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Year 11 - 12

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Glenunga International High School

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2026 OSA Scientific Inquiry
Subject: Biology

Bio-antiseptics; A Cross-Cultural Comparative Analysis of Thai and Australian Botanicals in the Treatment of Staphylococcus-Infected Eczema

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To what extent do ethanol-based extracts of traditional Thai and Australian herbs (Curcuma longa; Melaleuca alternifolia; Backhousia citriodora; Biancaea sappan) inhibit the growth of Staphylococcus aureus, a primary colonizer of eczema-prone skin, and to what extent can their properties be combined to produce a synergistic antimicrobial effect?

Abstract

*Staphylococcus aureu*s colonises up to 90% of eczema patients, exacerbating skin barrier damage and inflammation. This investigation compared the antimicrobial efficacy of ethanol-based extracts from four traditional Thai and Australian botanicals: *Curcuma longa, Biancaea sappan, Melaleuca alternifolia, and Backhousia citriodor*a, against *Staphylococcus epidermidis*, a safe model organism, using agar disc diffusion. Zones of inhibition were measured against ethanol (negative) and potassium triiodide (positive) controls. *B. sappan's* polyphenol compounds produced the greatest individual inhibition (5.55 ± 1.10 mm), while Australian monoterpene extracts performed poorly. A three-extract combination produced the largest combined inhibition (8.95 ± 2.28 mm), suggesting a possible synergistic antimicrobial effect warranting further investigation.

Background

Ethnobotany and Pharmocognosy

Ethnobotany is the interdisciplinary study of the relationships between human cultures and plants. In the context of drug discovery, knowledge in this field serves as a primary lead for pharmacognosy, the study of medicinal drugs derived from natural sources. Historically, secondary metabolites identified in traditional medicine have provided the chemical basis for numerous pharmaceutical agents, including salicylic acid and artemisinin (Veeresham, 2012).

Atopic Dermatitis (Eczema) and Staphylococcus aureus colonisation

Atopic Dermatitis (AD), commonly referred to as eczema, is a chronic and relapsing inflammatory skin disease characterized by dysfunction of the epidermal barrier. It involves a deficiency in filaggrin, a protein essential for maintaining the integrity of the stratum corneum [2]. It increases transepidermal water loss and cutaneous pH to facilitate microbial colonisation (Seiti Yamada Yoshikawa et al., 2019). Symptoms include dry, itchy skin and causes scratching, which cracks the skin and increases likelihood of infection. Staphylococcus aureus (S. aureus) is a Gram-positive, common bacterium which acts as a primary opportunistic pathogen in AD, colonising 90% of AD patients compared to 5–30% of healthy individuals (Nemeth and Evans, 2022). The bidirectional exacerbation of the disease is caused by AD weakening epidermal integrity, which enhances bacterial adherence to corneocytes. Concurrently, overexpression of Th2 and Th17 [3] cytokines, microbial dysbiosis and altered lipid profiles weaken the skin's defense against pathogens. Once S. Aureus settles on the dermis, the bacterium secretes three distinct types of Phenol-Soluble Modulins (PSMs) [4], in order to degrade the skin barrier. Secreted PSMα, PSMβ and δ-Toxin damages keratinocytes, triggering inflammatory cytokine release and disrupts the skin barrier, allowing pathogens to penetrate deeper into the dermis. See figure 1.

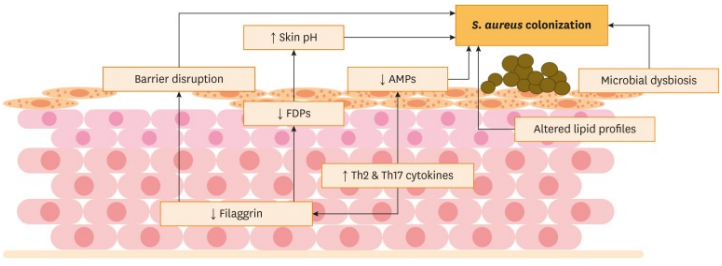


Fig. 1: Staphylococcus aureus colonization on the skin of an individual with AD (Kim et al., 2019)

Justification for Plant Selection

The selection of the four species for this investigation is based on their historical utility in traditional medicine, documented antimicrobial profiles, and direct relevance to the treatment of inflammatory skin conditions. In contemporary pharmacognosy, researchers are increasingly focused on identifying bioactive compounds of natural origin for development as therapeutic agents, owing to either the high cost of synthetic drugs or the adverse side effects associated with synthetic molecules (Anand et al., 2019; Atanasov et al., 2021). Consequently, there is a continuous search for bioactive molecules against non-medicated or chronic diseases. By comparing specific botanicals from Thailand and Australia, this study provides a cross-cultural perspective on approaches to managing the bacterial infections associated with AD without a total reliance on synthetic medication.

Thai Botanical Flora

***Curcuma longa* (Turmeric)**: Widely used in Thailand and throughout Asia. Its primary lipophilic polyphenol, curcumin, is notably recognized for its anti-microbial and anti-inflammatory activity (See fig. 2). Curcumin disrupts bacterial cell division by downregulating the expression of the filamentous temperature-sensitive mutant Z (FtsZ) protein, preventing protofilament formation and subsequent cytokinesis in *S. aureus* cells (Teow et al., 2016). Given that inflammation and bacterial colonisation are key factors in AD flare-ups, turmeric was selected as a representative Thai medicinal plant with potential therapeutic value.

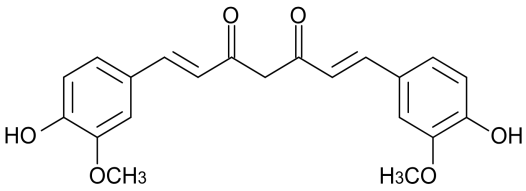


Fig. 2: Molecular structure of Curcumin, the relevant bioactive compound within Turmeric (Dai et al., 2022)

***Biancaea sappan* (Sappan Wood)**: Historically utilized in Thai medicinal practices to treat wounds and skin infections, the heartwood of *B. sappan* contains high concentrations of brazilin, along with its oxidized form, brazilein. These compounds exert antibacterial effects by disrupting the cell wall matrix and inducing plasma membrane permeabilization in Gram-positive organisms, making it a candidate of interest for targeting *S. aureus* (Nirmal et al., 2015).

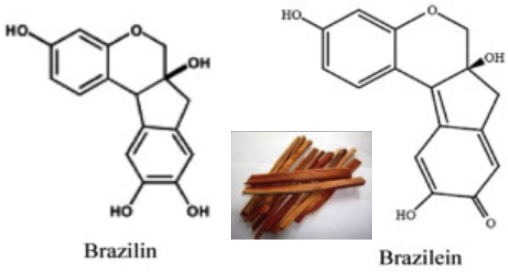
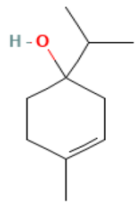


Fig. 3: Sappan heartwood, molecular structures of Brazilin and Brazilein (Nirmal et al., 2015)

Australian Botanical Flora



***Melaleuca alternifolia* (Australian Tea Tree)**: Indigenous to eastern Australia, the leaves of M. alternifolia have been utilized by Bundjalung Aboriginal populations for millennia to treat wounds. The primary bioactive monoterpene in tea tree oil is terpinen-4-ol (fig. 4). This compound destabilizes the structural integrity of the lipophilic bacterial plasma membrane, leading to the leakage of essential intracellular ions (such as K+) and eventual cell lysis (Carson et al., 2006).

