H9	H10	0,477	0,692	0,685	-45,1	-43,6	-44	1,0	-2 %	OK
284	H11	H12	0,477	0,431	0,379	9,6	20,5	15	7,7	51 %	⚠ HIGH VAR
▸ Set 3 (Samples 85–90) — E. coli | ODref (A3+A4 avg) = 0.453 (A3=0.452, A4=0.453)											
285	B1	B2	0,453	0,330	0,362	27,1	20,0	24	5,0	21 %	OK
288	B3	B4	0,453	0,596	0,642	-31,7	-41,9	-37	7,2	-20 %	⚠ HIGH VAR
289	B5	B6	0,453	0,477	0,476	-5,4	-5,2	-5	0,2	-3 %	OK
290	B7	B8	0,453	0,560	0,728	-23,8	-60,9	-42	26,3	-62 %	⚠ HIGH VAR
291	B9	B10	0,453	0,457	0,452	-1,0	0,1	0	0,8	-177 %	OK
293	B11	B12	0,453	0,485	0,417	-7,2	7,8	0	10,6	3206 %	⚠ HIGH VAR

5.1.2.5): Broad Spectrum of the Strains Results:

The clearest way to see the effect is to put each strong untreated inhibitor next to its oxidized self. Most of the bars barely shift, which tells me those extracts keep working after oxidation. Two break the pattern hard. #110 (*Plumbago auriculata*), the best inhibitor in the whole control set, falls from 91% down to 26%. #284 (*Tectaria zeylanica*) drops from 87% to around 30%. Whatever does the work in those two gets destroyed by oxidation, and that is a useful thing to know rather than a disappointment.

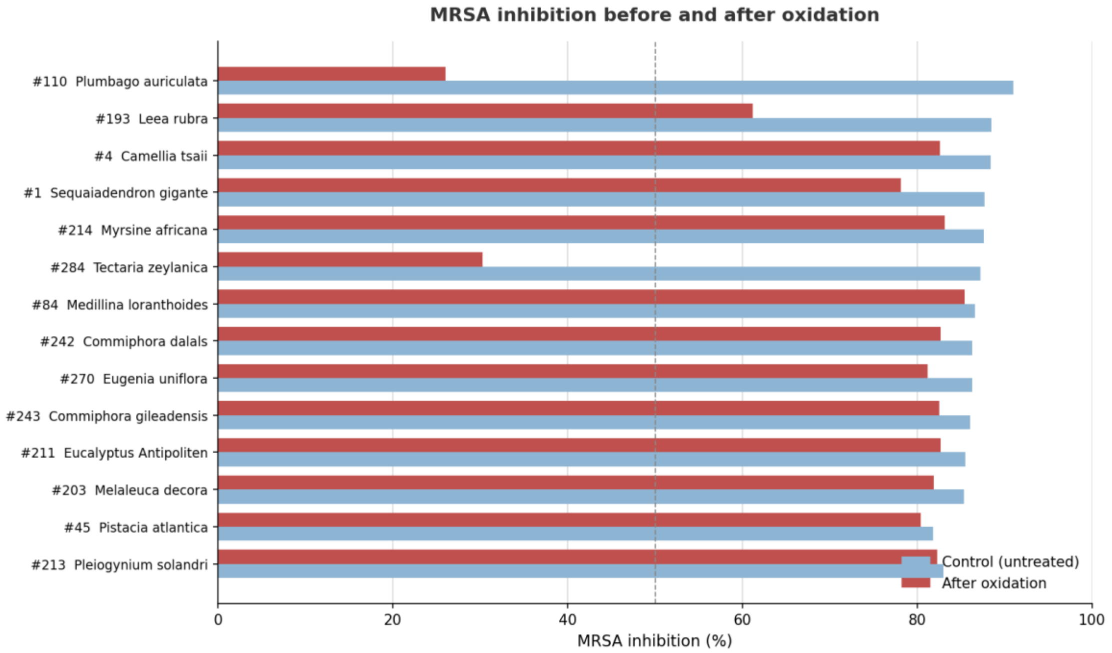


Figure 13: MRSA inhibition before oxidation (blue) and after (red), for the strongest untreated inhibitors. Most hold; #110 and #284 drop sharply.

Looking at the oxidized numbers on their own, the radar below takes the ten strongest oxidized MRSA inhibitors and plots all three strains together. MRSA is the outer red ring; the two references sit inside it.

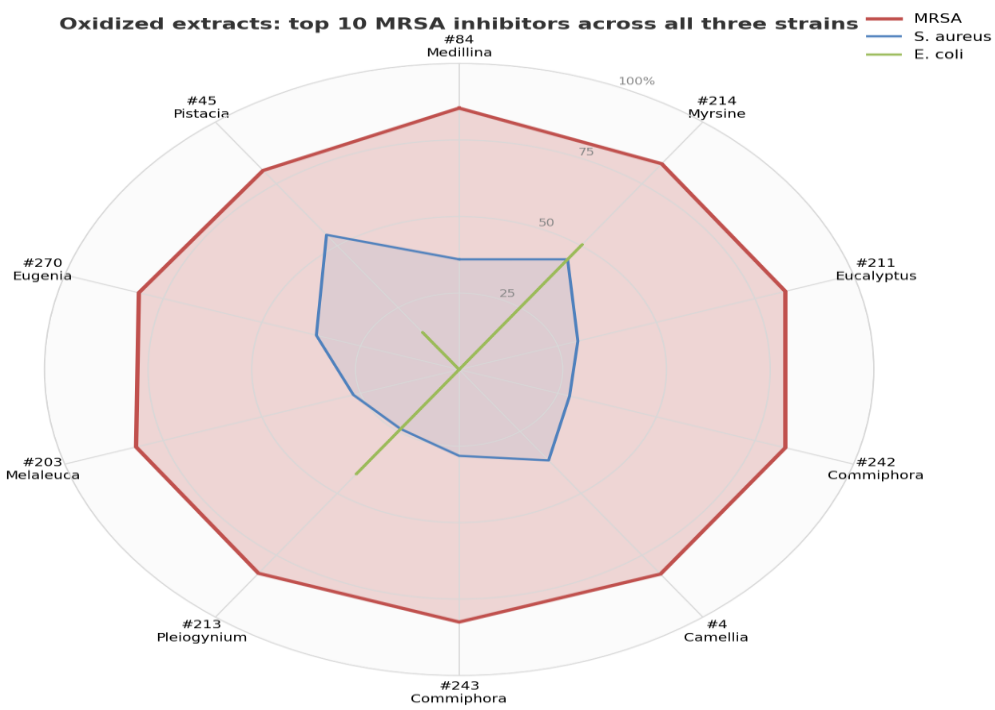


Figure 14: Top 10 MRSA inhibitors after oxidation, across all three strains. Negative reference values are drawn as zero to keep the shape readable.

5.1.3) Nylon Filtration (C) Samples Results:

5.1.3.1) Calculation of Activity:

Same method as the control and oxidation screens, so all three compare directly. Each filtered extract grew with bacteria for 24 hours while I followed optical density at 600 nm, and I read inhibition at the ultimate point:

$$\text{Inhibition (\%)} = [(\text{ODref} - \text{ODsample}) \div \text{ODref}] \times 100$$

ODref is the average final density of the A3 and A4 solvent wells, ODsample the final density of the treated well. Duplicates, averaged, spread reported as a standard deviation, pairs disagreeing by more than 10 points flagged. Above 50% is strong, 20 to 50% moderate, 0 to 20% weak, below zero is growth promotion.

Filtration hit MRSA harder than either of the other treatments. Only 10 of the 90 extracts still inhibited strongly, the average sank to -2.2%, and 65 samples tipped into promotion. Put the three side by side and the trend is stark: the MRSA average ran about 29% in the control, fell to 4% after oxidation, and now sits just below zero after filtration. That is exactly what I would expect if the big polyphenols nylon removes were carrying most of the antibacterial punch. Strip them out and the activity mostly goes with them.