Fig. 4: Terpinen-4-ol compound structure (PubChem, 2019)

***Backhousia citriodora* (Lemon Myrtle)**: Native to south-eastern Australia, the oil from B. citriodora leaves predominantly possess high concentration of aldehyde citrals (the isomers geranial and neral, fig. 5). Citral acts as an antimicrobial agent by penetrating the bacterial cell membrane, disrupting intracellular ATP synthesis, and altering cell surface hydrophobicity (Nirmal et al., 2018)

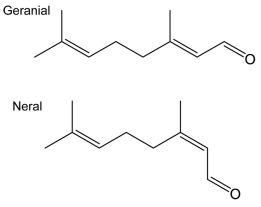


Fig. 5: Structures of Neral and Geranial isomers (Researchgate.net, n.d.)

Together, these four species represent both Thai and Australian ethnobotanical traditions and contain bioactive compounds reported to inhibit bacterial growth. Their selection allows for a meaningful comparison of traditional medicinal plants from two distinct cultural backgrounds while investigating their potential effectiveness against *S. aureus*.

Questioning and Predicting

Question: To what extent do ethanol-based extracts of traditional Thai and Australian herbs (Curcuma longa; Melaleuca alternifolia; Backhousia citriodora;; Biancaea sappan) inhibit the growth of Staphylococcus aureus, a primary colonizer of eczema-prone skin, and to what extent can their properties be combined to produce a synergistic antimicrobial effect?

Aim: To evaluate and compare the individual antimicrobial efficacy of ethanol-based extracts from traditional Thai and Australian herbs against Staphylococcus aureus, and to investigate whether a combination of these botanical extracts produce a synergistic inhibitory effect.

Prediction and Hypothesis: The antimicrobial efficacy of the selected plant extracts against Staphylococcus aureus is determined by the chemical structures and mechanisms of their primary secondary metabolites. Monoterpene compounds like citral (*B. citriodora*) and terpinen-4-ol (*M. alternifolia)* exhibit highly lipophilic properties that allow them to rapidly diffuse into and disrupt bacterial cytoplasmic membrane, in order to cause cell lysis (Carson et al., 2006; Hayes & Markovic, 2002). In comparison, the more complex polyphenolic structures (curcumin from *C. longa* and brazilin from *B. sappan)* primarily target intracellular processes (Nirmal et al., 2015; Teow et al., 2016).

In agar disc diffusion, the radial expansion of an antimicrobial agent is influenced by molecular weight and hydrophobicity, with smaller monoterpenoids being able to diffuse through the hydrophilic agar surface more rapidly than larger, phenolic structures. (American Society for Microbiology, 2009). When these extracts are combined, the application of multiple chemical compounds which target different biochemical avenues (e.g. a membrane disrupting monoterpene compound paired with an enzyme disrupting polyphenol) may result in a synergistic antimicrobial effect which better disrupts bacterial function.

Hence, it is predicted that individual treatments of Backhousia citriodora and Melaleuca alternifolia will produce the largest quantitative Zones of Inhibition (ZoI) due to their superior diffusion rate. It is further predicted that a combination pairing an Australian monoterpenoid extract (e.g., B. citriodora) with a Thai phenolic extract (e.g., B. sappan) will exhibit a notable synergistic effect, yielding a mean ZoI diameter greater than each individual component tested in isolation.

Planning and Conducting

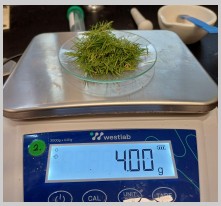
Variables

1. **<u>Independent variable:</u>**

   1.1 Species of Herb Extract

   - 4.0 grams (g) of relevant botanical tissue crushed and mixed in 20 millilitres (mL) of 70% ethanol solvent over a 24 hour maceration period (fig. 6)

Table 1: Species appearance and weighing

			
Curcuma longa **(Turmeric)**	***Biancaea sappan*** **(Sappan Wood)**:	***Melaleuca alternifolia*** **(Australian Tea Tree)**	***Backhousia citriodora*** **(Lemon Myrtle)**

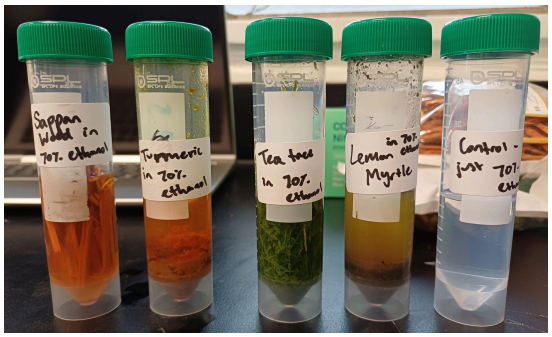


Fig. 6: Samples after being crushed via mortar and pestle. Mixed with 20mL of 70% ethanol in 50mL centrifuge tubes and left for maceration

1.2 Negative Control: Pure 70% ethanol containing no plant matter

*Essential to prove that any observed ZoI is caused by the active plant metabolites themselves, rather than the ethanol solvent. Since the ethanol evaporates from the disc during the 5-minute drying phase, the negative control should ideally yield a total diameter of 6.5 mm and indicate a net inhibition zone of 0 mm beyond the disc diameter.

1.3 Positive Control: Potassium Triiodide (KI_3), a standardized iodine-based antiseptic solution used in place of commercial Betadine.

*Used to confirm the susceptibility of the tested bacteria to antibacterial methods and that a ZoI can be created. See fig. 7

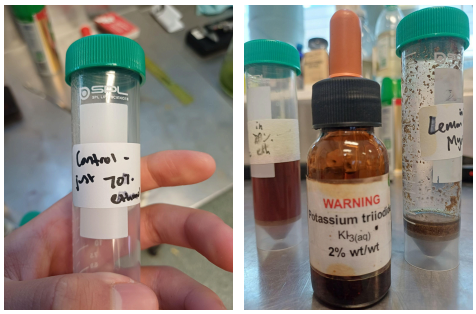


Fig. 7: Ethanol (left) and KI_3 (right) used as negative and positive control

1.4 A 1:1 volumetric blend of the prepared *B. sappan* and *C. longa* filtrate solutions, along with a 1:1:1 volumetric blend of *B. sappan*, *C. longa*, and *B. citriodora* filtrate solutions, each mixed in a separate vial before disc immersion prior to the secondary synergistic effect trial

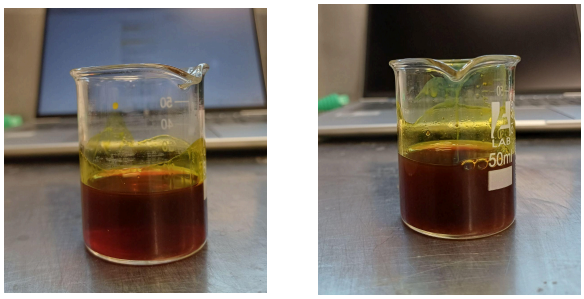


Fig. 8: Experimental setup for the secondary synergy trials, evaluating a dual polyphenolic combination (left, *B. sappan*, *C. longa*) alongside a monoterpenoid + polyphenolic tripartite combination (right, *B. sappan*, *C. longa*, and *B. citriodora*).

*Note: These specific combinations were chosen to observe potential synergistic effects in intracellular inhibition. The dual mixture combines the two highest-performing baseline extract, while the tripartite mixture incorporates Lemon Myrtle to evaluate if monoterpenoids can disrupt the bacterial membrane and aid effectiveness of the active Thai polyphenols. M. alternifolia was omitted from this stage due to its similarly negligible baseline efficacy compared to B. citriodora in primary trials, alongside resource constraints regarding viable fresh biomass for maceration.

2. Dependent variables:

2.1 Zone of Inhibition

- Measure the absolute diameter of the bacterial clearance zone around each 6.5mm absorbent disc by calculating the mean between the perpendicular X and Y axes using a digital vernier caliper (mm).

  *The digital vernier caliper produces a highly precise quantitative reading (±0.01 mm), which is significantly more precise than a standard manual ruler and improves experimental accuracy. Measurement was taken by placing the caliper against the bottom of the plate in order to minimize human parallax error from readings.

- Three sterile absorbent paper discs soaked with their respective samples are allocated per nutrient agar plate to provide larger sample size and reduce impact of random error for each treatment group.

  *Averaging the X and Y axes accounts for asymmetrical radial expansion of the extracts when inhibiting bacterial growth. Utilizing three duplicate discs per plate lowers the margin for error and mitigates the impact of outlying data.

- The method of conducting the experiment and measurement were chosen for their relative simplicity and use of accessible equipment within a school lab setting, which were factors prioritized due to the time constraints and limited availability of both the school lab and staff supervision.

Controlled variables:

Methodological Note:

Staphylococcus epidermis was used as a close substitute for *S. aureus* for the experiment due to lab safety constraints prohibiting use of the latter, *S. epidermidis* specifically was chosen due to its similar growth patterns to *S. aureus* and natural area of habitation, usually being found on human skin, including areas affected by AD (Stewart et al., 2017)

Table 2: Controlled variables, experimental effects and method of control

Variable	Effect on results	Method of control
Bacterial model	Different species possess different biology and varying levels of susceptibility to antibiotic treatment	*S. epidermidis* used exclusively as a uniform broth culture standard across all trials.
Bacterial inoculum volume (µL)	Excess volume obscures ZoI and makes sample effectiveness unclear Inadequate volume fails to yield a uniform bacterial lawn	Exactly 10µL pipetted via a single onto every agar plate before streaking, quantity suggested by lab supervisor for a balanced and uniform lawn
Sample mass (g)	Greater biomass increases the yield of extracted metabolites, may artificially inflate zone sizes	Calibrated digital electronic scale used to measure exactly 4.0g per herb.
Solvent parameters (mL)	Higher solvent volumes or lower alcohol concentration would dilute the extract and reduce result clarity	Volume: 20mL measuring cylinder. Concentration: 70% ethanol solvent batch.
Maceration duration (hours)	Shorter periods may lead to under-extraction	Mixtures left in 50mL centrifuge tubes and sealed with a screw-on lid for the same amount of time (24 hours)
Absorbent disc size	Larger disc surfaces absorb higher total fluid volumes and increase the starting physical footprint on the agar gel	Standardized 6.5mm blank absorbent paper discs used across all treatment groups, cut from the same hole punch
Disc soaking & drying times	Varied immersion alters the amount of liquid absorption via capillary action Unequal or insufficient drying time leaves 70% ethanol to interfere with bacteria.	Discs submerged for exactly 15 seconds, then air-dried for exactly 5 minutes on sanitized watch glasses. Procedure conducted as quickly as possible to minimize

		difference in drying time between discs
Agar depth and volume	Deeper agar allows extracts to diffuse vertically downwards instead of radially outwards and affect ZoI	Use agar plates from the same batch for each trial, observe plates before use for irregularities
Incubation conditions	Affects the rate of growth for S. epidermidis compared to how quickly the extract can spread throughout the agar	Lab incubator set to a constant 30°C for a fixed duration of exactly 24 hours
Aseptic sanitization	Contaminated equipment or surfaces may introduce competing bacteria or fungal colonies which obscure ZoI boundaries	Workspace and all tools sprayed completely with GLEN20 disinfectant prior to handling.

Extraneous variables and limitations:

Table 3: Extraneous variables/limitations of experiment and the steps taken during the experiment to mitigate their impact on the data

Extraneous variable / Limitation	Impact on Data	Mitigation protocol used
Evaporation of Active Compounds	Highly volatile essential oils (e.g., tea tree or lemon myrtle components) can rapidly evaporate into the air during the 5 minute drying phase, reducing the active chemical concentration on the disc and artificially shrinking the ZoI.	Strictly standardized the air-drying duration to exactly 5 minutes using a phone timer across all treatment groups, transferred cellulose discs onto the surface of the agar plates as quickly as possible using sterilised forceps
Residual Ethanol Interference	If trace amounts of the 70% ethanol solvent do not evaporate fully, the alcohol itself may act as an antiseptic, falsely inflating the size of the ZoI	Before conducting trials, decided to utilize a dedicated negative control disc (pure 70% ethanol dried for 5 minutes) to test involvement of solvent ethanol. The resulting 0.00 mm net ZoI demonstrated that the solvent had no independent impact on the results.
Molecular Diffusion Restrictions	Heavy molecular weight or hydrophobic bioactive compounds (curcumin) cannot diffuse easily through the agar gel, and may trap active agents within the paper fibres to create an inaccurately small ZoI	Formally separated the visible pigment diffusion ("Turmeric Stains") from the true clear zones during data analysis and addressed this physical constraint as an alternative hypothesis in the evaluation.
Suboptimal Incubation Temperature	Restricting incubation to 30°C due to school biosafety requirements (instead of the optimal human body temperature of 37°C) slows bacterial growth. Consequently, the experiment may not perfectly replicate real-world human skin infections.	Maintained a constant environment using a digital incubator fixed at 30°C to keep a high internal validity
Direct Inoculation / Unknown CFU Load	As information from the supplier was unavailable, inoculating directly from a stock broth culture without standardizing bacterial concentration means the exact Colony Forming Unit (CFU) density is unknown, introducing potential variation in lawn thickness.	Sourced all bacterial samples from the same culture flask and used a pipette to dispense a standardized volume of exactly 10 µL onto every agar plate. Conducted each round of trials in the same session.
Variation in botanical sample form	See below	See below
Variation in commercial processing history	The dried samples (turmeric, sappan, lemon myrtle) were purchased from commercial suppliers. Their drying methods and amount of time after production were unknown. Exposure to heat, oxygen or light during processing may have degraded some amount of bioactive compounds, potentially reducing extract efficiency and ZoI	All commercial botanical materials were sourced from reputable herbal suppliers and stored under identical laboratory conditions after purchase. Samples were processed shortly after acquisition (~18 hours) and extracted using the same ethanol concentration, extraction time and methodology to minimise additional degradation.

Variation in botanical sample form - Limitation acknowledgement

Due to time, financial and availability constraints, the botanical species could not all be obtained in equivalent forms. *M. alternifolia* was collected as fresh leaves from tube stock, whereas *B. sappan* and *C. longa* were sourced as dried herbal material and *B. citriodora* was only commercially available as a powdered product. These differences in sample form may have altered the concentration and availability of secondary metabolites during ethanol extraction, particularly volatile compounds such as essential oils, thereby influencing the antimicrobial potency of each extract and altering the resulting

Zone of Inhibition. Consequently, some variation between treatments may reflect differences in sample processing rather than the antimicrobial properties of each of the species (Ashs.org, 2025; Carson et al., 2006).

Mitigation procedure

The experiment was conducted with the exact same procedure and conditions for all samples to maximise reliability of comparisons between treatments.