Table 17: Activity across all 90 nylon-filtered samples, by strain. Reference-strain averages are affected by the low

Strain	Avg inh %	Strong ≥50%	Moderate 20–50%	Weak 0–20%	Promoter <0%
MRSA (DSM 21979), primary target	**-2.2%**	**10 (11%)**	**9 (10%)**	**6 (7%)**	**65 (72%)**
S. aureus (DSM 1104) †	-62.6%	3 (3%)	14 (16%)	1 (1%)	72 (80%)
E. coli (DSM 682) †	-7.4%	2 (2%)	10 (11%)	7 (8%)	71 (79%)

5.1.3.2) MRSA Results:

Ten extracts held strong against MRSA even after filtration, and those ten matter, because they are the ones whose activity does not depend on the big nylon-bound molecules. The leaders were #213 (*Pleiogynium solandri*) at 84.1%, #208 (*Acaena Pallida*) at 82.6%, #113 (*Hydnophytum formucarum*) at 75.4%, and #245 (*Crassula ovata*) at 73.5%. One of those is worth a second look. #245 (*Crassula ovata*) climbed after filtration, from about 51% in the control to 74% here, which is odd and means filtration removed something that was interfering rather than something that was helping. The rest of the strong group, #211 (*Eucalyptus Antipolitenis*), #193 (*Leea rubra*), #206 (*Cissus antarctica*) and #126 (*Ficus lutea -fruits*), all held above 50%.

Inhibition % Calculations — MRSA (Control samples, 24 h endpoint)

Inhibition (%) Formula = [(ODreference − ODsample) / ODreference] × 100 | ODreference = AVERAGE (A3, A4) | SD% = (Std Dev / |Mean Inh%|) × 100 | CV% flag >10% = ⚠ HIGH VAR

>50% Strong inhibitor = Sage Green

20–50% Moderate inhibitor= yellow green

0–20% Weak/negligible inhibitor= sand

<0% Growth promoter=clay

Table 18: MRSA inhibition for all 90 nylon-filtered samples

Sample #	Rep 1 Well	Rep 2 Well	ODref (avg A3+A4)	Rep 1 Abs (24h)	Rep 2 Abs (24h)	Inh % Rep 1	Inh % Rep 2	Ave Inh %	Std Dev (±)	SD % (StdDev/Mean)	CV% / Flag
▸ Set 1 (Samples 1–42) — MRSA | ODref (A3+A4 avg) = 0.954 (A3=0.955, A4=0.953)											
1	B1	B2	0,954	0,777	0,823	18,6	13,7	16	3,4	21 %	OK
4	B3	B4	0,954	0,571	0,568	40,1	40,5	40	0,2	1 %	OK
19	B5	B6	0,954	1,053	1,032	-10,4	-8,2	-9	1,6	-17 %	OK
25	B7	B8	0,954	1,150	1,143	-20,5	-19,8	-20	0,5	-3 %	OK
34	B9	B10	0,954	1,160	1,151	-21,6	-20,6	-21	0,7	-3 %	OK
44	B11	B12	0,954	1,191	1,194	-24,8	-25,2	-25	0,2	-1 %	OK
45	C1	C2	0,954	0,785	0,787	17,7	17,5	18	0,1	1 %	OK
48	C3	C4	0,954	1,137	1,114	-19,2	-16,8	-18	1,7	-9 %	OK
53	C5	C6	0,954	1,084	1,074	-13,6	-12,6	-13	0,7	-6 %	OK
55	C7	C8	0,954	1,138	1,553	-19,3	-62,8	-41	30,8	-75 %	⚠ HIGH VAR
56	C9	C10	0,954	0,762	0,765	20,1	19,8	20	0,2	1 %	OK
58	C11	C12	0,954	1,095	1,084	-14,8	-13,6	-14	0,8	-6 %	OK
62	D1	D2	0,954	1,179	1,178	-23,6	-23,5	-24	0,1	0 %	OK
63	D3	D4	0,954	1,148	1,177	-20,3	-23,4	-22	2,1	-10 %	OK
70	D5	D6	0,954	1,189	1,193	-24,6	-25,1	-25	0,3	-1 %	OK
74	D7	D8	0,954	1,090	1,099	-14,3	-15,2	-15	0,7	-5 %	OK
80	D9	D10	0,954	1,058	1,039	-10,9	-8,9	-10	1,4	-14 %	OK
84	D11	D12	0,954	1,069	1,065	-12,1	-11,6	-12	0,3	-3 %	OK
86	E1	E2	0,954	1,042	0,991	-9,2	-3,9	-7	3,8	-58 %	OK
88	E3	E4	0,954	1,179	1,184	-23,6	-24,1	-24	0,4	-2 %	OK
89	E5	E6	0,954	1,086	1,086	-13,8	-13,8	-14	0,0	0 %	OK
92	E7	E8	0,954	1,136	1,136	-19,1	-19,1	-19	0,0	0 %	OK
96	E9	E10	0,954	1,073	1,070	-12,5	-12,2	-12	0,2	-2 %	OK
100	E11	E12	0,954	1,060	1,017	-11,1	-6,6	-9	3,2	-36 %	OK
106	F1	F2	0,954	1,087	1,083	-13,9	-13,5	-14	0,3	-2 %	OK
110	F3	F4	0,954	0,541	0,513	43,3	46,2	45	2,1	5 %	OK

111	F5	F6	0,954	1,143	1,151	-19,8	-20,6	-20	0,6	-3 %	OK
112	F7	F8	0,954	1,092	1,036	-14,5	-8,6	-12	4,2	-36 %	OK
113	F9	F10	0,954	0,241	0,228	74,7	76,1	75	1,0	1 %	OK
120	F11	F12	0,954	1,218	1,232	-27,7	-29,1	-28	1,0	-4 %	OK
125	G1	G2	0,954	1,170	1,181	-22,6	-23,8	-23	0,8	-4 %	OK
126	G3	G4	0,954	0,472	0,393	50,5	58,8	55	5,9	11 %	OK
129	G5	G6	0,954	1,147	1,158	-20,2	-21,4	-21	0,8	-4 %	OK
133	G7	G8	0,954	1,138	1,135	-19,3	-19,0	-19	0,2	-1 %	OK
137	G9	G10	0,954	1,136	1,135	-19,1	-19,0	-19	0,1	0 %	OK
148	G11	G12	0,954	1,147	1,153	-20,2	-20,9	-21	0,4	-2 %	OK
159	H1	H2	0,954	1,017	1,035	-6,6	-8,5	-8	1,3	-18 %	OK
161	H3	H4	0,954	1,157	1,147	-21,3	-20,2	-21	0,7	-4 %	OK
163	H5	H6	0,954	1,157	1,152	-21,3	-20,8	-21	0,4	-2 %	OK
164	H7	H8	0,954	1,141	1,139	-19,6	-19,4	-19	0,1	-1 %	OK
165	H9	H10	0,954	1,213	1,213	-27,1	-27,1	-27	0,0	0 %	OK
166	H11	H12	0,954	1,145	1,153	-20,0	-20,9	-20	0,6	-3 %	OK
▸ Set 2 (Samples 43–84) — MRSA | ODref (A3+A4 avg) = 0.962 (A3=0.958, A4=0.965)											
169	B1	B2	0,962	1,161	1,160	-20,7	-20,6	-21	0,1	0 %	OK
172	B3	B4	0,962	1,159	1,146	-20,5	-19,2	-20	1,0	-5 %	OK
176	B5	B6	0,962	1,089	1,131	-13,3	-17,6	-15	3,1	-20 %	OK
188	B7	B8	0,962	1,127	1,124	-17,2	-16,9	-17	0,2	-1 %	OK
190	B9	B10	0,962	1,103	1,109	-14,7	-15,3	-15	0,4	-3 %	OK
191	B11	B12	0,962	1,141	1,149	-18,7	-19,5	-19	0,6	-3 %	OK
193	C1	C2	0,962	0,422	0,420	56,1	56,3	56	0,1	0 %	OK
194	C3	C4	0,962	1,076	1,091	-11,9	-13,5	-13	1,1	-9 %	OK
195	C5	C6	0,962	1,209	1,190	-25,7	-23,8	-25	1,4	-6 %	OK
197	C7	C8	0,962	1,152	1,140	-19,8	-18,6	-19	0,9	-5 %	OK
198	C9	C10	0,962	1,139	1,151	-18,5	-19,7	-19	0,9	-5 %	OK
199	C11	C12	0,962	1,074	1,108	-11,7	-15,2	-13	2,5	-19 %	OK
203	D1	D2	0,962	0,179	0,888	81,4	7,6	45	52,1	117 %	⚠ HIGH VAR
206	D3	D4	0,962	0,631	0,232	34,4	75,9	55	29,3	53 %	⚠ HIGH VAR
208	D5	D6	0,962	0,173	0,162	82,0	83,2	83	0,8	1 %	OK
210	D7	D8	0,962	0,977	0,921	-1,6	4,2	1	4,1	317 %	OK
211	D9	D10	0,962	0,251	0,307	73,9	68,1	71	4,1	6 %	OK
212	D11	D12	0,962	1,122	1,128	-16,7	-17,3	-17	0,4	-3 %	OK
213	E1	E2	0,962	0,159	0,147	83,5	84,7	84	0,9	1 %	OK
214	E3	E4	0,962	0,480	0,434	50,1	54,9	52	3,4	6 %	OK
225	E5	E6	0,962	1,137	1,120	-18,3	-16,5	-17	1,3	-7 %	OK

228	E7	E8	0,962	1,191	1,175	-23,9	-22,2	-23	1,2	-5 %	OK
229	E9	E10	0,962	1,051	1,065	-9,3	-10,8	-10	1,0	-10 %	OK
238	E11	E12	0,962	1,161	1,162	-20,7	-20,9	-21	0,1	0 %	OK
242	F1	F2	0,962	1,127	1,105	-17,2	-14,9	-16	1,6	-10 %	OK
243	F3	F4	0,962	1,170	1,144	-21,7	-19,0	-20	1,9	-9 %	OK
245	F5	F6	0,962	0,296	0,213	69,2	77,8	74	6,1	8 %	OK
249	F7	F8	0,962	1,118	1,139	-16,3	-18,5	-17	1,5	-9 %	OK
253	F9	F10	0,962	1,118	1,125	-16,3	-17,0	-17	0,5	-3 %	OK
254	F11	F12	0,962	1,142	1,160	-18,8	-20,6	-20	1,3	-7 %	OK
257	G1	G2	0,962	0,906	0,911	5,8	5,3	6	0,4	7 %	OK
258	G3	G4	0,962	1,190	1,189	-23,8	-23,7	-24	0,1	0 %	OK
264	G5	G6	0,962	1,165	1,150	-21,2	-19,6	-20	1,1	-5 %	OK
269	G7	G8	0,962	1,136	1,136	-18,1	-18,1	-18	0,0	0 %	OK
270	G9	G10	0,962	0,443	0,517	53,9	46,2	50	5,4	11 %	OK
271	G11	G12	0,962	1,159	1,160	-20,5	-20,6	-21	0,1	0 %	OK
273	H1	H2	0,962	1,084	1,111	-12,7	-15,5	-14	2,0	-14 %	OK
275	H3	H4	0,962	1,053	1,048	-9,5	-9,0	-9	0,4	-4 %	OK
276	H5	H6	0,962	1,153	1,142	-19,9	-18,8	-19	0,8	-4 %	OK
279	H7	H8	0,962	1,154	1,149	-20,0	-19,5	-20	0,4	-2 %	OK
281	H9	H10	0,962	1,152	1,159	-19,8	-20,5	-20	0,5	-3 %	OK
284	H11	H12	0,962	0,763	0,861	20,6	10,5	16	7,2	46 %	⚠ HIGH VAR
▸ Set 3 (Samples 85–90) — MRSA | ODref (A3+A4 avg) = 1.225 (A3=1.22, A4=1.23)											
285	B1	B2	1,225	0,946	0,988	22,8	19,3	21	2,4	12 %	OK
288	B3	B4	1,225	0,962	0,955	21,5	22,0	22	0,4	2 %	OK
289	B5	B6	1,225	0,954	0,952	22,1	22,3	22	0,1	1 %	OK
290	B7	B8	1,225	0,972	0,966	20,7	21,1	21	0,3	2 %	OK
291	B9	B10	1,225	0,944	0,957	22,9	21,9	22	0,8	3 %	OK
293	B11	B12	1,225	0,972	1,019	20,7	16,8	19	2,7	14 %	OK

5.1.3.3) S. aureus Results:

Inhibition % Calculations — S. aureus (Control samples, 24 h endpoint)

Inhibition (%) Formula = [(ODreference − ODsample) / ODreference] × 100 | ODreference = AVERAGE (A3, A4) |

SD% = (Std Dev / |Mean Inh%|) × 100 | CV% flag >10% = ⚠ HIGH VAR

>50% Strong inhibitor = Sage Green

20–50% Moderate inhibitor= yellow green

0–20% Weak/negligible inhibitor= sand

<0% Growth promoter=clay

Table 19: S. aureus (DSM 1104) inhibition for all 90 nylon-filtered samples

Sample #	Rep 1 Well	Rep 2 Well	ODref (avg A3+A4)	Rep 1 Abs (24h)	Rep 2 Abs (24h)	Inh % Rep 1	Inh % Rep 2	Ave Inh %	Std Dev (±)	SD % (Std Dev /Mean)	CV % / Flag
▸ Set 1 (Samples 1–42) — S. aureus | ODref (A3+A4 avg) = 0.263 (A3=0.267, A4=0.258)											
1	B1	B2	0,263	0,143	0,646	45,5	-146,1	-50	135,5	-269 %	⚠ HIGH VAR
4	B3	B4	0,263	0,146	0,143	44,4	45,5	45	0,8	2 %	OK
19	B5	B6	0,263	0,633	0,630	-141,1	-140,0	-141	0,8	-1 %	OK
25	B7	B8	0,263	0,445	0,432	-69,5	-64,6	-67	3,5	-5 %	OK
34	B9	B10	0,263	0,436	0,460	-66,1	-75,2	-71	6,5	-9 %	OK
44	B11	B12	0,263	0,513	0,500	-95,4	-90,5	-93	3,5	-4 %	OK
45	C1	C2	0,263	0,587	0,569	-123,6	-116,8	-120	4,8	-4 %	OK
48	C3	C4	0,263	0,438	0,456	-66,9	-73,7	-70	4,8	-7 %	OK
53	C5	C6	0,263	0,844	0,856	-221,5	-226,1	-224	3,2	-1 %	OK
55	C7	C8	0,263	0,414	0,422	-57,7	-60,8	-59	2,2	-4 %	OK
56	C9	C10	0,263	0,766	0,773	-191,8	-194,5	-193	1,9	-1 %	OK
58	C11	C12	0,263	0,593	0,551	-125,9	-109,9	-118	11,3	-10 %	⚠ HIGH VAR
62	D1	D2	0,263	0,451	0,458	-71,8	-74,5	-73	1,9	-3 %	OK
63	D3	D4	0,263	0,384	0,360	-46,3	-37,1	-42	6,5	-15 %	OK
70	D5	D6	0,263	0,431	0,426	-64,2	-62,3	-63	1,3	-2 %	OK
74	D7	D8	0,263	0,364	0,416	-38,7	-58,5	-49	14,0	-29 %	⚠ HIGH VAR
80	D9	D10	0,263	0,491	0,495	-87,0	-88,6	-88	1,1	-1 %	OK
84	D11	D12	0,263	0,366	0,343	-39,4	-30,7	-35	6,2	-18 %	OK
86	E1	E2	0,263	0,910	0,866	-246,7	-229,9	-238	11,9	-5 %	⚠ HIGH VAR
88	E3	E4	0,263	0,437	0,439	-66,5	-67,2	-67	0,5	-1 %	OK
89	E5	E6	0,263	0,433	0,406	-65,0	-54,7	-60	7,3	-12 %	⚠ HIGH VAR