Apparatus list:

PPE - Apron, safety goggles, gloves, face mask

Safety equipment - Disinfectant spray (GLEN20), paper towel, dustpan and brush

Table 4: Apparatus required for the experiment, along with quantities, notes and uncertainty where applicable

Material	Quantity (including units)	Notes	Uncertainty
S. epidermis	1x 10mL broth culture	Sourced from a uniform stock flask provided by the school lab to act as a safe and structurally sound substitute for S. aureus. Controlled variable	N/A
Relevant botanicals (C. longa, B. sappan, M. alternifolia, B.citriodora)	4.00 g each	Independent variable to test different ZoI	±0.01 g (Electronic scale)
70% Ethanol solvent	~200 mL total (20 mL per sample)	Used as the primary liquid extraction medium for maceration. Also used as negative control group	±0.5 mL (per measuring cylinder use)
2% wt/wt Potassium triiodide (KI_3)	1x small bottle (~100 mL)	Positive control antiseptic solution to validate bacterial susceptibility	N/A (Standard solution provided by lab)
Nutrient agar plates	18x plates total	Premade to a uniform standard by the lab; 12 plates allocated for Trial 1 and 6 plates for Trial 2.	N/A
No.1 filter paper	1x pack of 100	Used to filter out biological matter after maceration, and cut out using the 6.5mm diameter hole punch to be used as absorbent paper discs during trials	N/A
6.5mm diameter hole punch	1x	Used to cut standardized paper discs	N/A
25mL measuring cylinder	1x unit	Used to measure volume of ethanol solvent (20mL) for each sample	±0.5 mL
25mL beaker	6x units	Used for holding filtered extracts after maceration stage	N/A (volume measured via 25mL measuring cylinders)
50mL centrifuge tubes with screw-on lids	6x units	Dedicated individually to each sample extract (see section 1.1) to eliminate cross-contamination. Used to hold ethanol solution with crushed herbal samples and sealed with lid to leave for maceration	N/A (volume measured via 25mL measuring cylinders)
Pipette with 10µl extended-length tips	1x unit (3mL capacity)	Sanitized with GLEN20. Used in combination with the calibrated tips to dispense precise volumes (10 µL) of bacterial culture onto agar	±0.1 mL (Graduation marks)
Plastic L-shaped spreader	1x unit	Sanitized with GLEN20. Used to spread the 10µl bacterial solution evenly across the agar face using a three-step streaking technique to create a uniform confluent lawn.	N/A
Watch glasses	4x units	Sanitized with GLEN20. Used to hold plant samples during weighing,	N/A

		used to lay out soaked cellulose discs during the air-drying stage	
Mortar and pestle	1x unit	Used to manually crush raw plant materials into a fine paste to facilitate maceration	N/A
Forceps	1x unit	Sanitized with GLEN20. Used for aseptic transport of discs to soak in beakers, dry on watch glasses and place on agar plate surfaces.	N/A
Spatula	1x unit	Sanitized with GLEN20. Used to cleanly transfer crushed plant paste into the centrifuge maceration tubes.	N/A
Digital vernier caliper	1x unit	Precise instrument used to measure perpendicular axes of the zones of inhibition.	±0.01 mm
Electronic scale	1x unit	Used to weigh precise quantities of raw botanical matter and samples	±0.01 g
Mobile phone clock	1x unit	Used to standardize the 15-second immersion phase and 5-minute drying window	~±0.01s (digital precision), but mostly irrelevant compared to the time required to transport all the paper discs during the method

Method

1. **Preparation of Botanical Extracts**

   1.1 Weigh 4.00 g of each sample with electronic balance.

   1.2 Crush samples into a paste with a mortar and pestle (table 1).

   1.2 Add samples to separate 50 mL centrifuge tubes and label, add 20 mL of 70% ethanol added to each tube.

   1.3 Add a fifth centrifuge tube containing only 20 mL of 70% ethanol as negative control.

   1.4 Seal tubes and leave to macerate for 24 hours (figure 6).

   1.3 After 24 hours, filter extract through filter paper into clean beakers to separate solid material

2. **Preparation of Bacterial Lawns**

   2.1. Dispense 10 µL of bacterial culture onto the centre of each nutrient agar plate using a micropipette.

   2.2. Evenly distribute inoculum across the agar surface to produce a uniform bacterial lawn with L-shaped spreader. *S. epidermidis* was selected as a safe substitute for *S. aureus* (see methodological note)

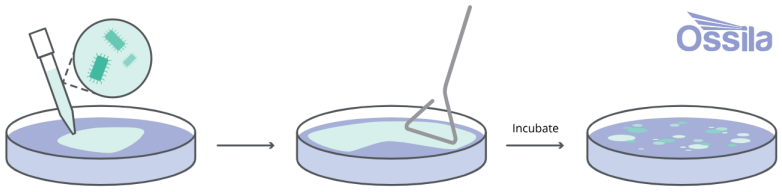


Fig. 9: Spread plate method of bacteria across agar (Ossila, 2025).

3. **Preparation of Antimicrobial Discs**

   3.1. Immerse sterile absorbent paper discs into each extract for 15 seconds. Soak 6 discs in pure ethanol and 6 in KI_3 solution.

   3.2. Air dry discs on watchglasses for 5 minutes.

4. **Placement of discs and incubation**

   4.1. Transfer discs onto the surface of each agar plate using forceps, 6 discs across 2 plates each. Disinfect between each sample class.

   4.2 Seal plates and incubate at 30°C for 24 hours.

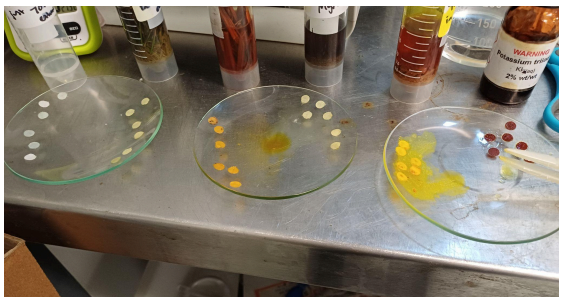


Fig. 10: Paper discs left to dry after being soaked within extracts

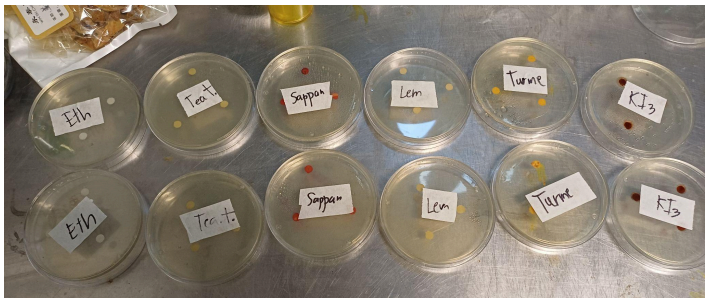


Fig. 11: All extract discs placed within their respective inoculated agar plate and labelled, ready for incubation

Fig. 12: Incubation of agar plates, primary trial

5. Measurement of Antimicrobial Activity

5.1. Measuring bacterial growth following incubation with a digital caliper. Record two perpendicular diameters (X, Y-axis) for every ZoI. Take measurements from beneath the plate to minimise parallax error.

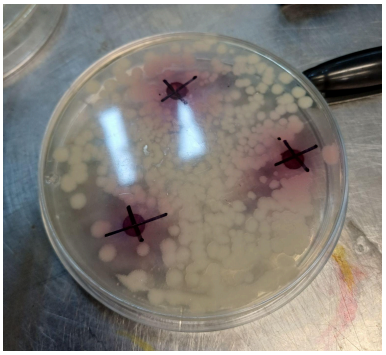


Fig. 12. Lines across the X and Y-axes with a ruler and marker were drawn in order to identify the edge of bacterial growth inhibition prior to measurement with caliper.