92	E7	E8	0,263	0,457	0,488	-74,1	-85,9	-80	8,4	-10 %	⚠ HIGH VAR
96	E9	E10	0,263	0,491	0,460	-87,0	-75,2	-81	8,4	-10 %	⚠ HIGH VAR
100	E11	E12	0,263	0,873	0,841	-232,6	-220,4	-226	8,6	-4 %	⚠ HIGH VAR
106	F1	F2	0,263	0,838	0,848	-219,2	-223,0	-221	2,7	-1 %	OK
110	F3	F4	0,263	0,100	0,104	61,9	60,4	61	1,1	2 %	OK
111	F5	F6	0,263	0,406	0,438	-54,7	-66,9	-61	8,6	-14 %	⚠ HIGH VAR
112	F7	F8	0,263	0,683	0,735	-160,2	-180,0	-170	14,0	-8 %	⚠ HIGH VAR
113	F9	F10	0,263	0,225	0,216	14,3	17,7	16	2,4	15 %	OK
120	F11	F12	0,263	0,531	0,501	-102,3	-90,9	-97	8,1	-8 %	⚠ HIGH VAR
125	G1	G2	0,263	0,456	0,445	-73,7	-69,5	-72	3,0	-4 %	OK
126	G3	G4	0,263	0,373	0,426	-42,1	-62,3	-52	14,3	-27 %	⚠ HIGH VAR
129	G5	G6	0,263	0,433	0,437	-65,0	-66,5	-66	1,1	-2 %	OK
133	G7	G8	0,263	0,429	0,457	-63,4	-74,1	-69	7,5	-11 %	⚠ HIGH VAR
137	G9	G10	0,263	0,510	0,515	-94,3	-96,2	-95	1,3	-1 %	OK
148	G11	G12	0,263	0,486	0,458	-85,1	-74,5	-80	7,5	-9 %	⚠ HIGH VAR
159	H1	H2	0,263	0,680	0,703	-159,0	-167,8	-163	6,2	-4 %	OK
161	H3	H4	0,263	0,417	0,410	-58,9	-56,2	-58	1,9	-3 %	OK
163	H5	H6	0,263	0,418	0,405	-59,2	-54,3	-57	3,5	-6 %	OK
164	H7	H8	0,263	0,428	0,493	-63,0	-87,8	-75	17,5	-23 %	⚠ HIGH VAR
165	H9	H10	0,263	0,502	0,506	-91,2	-92,8	-92	1,1	-1 %	OK
166	H11	H12	0,263	0,388	0,371	-47,8	-41,3	-45	4,6	-10 %	OK
▸ Set 2 (Samples 43–84) — S. aureus | ODref (A3+A4 avg) = 0.261 (A3=0.263, A4=0.259)											
169	B1	B2	0,261	0,419	0,432	-60,5	-65,5	-63	3,5	-6 %	OK
172	B3	B4	0,261	0,379	0,387	-45,2	-48,3	-47	2,2	-5 %	OK

176	B5	B6	0,261	0,484	0,545	-85,4	-108,8	-97	16,5	-17 %	⚠ HIGH VAR
188	B7	B8	0,261	0,427	0,444	-63,6	-70,1	-67	4,6	-7 %	OK
190	B9	B10	0,261	0,411	0,404	-57,5	-54,8	-56	1,9	-3 %	OK
191	B11	B12	0,261	0,410	0,382	-57,1	-46,4	-52	7,6	-15 %	⚠ HIGH VAR
193	C1	C2	0,261	0,114	0,115	56,3	55,9	56	0,3	0 %	OK
194	C3	C4	0,261	0,407	0,464	-55,9	-77,8	-67	15,4	-23 %	⚠ HIGH VAR
195	C5	C6	0,261	0,430	0,445	-64,8	-70,5	-68	4,1	-6 %	OK
197	C7	C8	0,261	0,444	0,468	-70,1	-79,3	-75	6,5	-9 %	OK
198	C9	C10	0,261	0,449	0,424	-72,0	-62,5	-67	6,8	-10 %	OK
199	C11	C12	0,261	0,529	0,450	-102,7	-72,4	-88	21,4	-24 %	⚠ HIGH VAR
203	D1	D2	0,261	0,147	0,149	43,7	42,9	43	0,5	1 %	OK
206	D3	D4	0,261	0,571	0,600	-118,8	-129,9	-124	7,9	-6 %	⚠ HIGH VAR
208	D5	D6	0,261	0,156	0,165	40,2	36,8	39	2,4	6 %	OK
210	D7	D8	0,261	0,843	0,877	-223,0	-236,0	-230	9,2	-4 %	⚠ HIGH VAR
211	D9	D10	0,261	0,148	0,148	43,3	43,3	43	0,0	0 %	OK
212	D11	D12	0,261	0,446	0,365	-70,9	-39,8	-55	21,9	-40 %	⚠ HIGH VAR
213	E1	E2	0,261	0,204	0,170	21,8	34,9	28	9,2	32 %	⚠ HIGH VAR
214	E3	E4	0,261	0,336	0,539	-28,7	-106,5	-68	55,0	-81 %	⚠ HIGH VAR
225	E5	E6	0,261	0,404	0,419	-54,8	-60,5	-58	4,1	-7 %	OK
228	E7	E8	0,261	0,583	0,580	-123,4	-122,2	-123	0,8	-1 %	OK
229	E9	E10	0,261	0,617	0,707	-136,4	-170,9	-154	24,4	-16 %	⚠ HIGH VAR
238	E11	E12	0,261	0,483	0,408	-85,1	-56,3	-71	20,3	-29 %	⚠ HIGH VAR

242	F1	F2	0,261	0,136	0,137	47,9	47,5	48	0,3	1 %	OK
243	F3	F4	0,261	0,134	0,137	48,7	47,5	48	0,8	2 %	OK
245	F5	F6	0,261	0,290	0,343	-11,1	-31,4	-21	14,4	-68 %	⚠ HIGH VAR
249	F7	F8	0,261	0,455	0,467	-74,3	-78,9	-77	3,3	-4 %	OK
253	F9	F10	0,261	0,432	0,424	-65,5	-62,5	-64	2,2	-3 %	OK
254	F11	F12	0,261	0,417	0,412	-59,8	-57,9	-59	1,4	-2 %	OK
257	G1	G2	0,261	0,427	0,210	-63,6	19,5	-22	58,8	-267 %	⚠ HIGH VAR
258	G3	G4	0,261	0,461	0,461	-76,6	-76,6	-77	0,0	0 %	OK
264	G5	G6	0,261	0,434	0,444	-66,3	-70,1	-68	2,7	-4 %	OK
269	G7	G8	0,261	0,478	0,480	-83,1	-83,9	-84	0,5	-1 %	OK
270	G9	G10	0,261	0,145	0,143	44,4	45,2	45	0,5	1 %	OK
271	G11	G12	0,261	0,452	0,455	-73,2	-74,3	-74	0,8	-1 %	OK
273	H1	H2	0,261	0,410	0,464	-57,1	-77,8	-67	14,6	-22 %	⚠ HIGH VAR
275	H3	H4	0,261	0,776	0,728	-197,3	-178,9	-188	13,0	-7 %	⚠ HIGH VAR
276	H5	H6	0,261	0,424	0,436	-62,5	-67,0	-65	3,3	-5 %	OK
279	H7	H8	0,261	0,412	0,416	-57,9	-59,4	-59	1,1	-2 %	OK
281	H9	H10	0,261	0,416	0,416	-59,4	-59,4	-59	0,0	0 %	OK
284	H11	H12	0,261	0,113	0,117	56,7	55,2	56	1,1	2 %	OK
▸ **Set 3 (Samples 85–90) — S. aureus | ODref (A3+A4 avg) = 0.435 (A3=0.447, A4=0.422)**											
285	B1	B2	0,435	0,252	0,259	42,0	40,4	41	1,1	3 %	OK
288	B3	B4	0,435	0,269	0,263	38,1	39,5	39	1,0	3 %	OK
289	B5	B6	0,435	0,275	0,265	36,7	39,0	38	1,6	4 %	OK
290	B7	B8	0,435	0,274	0,272	36,9	37,4	37	0,3	1 %	OK
291	B9	B10	0,435	0,281	0,266	35,3	38,8	37	2,4	7 %	OK
293	B11	B12	0,435	0,282	0,280	35,1	35,6	35	0,3	1 %	OK

5.1.3.4) E. Coli Results:

Inhibition % Calculations — E. Coli (Control samples, 24 h endpoint)

Inhibition (%) Formula = [(ODreference − ODsample) / ODreference] × 100 | ODreference = AVERAGE (A3, A4) | SD% = (Std Dev / |Mean Inh%|) × 100 | CV% flag >10% = ⚠ HIGH VAR

>50% Strong inhibitor = Sage Green

20–50% Moderate inhibitor= yellow green
0–20% Weak/negligible inhibitor= sand
<0% Growth promoter=clay

Table 20: E. coli (DSM 682) inhibition for all 90 nylon-filtered samples

Sample #	Rep 1 Well	Rep 2 Well	ODref (avg A3+A4)	Rep 1 Abs (24h)	Rep 2 Abs (24h)	Inh % Rep 1	Inh % Rep 2	Ave Inh %	Std Dev (±)	SD % (StdDev/Mean)	CV% / Flag
▸ Set 1 (Samples 1–42) — E. coli | ODref (A3+A4 avg) = 0.592 (A3=0.609, A4=0.575)											
1	B1	B2	0,592	0,433	0,457	26,9	22,8	25	2,9	12 %	OK
4	B3	B4	0,592	0,663	0,659	-12,0	-11,3	-12	0,5	-4 %	OK
19	B5	B6	0,592	0,660	0,645	-11,5	-9,0	-10	1,8	-18 %	OK
25	B7	B8	0,592	0,729	0,723	-23,1	-22,1	-23	0,7	-3 %	OK
34	B9	B10	0,592	0,713	0,712	-20,4	-20,3	-20	0,1	-1 %	OK
44	B11	B12	0,592	0,561	0,625	5,2	-5,6	0	7,6	-4525 %	⚠ HIGH VAR
45	C1	C2	0,592	0,413	0,444	30,2	25,0	28	3,7	13 %	OK
48	C3	C4	0,592	0,640	0,641	-8,1	-8,3	-8	0,1	-1 %	OK
53	C5	C6	0,592	0,740	0,716	-25,0	-20,9	-23	2,9	-12 %	OK
55	C7	C8	0,592	0,729	0,737	-23,1	-24,5	-24	1,0	-4 %	OK
56	C9	C10	0,592	0,428	0,446	27,7	24,7	26	2,1	8 %	OK
58	C11	C12	0,592	0,802	0,744	-35,5	-25,7	-31	6,9	-23 %	OK
62	D1	D2	0,592	0,693	0,706	-17,1	-19,3	-18	1,6	-9 %	OK
63	D3	D4	0,592	0,746	0,724	-26,0	-22,3	-24	2,6	-11 %	OK
70	D5	D6	0,592	0,706	0,647	-19,3	-9,3	-14	7,0	-49 %	OK
74	D7	D8	0,592	0,699	0,685	-18,1	-15,7	-17	1,7	-10 %	OK
80	D9	D10	0,592	0,585	0,618	1,2	-4,4	-2	3,9	-246 %	OK
84	D11	D12	0,592	0,679	0,669	-14,7	-13,0	-14	1,2	-9 %	OK
86	E1	E2	0,592	0,632	0,607	-6,8	-2,5	-5	3,0	-64 %	OK
88	E3	E4	0,592	0,695	0,684	-17,4	-15,5	-16	1,3	-8 %	OK
89	E5	E6	0,592	0,785	0,754	-32,6	-27,4	-30	3,7	-12 %	OK
92	E7	E8	0,592	0,758	0,643	-28,0	-8,6	-18	13,7	-75 %	⚠ HIGH VAR
96	E9	E10	0,592	0,685	0,705	-15,7	-19,1	-17	2,4	-14 %	OK
100	E11	E12	0,592	0,599	0,609	-1,2	-2,9	-2	1,2	-59 %	OK
106	F1	F2	0,592	0,568	0,575	4,1	2,9	3	0,8	24 %	OK
110	F3	F4	0,592	0,252	0,196	57,4	66,9	62	6,7	11 %	OK
111	F5	F6	0,592	0,730	0,714	-23,3	-20,6	-22	1,9	-9 %	OK
112	F7	F8	0,592	0,544	0,520	8,1	12,2	10	2,9	28 %	OK
113	F9	F10	0,592	0,646	0,738	-9,1	-24,7	-17	11,0	-65 %	⚠ HIGH VAR
120	F11	F12	0,592	0,727	0,675	-22,8	-14,0	-18	6,2	-34 %	OK

125	G1	G2	0,592	0,659	0,707	-11,3	-19,4	-15	5,7	-37 %	OK
126	G3	G4	0,592	0,387	0,364	34,6	38,5	37	2,7	8 %	OK
129	G5	G6	0,592	0,709	0,707	-19,8	-19,4	-20	0,2	-1 %	OK
133	G7	G8	0,592	0,731	0,731	-23,5	-23,5	-23	0,0	0 %	OK
137	G9	G10	0,592	0,750	0,740	-26,7	-25,0	-26	1,2	-5 %	OK
148	G11	G12	0,592	0,728	0,702	-23,0	-18,6	-21	3,1	-15 %	OK
159	H1	H2	0,592	0,623	0,644	-5,2	-8,8	-7	2,5	-36 %	OK
161	H3	H4	0,592	0,692	0,701	-16,9	-18,4	-18	1,1	-6 %	OK
163	H5	H6	0,592	0,525	0,700	11,3	-18,2	-3	20,9	-604 %	⚠ HIGH VAR
164	H7	H8	0,592	0,728	0,732	-23,0	-23,6	-23	0,5	-2 %	OK
165	H9	H10	0,592	0,679	0,666	-14,7	-12,5	-14	1,6	-11 %	OK
166	H11	H12	0,592	0,620	0,563	-4,7	4,9	0	6,8	8061 %	OK
▸ Set 2 (Samples 43–84) — E. coli | ODref (A3+A4 avg) = 0.618 (A3=0.604, A4=0.632)											
169	B1	B2	0,618	0,655	0,701	-6,0	-13,4	-10	5,3	-54 %	OK
172	B3	B4	0,618	0,713	0,720	-15,4	-16,5	-16	0,8	-5 %	OK
176	B5	B6	0,618	0,655	0,665	-6,0	-7,6	-7	1,1	-17 %	OK
188	B7	B8	0,618	0,717	0,747	-16,0	-20,9	-18	3,4	-19 %	OK
190	B9	B10	0,618	0,744	0,785	-20,4	-27,0	-24	4,7	-20 %	OK
191	B11	B12	0,618	0,721	0,785	-16,7	-27,0	-22	7,3	-34 %	⚠ HIGH VAR
193	C1	C2	0,618	0,519	0,406	16,0	34,3	25	12,9	51 %	⚠ HIGH VAR
194	C3	C4	0,618	0,763	0,759	-23,5	-22,8	-23	0,5	-2 %	OK
195	C5	C6	0,618	0,698	0,677	-12,9	-9,5	-11	2,4	-21 %	OK
197	C7	C8	0,618	0,727	0,734	-17,6	-18,8	-18	0,8	-4 %	OK
198	C9	C10	0,618	0,724	0,755	-17,2	-22,2	-20	3,5	-18 %	OK
199	C11	C12	0,618	0,790	0,755	-27,8	-22,2	-25	4,0	-16 %	OK
203	D1	D2	0,618	0,693	0,666	-12,1	-7,8	-10	3,1	-31 %	OK
206	D3	D4	0,618	0,347	0,331	43,9	46,4	45	1,8	4 %	OK
208	D5	D6	0,618	0,565	0,583	8,6	5,7	7	2,1	29 %	OK
210	D7	D8	0,618	0,640	0,596	-3,6	3,6	0	5,0	#DIV/0!	OK
211	D9	D10	0,618	0,748	0,739	-21,0	-19,6	-20	1,0	-5 %	OK
212	D11	D12	0,618	0,684	0,745	-10,7	-20,6	-16	7,0	-45 %	OK
213	E1	E2	0,618	0,439	0,383	29,0	38,0	33	6,4	19 %	OK
214	E3	E4	0,618	0,261	0,340	57,8	45,0	51	9,0	18 %	⚠ HIGH VAR
225	E5	E6	0,618	0,731	0,710	-18,3	-14,9	-17	2,4	-14 %	OK
228	E7	E8	0,618	0,828	0,813	-34,0	-31,6	-33	1,7	-5 %	OK
229	E9	E10	0,618	0,673	0,678	-8,9	-9,7	-9	0,6	-6 %	OK
238	E11	E12	0,618	0,788	0,738	-27,5	-19,4	-23	5,7	-24 %	OK
242	F1	F2	0,618	0,686	0,705	-11,0	-14,1	-13	2,2	-17 %	OK