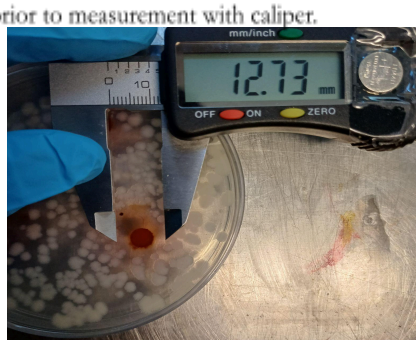


Fig. 13. Measurement of observable ZoI across two axes using digital caliper ($\pm$0.01mm uncertainty)

6. Secondary Synergy Investigation

6.1 Blend B. sappan and C. longa filtrates in a 1:1 volumetric ratio.

6.2 Blend B. sappan, C. longa, and B. citriodora filtrates in a 1:1:1 volumetric ratio.

6.3 Follow methodology from Sections 1–5, accounting for 9 discs and 3 plates

6.4 Measure and record results

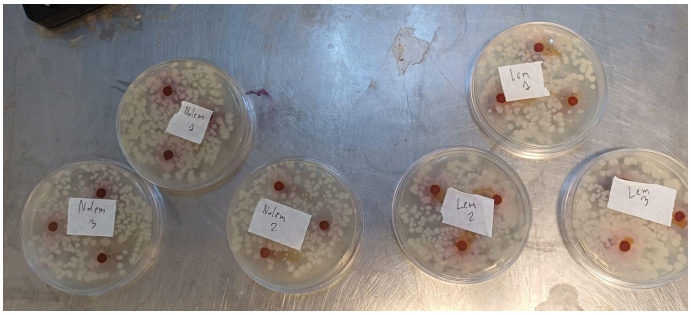


Fig. 14. Methods step 1-5 followed when handling secondary trial discs, before incubation. Because of the lower amount of samples to test, a higher number of agar plates was secured for the experiment

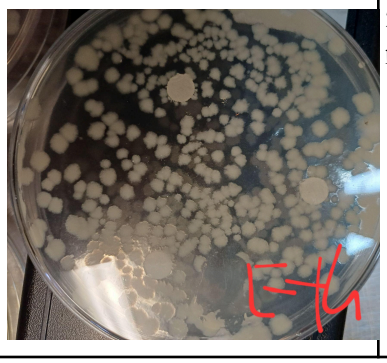
Risk Assessment

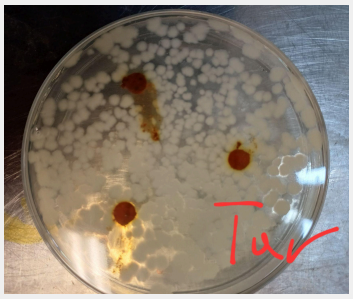
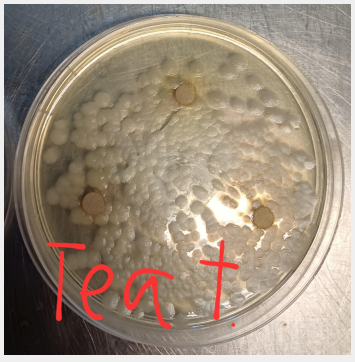
Table 5: Apparatus required for the experiment, along with quantities, notes and uncertainty where applicable

Risk identification	Potential harm	Procedure for mitigation
Exposure to *S. epidermidis* bacterial culture	Although *S. epidermidis* is a lower-risk skin bacterium than *S. aureus*, accidental exposure may cause contamination of surfaces or potential infection if introduced into open wounds or compromised skin, as it is still an opportunistic pathogen.	Wear PPE including gloves, safety goggles, lab coat/apron and face mask. Maintain aseptic technique throughout the investigation. Disinfect work surfaces and equipment before and after use
Use of ethanol-based extracts (70% ethanol)	Ethanol and concentrated herbal extracts (particularly from Lemon Myrtle and Tea Tree oils) can cause irritation or allergic reactions in the eyes and skin. Ethanol vapours may ignite near heat sources	Keep ethanol containers away from flames and ignition sources. Use only small volumes during extraction. Wear gloves and goggles when handling ethanol solutions. Clean spills immediately.
Cuts from glassware and laboratory equipment	Broken glass shards from beakers/vials/dishes or sharp edges of forceps used for herb preparation can cause lacerations.	Inspect glassware before use. Handle forceps, mortar and pestle, and other equipment carefully. Dispose of broken glass with dustpan and brush

Results

Table 6: Qualitative observations - Primary trial

Species	Appearance	Comment
Ethanol (negative control)		Zero observable ZOI beyond disc across both plates, negative control successful

Potassium triiodide (positive control)		Clear and large ZOI around every disc, consistent across both trials, positive control successful
Biancaea sappan		Small to moderately sized ZOI observed, colour stains from maceration extract. Effective
Curcuma longa		Very small ZOI in some discs to no effect. Notably traces of turmeric outside of discs seemed to inhibit growth. Out of the three plates, four of the six discs were identified as having turmeric stains with an observable ZOI
Backhousia citriodora		Small ZOI in some discs to no effect, slightly better than turmeric but overall ineffective
Melaleuca alternifolia		Slight greenish tint likely from leaf maceration, roughly equal ZOI to lemon myrtle, and overall ineffective

Secondary trial - *B. sappan* + *C. longa*		Small to moderately sized ZOI observed, results roughly the same as in Sappan wood trial
Secondary trial - *B. sappan* + *C. longa* + *B. citradoria*		Medium sized ZOI in majority of the plates, larger than each of the components individually but smaller than positive control

Table 6: Recorded raw data

Species	**Disc 1 (mm)**	**Disc 2 (mm)**	**Disc 3 (mm)**	**Disc 4 (mm)**	**Disc 5 (mm)**	**Disc 6 (mm)**
Ethanol (negative control)	6.5, 6.5	6.5, 6.5	6.5, 6.5	6.5, 6.5	6.5, 6.5	6.5, 6.5
Potassium triiodide (positive control)	21.31, 18.33	15.76, 18.44	19.68, 17.4	13.75, 18.7	19.73, 18.7	15.86, 18.01
Biancaea sappan	9.92, 14.8	12.43, 14.24	10.71, 11.79	15.02, 11.55	12.51, 9.94	11.96, 9.77
Curcuma longa	6.95, 6.5	6.5, 6.54	6.5, 8.98	6.5, 6.5	6.5, 6.65	6.5, 6.5
Curcuma longa, observable stains	13.07, 4.33	N/A	12.86, 7.6	7.97, 19.75	5.64, 4.99	N/A
Backhousia citriodora	7.79, 7.78	7.7, 6.5	7.74, 7.22	6.57, 7.3	7.83, 6.83	7.36, 6.93
Melaleuca alternifolia	6.79, 6.8	6.5, 7.2	6.5, 6.9	7.24, 6.53	6.54, 6.5	7.08, 6.5

Table 7: Recorded raw data - secondary synergistic trials

Species	**Disc number**	**Measurements**
B. sappan + *C. longa*	**1**	10.47, 12.08
B. sappan + *C. longa*	**2**	10.13, 10.42
B. sappan + *C. longa*	**3**	8.79, 9.52
B. sappan + *C. longa*	**4**	12.55, 7.76
B. sappan + *C. longa*	**5**	16.65, 10.9
B. sappan + *C. longa*	**6**	14.33, 12.03

B. sappan + *C. longa*	**7**	14.9, 7.9
B. sappan + *C. longa*	**8**	9.14, 9.44
B. sappan + *C. longa*	**9**	9, 6.8
B. sappan + *C. longa* + *B. citradoria*	**1**	12.6, 16.57
B. sappan + *C. longa* + *B. citradoria*	**2**	18.95, 12.75
B. sappan + *C. longa* + *B. citradoria*	**3**	11.6, 26.26
B. sappan + *C. longa* + *B. citradoria*	**4**	14.28, 10.13
B. sappan + *C. longa* + *B. citradoria*	**5**	14.27, 17.13
B. sappan + *C. longa* + *B. citradoria*	**6**	16.61, 12.73
B. sappan + *C. longa* + *B. citradoria*	**7**	12.61, 18.97
B. sappan + *C. longa* + *B. citradoria*	**8**	22.26, 14.9
B. sappan + *C. longa* + *B. citradoria*	**9**	12.86, 12.58

Processed Data Analysis

Sample calculation: Mean diameter and standard deviation of KI_3

To transform the raw X and Y caliper measurements into plottable values, the following standard microbiology calculations were applied to each treatment group:

1. Mean disc diameter (D): Accounts for directional asymmetry

$$D_m = \frac{X + Y}{2}$$

Table 8: Mean disc diameter calculations

Disc #	Measurements (mm)	Calculations (mm)	Result (mm) (D_m to 3 s.f.)
1	21.31, 18.33	$D_m = \frac{21.31 + 18.33}{2}$	D_m = 19.8
2	15.76, 18.44	$D_m = \frac{15.76 + 18.44}{2}$	D_m = 17.1
3	19.68, 17.4	$D_m = \frac{19.68 + 17.4}{2}$	D_m = 18.5
4	13.75, 18.7	$D_m = \frac{13.75 + 18.7}{2}$	D_m = 16.2
5	19.73, 18.7	$D_m = \frac{19.73 + 18.7}{2}$	D_m = 19.2
6	15.86, 18.01	$D_m = \frac{15.86 + 18.01}{2}$	D_m = 16.9

2. Overall Mean Diameter (D_t): Across replicate discs (n) per each sample

$$D_t = \frac{\Sigma D_m}{n}$$

$$\text{Working: } D_t = \frac{19.8 + 17.1 + 18.5 + 16.2 + 19.2 + 16.9}{6}$$

$$D_t = \frac{107.835}{6} = 17.97 \text{ mm} \approx 18.0 \text{ mm (3 s.f.)}$$

3. Net ZoI: Subtract from physical disc footprint (6.5 mm) to determine the actual extension of extract beyond the diameter of the carrier cellulose discs

$$Net\ ZoI = D_t - 6.5\ mm$$

$$Net\ ZoI = 17.97\ mm - 6.5\ mm$$

$$Net\ ZoI = 11.47\ mm \approx 11.5\ mm\ (3\ s.f.)$$

4. Sample standard deviation (*Sd*): Calculate individual disc variances relative to the calculated treatment mean of sample ($D_t = 17.97$ mm) to show data dispersion

$$Sd = \sqrt{\frac{\Sigma(D_m - D_t)^2}{n-1}}$$

Table 9: Calculation of squared differences for each disc

Disc #	Calculations (mm)
1	$(19.82 - 17.97)^2 = (1.85)^2 = 3.423$
2	$(17.10 - 17.97)^2 = (-0.87)^2 = 0.757$
3	$(18.54 - 17.97)^2 = (0.57)^2 = 0.325$
4	$(16.23 - 17.97)^2 = (-1.74)^2 = 3.028$
5	$(19.22 - 17.97)^2 = (1.25)^2 = 1.563$
6	$(16.94 - 17.97)^2 = (-1.03)^2 = 1.061$

4.2 Sum calculated variations (Σ) and divide by one less than the total sample size (using Bessel's correction)

$$\Sigma(D_m - D_t)^2 = 3.423 + 0.757 + 0.325 + 3.028 + 1.563 + 1.061$$

$$\Sigma(D_m - D_t)^2 = \frac{10.157}{5} = 2.0314$$

4.3. Take the square root of the result

$$Sd = \sqrt{(2.0314)} = 1.426 \approx 1.43\ mm\ (3\ s.f.)$$

Table 10: Processed Net Zone of Inhibition and Standard Deviation Calculations

Treatment Species / Control	Overall Mean Diameter (mm)	Net Zone of Inhibition (mm)	Standard Deviation (Sd) (mm)
Ethanol (Negative Control)	6.5	0	0
Potassium Triiodide (Positive Control)	17.97	11.47	1.426
Sappan Wood *(B. sappan)*	12.05	5.55	1.095
Turmeric *(C. longa)*	6.76	0.26	0.488
Turmeric Stains (Pigment Diffusion)	9.53	3.03	3.545
Lemon Myrtle *(B. citriodora)*	7.3	0.8	0.305
Tea Tree *(M. alternifolia)*	6.76	0.26	0.132
Secondary trial - *B. sappan* + *C. longa* + *B. citradoria*	10.71	4.21	1.908
Secondary trial - *B. sappan* + *C. longa* + *B. citradoria*	15.45	8.95	2.280

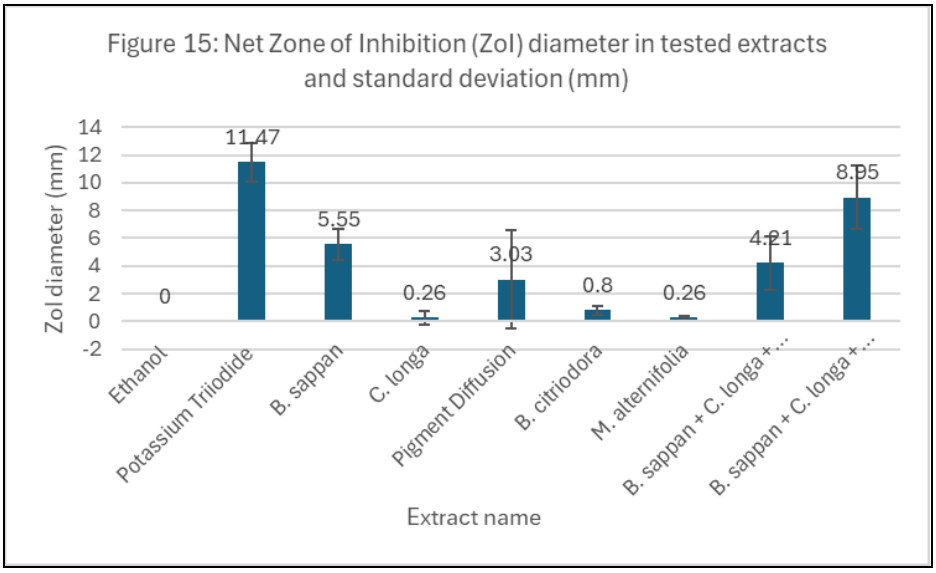
NOTE - Methodological Isolation of Staining vs. Biological Inhibition

During data collection, curcumin pigment diffused into the agar, leaving uneven stains extending beyond the true clear ZoI. These stains were analyzed separately from *C. longa* for two reasons:

- Incomplete data (n = 4): discs 2 and 6 were unmeasurable due to unclear boundaries, making caliper jaw placement impossible
- High variability: the stain group showed massive standard deviation (3.545) and percentage uncertainty (44.85%), caused by irregular, often oval shapes (e.g., disc 4 measured 19.75 mm by 7.97 mm).

Conclusion: Unclear if the staining reflects passive dye diffusion or true antimicrobial activity against S. epidermidis, so only the clear ZoI data was used as a reliable measure of *C. longa's* effectiveness. However, curcumin's hydrophobic nature and large molecular size may limit its diffusion through agar, meaning the small ZoI may underestimate its true antibacterial potential. Due to this possibility, C. longa was retained for further testing and included in secondary synergistic trials.