243	F3	F4	0,618	0,683	0,699	-10,5	-13,1	-12	1,8	-15 %	OK
245	F5	F6	0,618	0,353	0,346	42,9	44,0	43	0,8	2 %	OK
249	F7	F8	0,618	0,713	0,738	-15,4	-19,4	-17	2,9	-16 %	OK
253	F9	F10	0,618	0,699	0,732	-13,1	-18,4	-16	3,8	-24 %	OK
254	F11	F12	0,618	0,745	0,747	-20,6	-20,9	-21	0,2	-1 %	OK
257	G1	G2	0,618	0,444	0,447	28,2	27,7	28	0,3	1 %	OK
258	G3	G4	0,618	0,714	0,679	-15,5	-9,9	-13	4,0	-32 %	OK
264	G5	G6	0,618	0,686	0,708	-11,0	-14,6	-13	2,5	-20 %	OK
269	G7	G8	0,618	0,777	0,770	-25,7	-24,6	-25	0,8	-3 %	OK
270	G9	G10	0,618	0,709	0,695	-14,7	-12,5	-14	1,6	-12 %	OK
271	G11	G12	0,618	0,789	0,754	-27,7	-22,0	-25	4,0	-16 %	OK
273	H1	H2	0,618	0,670	0,716	-8,4	-15,9	-12	5,3	-43 %	OK
275	H3	H4	0,618	0,539	0,533	12,8	13,8	13	0,7	5 %	OK
276	H5	H6	0,618	0,675	0,535	-9,2	13,4	2	16,0	761 %	⚠ HIGH VAR
279	H7	H8	0,618	0,716	0,733	-15,9	-18,6	-17	1,9	-11 %	OK
281	H9	H10	0,618	0,723	0,750	-17,0	-21,4	-19	3,1	-16 %	OK
284	H11	H12	0,618	0,483	0,484	21,8	21,7	22	0,1	1 %	OK
▸ Set 3 (Samples 85–90) — E. coli | ODref (A3+A4 avg) = 0.535 (A3=0.555, A4=0.515)											
285	B1	B2	0,535	0,540	0,570	-0,9	-6,5	-4	4,0	-106 %	OK
288	B3	B4	0,535	0,514	0,559	3,9	-4,5	0	5,9	-2121 %	OK
289	B5	B6	0,535	0,583	0,585	-9,0	-9,3	-9	0,3	-3 %	OK
290	B7	B8	0,535	0,589	0,577	-10,1	-7,9	-9	1,6	-18 %	OK
291	B9	B10	0,535	0,575	0,570	-7,5	-6,5	-7	0,7	-9 %	OK
293	B11	B12	0,535	0,574	0,581	-7,3	-8,6	-8	0,9	-12 %	OK

5.1.3.5) Broad Spectrum of the Strains Results:

For each strong MRSA extract it shows all three treatments together: control, oxidation, filtration. Reading across the three bars for any sample indicates what its active compound is made of.

A few patterns jump out. #213 (*Pleiogynium solandri*) and #208 (*Acaena Pallida*) barely move across all three bars. Whatever they contain is tough, surviving both oxidation and filtration, and those are my most robust candidates. At the opposite end, #84 (*Medillina loranthoides*), #242 (*Commiphora dalals*) and #243 (*Commiphora gileadensis*) sail through oxidation but crash under filtration, going from strong inhibitors to slight promoters. Their active compounds must be the large polyphenols nylon strips out. And #110 (*Plumbago auriculata*), the control champion, shows a third pattern: it drops under oxidation, recovers part way under filtration, so its active is fragile to oxidation but not held by nylon.

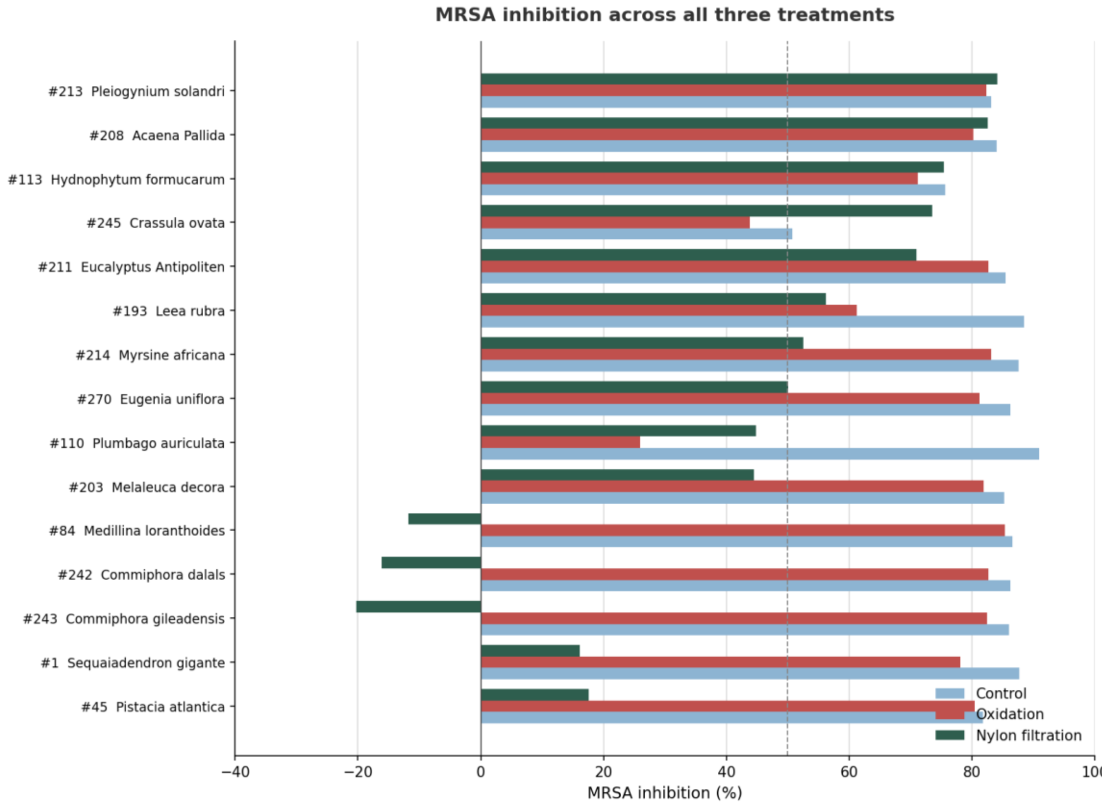


Figure 15: MRSA inhibition across all three treatments for the strongest inhibitors. Reading the three bars for each sample shows whether its active compound survives oxidation and filtration. The dashed line marks 50%.