Processed Data Graph



Interpretation of graph

The graph demonstrates clear differences in the antimicrobial effectiveness of the tested botanical extracts against *S. epidermidis*, used as a model organism for *S. aureus*. The positive control produced the largest mean net ZoI (11.47 mm), confirming that the bacterial culture was susceptible to antimicrobial agents. The negative control produced an observable net ZoI of 0.00 mm, demonstrating that the solvent itself did not inhibit bacterial growth and that any inhibition observed resulted from plant metabolites rather than residual ethanol. Among the individual botanical extracts, *B. sappan* produced the greatest inhibition (5.55 ± 1.10 mm), indicating the strongest antibacterial activity. *B. citriodora* (0.80 ± 0.31 mm), *C. longa* (0.26 ± 0.49 mm), and *M. alternifolia* (0.26 ± 0.13 mm) showed minimal inhibition. Although *C. longa* produced visible pigment diffusion beyond the paper disc, these regions displayed irregular boundaries and high variability (SD = 3.55 mm), meaning they could not be confidently interpreted as genuine inhibition.

The secondary investigation showed that combining *B. sappan* and *C. longa* produced a Net ZoI of 4.21 ± 1.91 mm, which was slightly lower than *B. sappan* alone (likely random variance or evaporation of antimicrobial compounds). However, the combination of *B. sappan, C. longa, and B. citriodora* interestingly produced a substantially larger inhibition zone (8.95 ± 2.28 mm). This suggests that combining extracts with different mechanisms of antibacterial action increased antimicrobial effectiveness.

Analysis and Discussion

Although individual results confirmed that each tested extract held some degree of antibacterial activity, *B. sappan* exhibited the greatest individual antibacterial activity, indicating that its extract contained sufficient potency to inhibit *S. epidermidis* growth under the experimental conditions. Brazilin and brazilein are known to damage Gram-positive bacterial cell walls and increase membrane permeability, which is consistent with the relatively large ZoI observed (Nirmal et al., 2015).

One explanation for the unexpectedly poor performance of the Australian botanicals is that effective inhibition requires both antimicrobial potency and the diffusion of compounds through agar. Although monoterpenes (terpinen-4-ol, citral) are relatively small molecules, ethanol maceration may have extracted lower concentrations of volatile essential oils than other commercial methods such as steam distillation or purified essential oil extraction. Furthermore, evaporation during the standardised drying stage may have reduced the quantity of active compounds remaining on the discs before incubation (Carson et al., 2006; Hayes & Markovic, 2002). Consequently, the measured ZoI may underestimate the true antimicrobial activity of these species. The comparatively poor performance of *C. longa* is also consistent with published literature. Curcumin possesses strong antibacterial activity but is highly hydrophobic, which limits its diffusion through agar (Teow et al., 2016; Dai et al., 2022). Although extensive yellow pigment diffused into the agar surrounding several discs, much of this stained region lacked clearly defined bacterial inhibition. This suggests that while some curcumin molecules were able to spread away from the disc, their concentration may have fallen below the minimum inhibitory concentration required to prevent bacterial growth.

Furthermore, the sample variation may also have contributed to the observed differences in antibacterial activity (see 'Extraneous variables and limitations'). The stronger inhibition produced by *B. sappan* may reflect differences in the concentration and preservation of extracted compounds rather than the species alone, meaning direct comparisons between treatments should be interpreted with discretion. In regards to the secondary investigation, the increased antibacterial activity observed for the three-extract combination is plausible because citral from *B. citriodora* disrupts bacterial membranes, potentially increasing cell permeability and allowing intracellular compounds (brazilin and curcumin) to act more effectively (Nirmal et al., 201).

Evaluation

Reliability: This investigation had high reliability. Variables such as extraction procedures (4.00 ± 0.01 g botanical matter, 20 ± 0.5 mL of 70% ethanol, 24-hour maceration), disc parameters (6.5 mm discs, 15-second immersion, 5-minute drying), and incubation conditions (30°C, 24 hours) were all standardised, and replicate discs per group reduced random error. This is reflected in the generally low standard deviations (e.g. Sd = 0.13 mm for M. alternifolia; Sd = 0.31 mm for B. citriodora), apart from the *C. longa* pigment-stain data (Sd = 3.55 mm, n = 4), which was excluded from the main analysis due to its high variability and unreadable boundaries.

Internal validity: Internal validity was also high. The negative control confirmed the ethanol solvent itself caused no inhibition, and the positive control confirmed *S. epidermidis* susceptibility to antimicrobial agents. Mitigation of extraneous variables (inoculum volume, agar batch, aseptic technique, incubation conditions) further improved reliability and strengthened causal link between the species of extract and resulting ZoI.

Construct validity: Construct validity was comparatively low, as the procedure may not fully represent real-world antimicrobial effectiveness against S. aureus on eczema-affected human skin. Limitations such as *S. epidermidis* being substituted for *S. aureus*, incubation occurring at 30°C rather than optimal skin temperature of 37°C, and botanical samples being obtained in inconsistent forms (fresh, dried, powdered), all introduced variation unrelated to species identity. Agar disc diffusion also inherently conflates antimicrobial potency with molecular weight and hydrophobicity (seen through curcumin's poor diffusion and ZoI), Therefore, the results of this investigation are best read as an indicator for antimicrobial potential under these specific conditions, and not as a definitive measure of compound effectiveness and synergy.

Conclusion

This investigation aimed to evaluate the antimicrobial efficacy of ethanol-based extracts from *C. longa, M. alternifolia, B. citriodora,* and *B. sappan* against *S. epidermidis*, and to investigate whether combining these extracts could produce a synergistic effect. The hypothesis was only partially supported. All four extracts produced measurable inhibition relative to the ethanol control (0.00 mm), confirming genuine antimicrobial activity. However, contrary to the prediction that the Australian monoterpene extracts would produce the largest Zones of Inhibition, B. sappan was the most effective individual extract (5.55 ± 1.10 mm), significantly ahead of B. citriodora (0.80 ± 0.31 mm), *C. longa* (0.26 ± 0.49 mm), and *M. alternifolia (*0.26 ± 0.13 mm).

For the secondary aim, the *B. sappan* + *C. longa* blend (4.21 ± 1.91 mm) did not exceed *B. sappan* alone, but the three-extract combination of *B. sappan, C. longa, and B. citriodor*a produced the largest combined result (8.95 ± 2.28 mm) out of all the extracts. This supports the prediction that pairing extracts with different mechanisms of action enhances antibacterial activity, although without a concentration-controlled method, this cannot be confirmed as true synergy rather than an additive effect (see evaluation). Despite these limitations, the investigation successfully narrows five candidate botanicals down to the most promising leads, B. sappan individually, and the three-extract combination overall. It suggests that a synergistic link between a monoterpenoid and phenolic compound may be possible. Further, more resource-intensive testing in the future should be conducted based on this link, including testing extract concentrations directly relevant to human skin application and using a more specialised method, such as a checkerboard dilution assay, to confirm whether this interaction is genuinely synergistic.

Background - Glossary

[2] Stratum corneum - the outermost layer of the skin

[3] Helper T cells that release specific cytokines to coordinate different types of immune responses (Immunology.org. , 2016)

[4] Phenol-Soluble Modulins (PSMs) - a family of peptides highly destructive to human cells

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Appendix: Calculation of Net ZoI, Sd

Guide to calculate for standard deviation: (Biology for Life, n.d.)

Note: The following calculations represent raw data transformation for all experimental botanical treatments and the negative control. Potassium Triiodide (KI_3) calculations included in sample calculation (Section 4.3).

1. Ethanol (Negative Control)

Because X and Y for all 6 replicates measured exactly 6.50 mm (no ZoI), all Dm values = 6.50 mm.