On its own, the radar below plots the ten strongest filtered MRSA inhibitors against all three strains at once, MRSA as the outer ring.

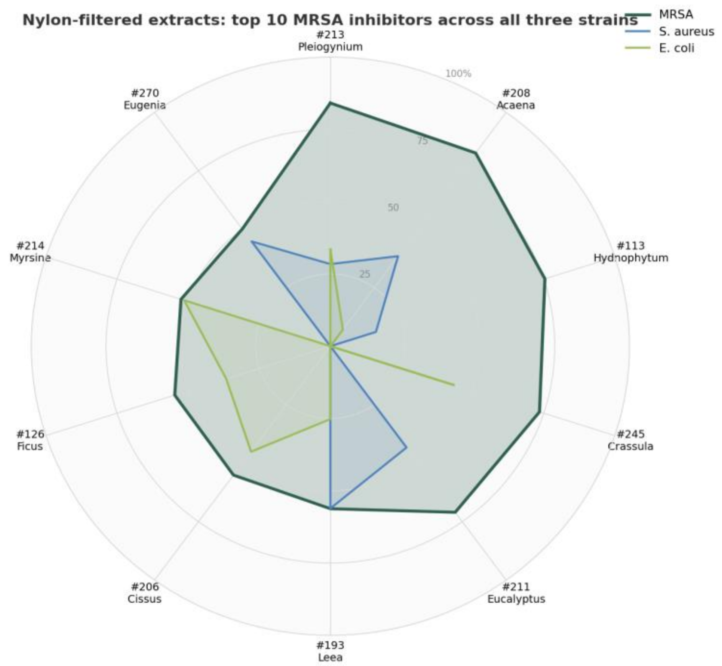


Figure 16: Top 10 MRSA inhibitors after nylon filtration, across all three strains. Negative reference values are drawn as zero for readability.

5.2) Discussion:

Performing the same 90 extracts through several chemical processes was never intended to improve the performance of the substances. The goal of the process was to discover which specific compounds were performing the activity. Because a crude plant extract is a combination of many microscopic particles from different structural classes, identifying the specific active part is difficult. Many people believe that the most difficult challenge in this scientific inquiry is identifying which specific compound causes the result. When one class of compound is removed or changed while observing the inhibition, the answer is found.

Three treatments were finished: the untreated control, enzymatic oxidation, and nylon filtration samples. It is observed that these three treatments function like a decision tree. If activity continues after nylon filtration, the large tannins are not the reason for the effect. Where the activity also continues after enzymatic oxidation, the active compound is not a catechol or pyrogallol which is easily oxidized. This outcome suggests that a smaller, more durable microscopic particle is present. When the activity stops during one of these procedures, that specific procedure identifies the compound class. The logic of the introduction was followed by the data in a clean way.

It has been observed that the library-wide averages show a stable narrative for MRSA. Untreated extracts showed a mean inhibition near 29%. This mean inhibition decreased to 4% after enzymatic oxidation occurred. When nylon filtration was used, the inhibition fell just below zero. The same direction was taken by the number of strong inhibitors. The count started at 28 in the control, moved to 16 after oxidation, and reached 10 after filtration.

The downward trend in activity is informative even though the result might seem disappointing. The fact that nylon filtration removed the most activity matches the prediction from the introduction. Large polyphenols, which include hydrolysable and condensed tannins, were responsible for much of the inhibition. The list of strong inhibitors became empty because the tannins were removed rather than oxidized. This outcome matches the well-known tannin challenge. Some activity in an untreated extract is caused by non-specific protein precipitation which is not a true medicine-like effect. Nylon filtration is the specific step created to reveal this fact. A tannin artefact is considered the honest first interpretation where activity disappears during filtration. Where activity survives the filtration, the inhibition is more likely to be real.

5.2.1). The standout Sample: (#213 Pleiogynium solandri)

This research shows that the stability of this specific botanical specimen makes Pleiogynium solandri worth building the story around. Pleiogynium solandri, this member of the Anacardiaceae (the cashew and sumac family) maintained a robust inhibition through every single procedure. These percentages were recorded as 83.1% untreated, 82.3% after enzymatic oxidation, and 84.1% after nylon filtration. A strong inhibition was maintained by #213 (Pleiogynium solandri) through every single procedure. Pleiogynium solandri did not just endure the procedures; Pleiogynium solandri barely noticed the changes.

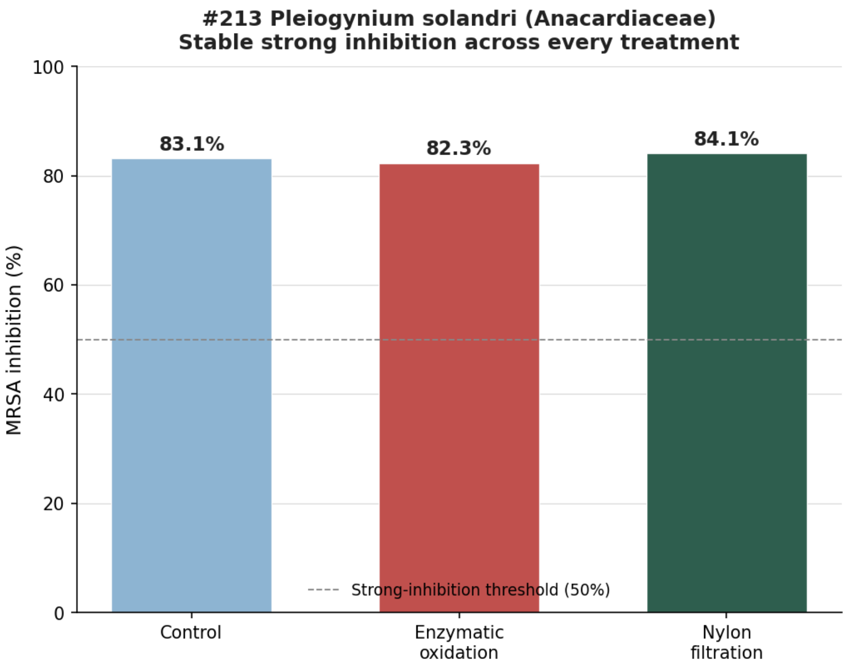


Figure 17: MRSA inhibition of #213 Pleiogynium solandri (Anacardiaceae) across the three completed treatments. The activity is essentially flat, which is what marks it out from every other strong inhibitor in the library.

It can be said that this lack of change provides the primary objective for the study. Because the activity endures nylon filtration, the decision tree from section 1 indicates that the result is not caused by large tannins or a precipitation artifact. This activity also endures enzymatic oxidation, which means the compound is not a readily oxidized catechol or pyrogallol substance of the kind that dominates so much of this library of botanical samples. A smaller or structurally more stable metabolite is left behind, which is the exact profile the opening section highlighted as the most promising for isolation. Within the compound-class framework of Table 1 in the opening section, this pattern does not match gallotannins, ellagitannins, or hydroxycinnamic acids. These other substances are predicted to decrease because of oxidation or filtration. The result indicates a flavonoid aglycone or another oxidation-resistant scaffold.

Analyzing the context of the botanical family confirms these findings. It has been observed that Anacardiaceae are well-known for stable, lipophilic phenolics such as alkylresorcinols and biflavonoids. This family possesses robust flavonoids instead of the delicate high-molecular-weight tannins that dominate families like Rosaceae or Onagraceae. Such a compound would be expected to ignore both oxidation and nylon binding, which is exactly what the numbers show. Biological research has not widely studied Pleiogynium solandri as a species, which makes this result more significant. A stable, broad-acting anti-MRSA metabolite is carried by Pleiogynium solandri from the collection of the botanical garden. This discovery is precisely the result that the bioprospecting strategy was built to find because the outcome shows high stability.

5.2.2) The next best samples:

The study of enzymatic and alkaline oxidative activities of plant phenolics, present each species as a stack of chromatograms, initial then enzymatic then pH 10, and read the compound behaviour by watching individual peaks shrink or survive across those panels. The same visual logic applies here, with nylon filtration standing in for their pH 10 panel. For a compound that matters, you want to see whether its peak, and the activity that tracks it, holds across the panels or drops away. Regarding #213 (Pleiogynium solandri), the antibacterial activity is seen to persist across all three experimental conditions. Many researchers believe that according to the Kim reading, this specific behavior serves as the signature of a metabolite which is durable and resistant to processing procedures.

This chemical unit is different from substances that are sensitive to oxidation or those that stay bound during the filtration process. If the UHPLC-MS/MS metabolite profiles for #213 (Pleiogynium solandri) are examined peak by peak, the specific chemical unit is the one that must be identified by a chromatographic peak that remains almost the same during the control, oxidation, and filtration stages. Because the peak for this metabolite stays unvarying, the antibacterial activity also remains at a prominent level throughout the testing. That peak, stable across every treatment, is the isolation target. Matching it to the group-specific MRM transitions described in the methodology should place it in a compound class, and given the activity pattern and the family, a stable flavonoid is the leading candidate to check first.

Four more samples held strong or near strong across all three treatments and deserve to travel with the champion into any follow-up work. #208 (Acaena Pallida) (Rosaceae) stayed at 84, 80 and 83% across control, oxidation and filtration, almost as flat as #213. #113 (Hydnophytum formucarum) held in the 70s throughout. #211 (Eucalyptus Antipolitenis) and #193 (Leea rubra) stayed strong through oxidation and dropped only partway under filtration, so they lean a little more on filtration-sensitive compounds but still retain real activity. These five together form the shortlist of treatment-robust anti-MRSA hits from the whole 90-species library.

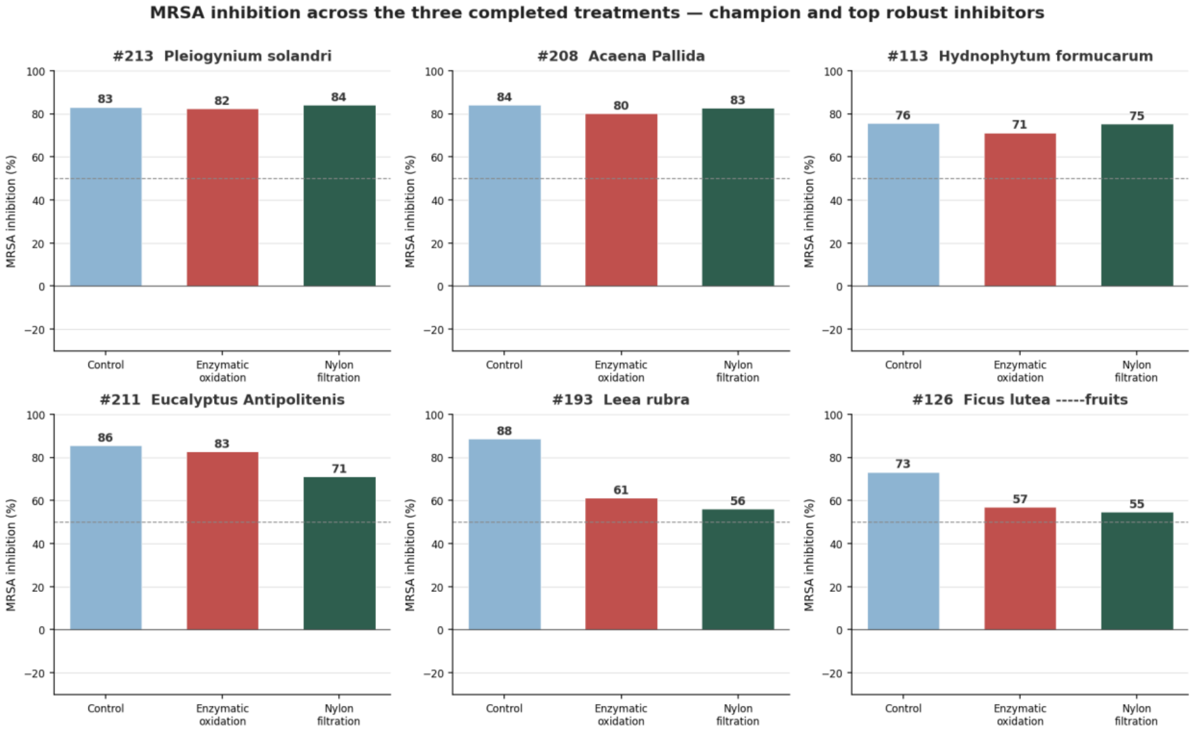


Figure 18: MRSA inhibition across the three completed treatments for the champion (#213) and the five most treatment-robust inhibitors. Bars that stay high across all three panels mark compounds whose activity does not depend on tannins or on oxidation-sensitive phenolics.

It is worth naming the opposite pattern too, because it is just as informative. Several strong control inhibitors, including #214 (Myrsine africana), #270 (Eugenia uniflora) and both Commiphora species from the oxidation chapter, held up through oxidation but collapsed under filtration. For those, the reading is the reverse: their activity was carried mainly by the large, nylon-bound polyphenols, and once those were stripped out, little was left. Whether that residual activity was ever a real drug-like effect or a tannin precipitation artefact is exactly the question the filtration step was designed to answer, and for these samples the honest answer leans toward the tannins.

5.2.3) Uncompleted treatments:

The study was originally designed around six treatments: the control, enzymatic oxidation, alkaline oxidation at pH 10, nylon filtration, and two combination treatments, enzymatic plus alkaline and nylon plus alkaline. In the end three of these, the pH 10 alkaline oxidation and both combination treatments that depended on it, were not carried out. The decision to stop at control, enzymatic oxidation, and nylon filtration was made deliberately, for reasons of time and for practical and technical constraints in running and reading the additional oxidation steps within the period available for the project.

This narrowing does not undermine the central logic of the screen. The three completed treatments already span the two most informative axes: nylon filtration isolates the contribution of the large tannins, and enzymatic oxidation isolates the contribution of the oxidation-sensitive catechol and pyrogallol phenolics. Between them they are enough to sort the library into treatment-robust hits and treatment-sensitive ones, and enough to identify #213 (Pleiogynium solandri) as a stable, broad-acting inhibitor worth isolating. The pH 10 and combination treatments would have added resolution, particularly in separating alkaline-sensitive compounds from enzymatically oxidised ones and in confirming assignments through a second oxidative route, and they remain a natural extension for future work. Their absence is a limit on completeness, not a flaw in the conclusions that the completed treatments support.

6- Conclusion:

Across three treatments and 90 rare species, the screen did what it was designed to do: it separated extracts whose anti-MRSA activity depends on removable or oxidisable compounds from the few whose activity does not. Most of the library lost its punch once the large tannins were filtered out, which is both a real finding about where the activity lives and a reminder of how much apparent tannin activity is non-specific. Against that background, #213 (Pleiogynium solandri) (Pleiogynium solandri, Anacardiaceae) stands out clearly as the single most promising candidate: strong, broad, and unmoved by either oxidation or filtration. It is the natural focus for the next stage of work, isolation and structural identification of the stable metabolite behind its activity, and it is the result the whole multi-treatment strategy was built to surface.

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