$Dt = (6.50 + 6.50 + 6.50 + 6.50 + 6.50 + 6.50) / 6 = 6.50$ mm

Net ZoI = 6.50 mm − 6.50 mm

= 0.00 mm

$\Sigma(Dm - Dt)^2 = 0$

$Sd = \sqrt{(0 / 5)}$

= 0.000 mm

2. Sappan Wood (Biancaea sappan)

$Dt = (12.36 + 13.34 + 11.25 + 13.29 + 11.23 + 10.87) / 6$

= 12.05 mm

Net ZoI = 12.05 mm − 6.50 mm

= 5.55 mm

$\Sigma(Dm - Dt)^2 = 5.997$

$Sd = \sqrt{(5.997 / (6 - 1))}$

$= 1.10$ mm

3. Turmeric (Curcuma longa) ZoI of discs

$Dt = (6.73 + 6.52 + 7.74 + 6.50 + 6.58 + 6.50) / 6$

$= 6.76$ mm

Net ZoI = 6.76 mm − 6.50 mm

= 0.26 mm

$\Sigma(Dm - Dt)^2 = 1.189$

$Sd = \sqrt{(1.189 / (6 - 1))}$

$= 0.488$ mm (3 s.f.)

4. Turmeric Stains

n = 4 due to unreadable margins for Discs 2 and 6.

$Dt = (8.70 + 10.23 + 13.86 + 5.32) / 4$

$= 9.53$ mm

Net ZoI = 9.53 mm − 6.50 mm

= 3.03 mm

$\Sigma(Dm - Dt)^2 = 37.694$

$Sd = \sqrt{(37.694 / (4 - 1))}$

$= 3.54$ mm (3 s.f.)

5. Lemon Myrtle (Backhousia citriodora)

$Dt = (7.79 + 7.10 + 7.48 + 6.94 + 7.33 + 7.15) / 6$

$= 7.30$ mm

Net ZoI = 7.30 mm − 6.50 mm

= 0.80 mm

$\Sigma(Dm - Dt)^2 = 0.466$

$Sd = \sqrt{(0.466 / (6 - 1))}$

$= 0.305$ mm (3 s.f.)

6. Tea Tree (Melaleuca alternifolia)

$Dt = (6.80 + 6.85 + 6.70 + 6.89 + 6.52 + 6.79) / 6$

$= 6.76$ mm

Net ZoI = 6.76 mm − 6.50 mm

= 0.26 mm

$\Sigma(Dm - Dt)^2 = 0.087$

$Sd = \sqrt{(0.087 / (6 - 1))}$

= 0.132 mm (3 s.f.)

Secondary trials

7. Sappan wood + Turmeric

$D_t = (11.275 + 10.275 + 9.155 + 10.155 + 13.775 + 13.180 + 11.4 + 9.290 + 7.900)/9$

= 10.71 mm

Net ZoI = 10.712 mm − 6.50 mm

= 4.21 mm

$\Sigma(Dm - Dt)^2 = 29.123$

$Sd = \sqrt{(29.123 / (9 - 1))}$

= 1.908 mm (3 s.f.)

8. Sappan wood + Turmeric + Lemon myrtle

Dt = (14.585 + 15.850 + 18.930 + 12.205 + 15.700 + 14.670 + 15.790 + 18.580 + 12.720) / 9

= 15.45 mm

Net ZoI = 15.448 mm − 6.50 mm

= 8.95 mm

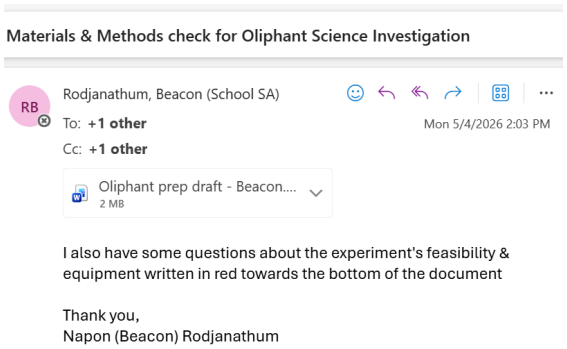
$\Sigma(Dm - Dt)^2 = 41.583$

$Sd = \sqrt{(41.583 / (9 - 1))}$

= 2.280mm (3 s.f.)

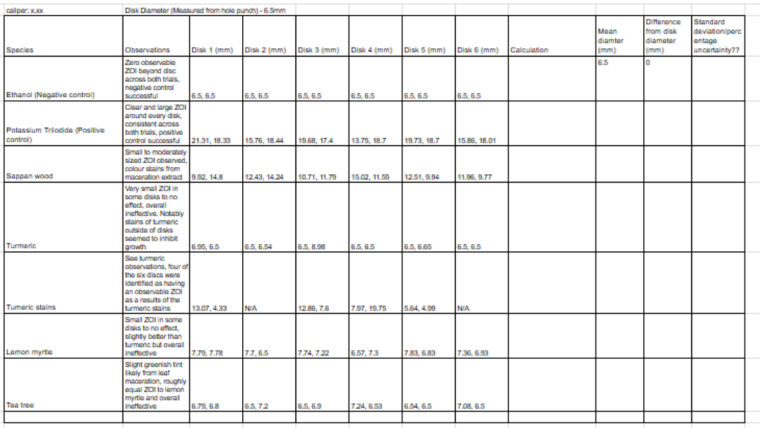
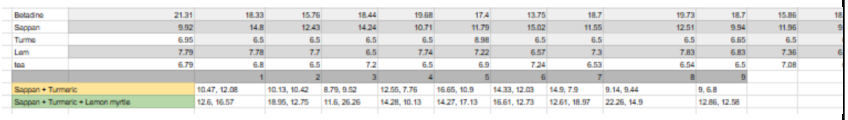
Scientific Journal Logbook

Date Written (DD/MM/YY)	**Content**
Log 1 12/04/26	After weeks of switching around multiple different ideas for my investigation, I think I've finally landed on one! Even though I participated in and received a 'Highly commended' award for my poster category in 2025, I felt like I was just chasing portfolio credit back then rather than actually doing something science-related which was worth my time. This is my second last year of high school, and since I hope to get into medical school in the scarily near future. I want to conduct an investigation which would be more than just something to reference in my CV. I would like this experience to teach me about formal academic writing, along with a real-world concept relevant to the field of pathology which could have potential applications in healthcare To ensure my investment in this project, I established a requirement that the inquiry must have personal relevance and potential practical applications I have chosen to investigate Atopic Dermatitis (Eczema) and how its associated risks of S. aureus infection can be mitigated by the antimicrobial properties of traditional herbs. This choice is especially personal to me compared to my previous entry. I've had chronic eczema since 3rd grade, and it has been a constant source of physical pain and annoyance. Over the years, the growing rashes have steadily creeped up both my legs. I was prescribed various conventional treatments throughout my childhood, including Triamcinolone Acetonide (TA) 0.1% (a topical corticosteroid) and several antifungal creams. While these provided temporary relief, they lacked a sustainable effect, and I am increasingly concerned about the long-term health implications of prolonged steroid use, such as skin thinning (atrophy) and systemic absorption. My lived experience with AD and seeing how moments of severe itching had led to accidental scratching and consequent progression of the condition drives my desire to understand the pathology of AD and find natural, sustainable alternatives. I want to understand why the damaged skin barrier is so susceptible to persistent bacterial colonization and if botanical phytochemicals can offer a safer long-term management strategy compared to commonly prescribed treatments. I went to the state library for around two and a half hours today to start on my investigation. I ended up doing research into the relevant concepts in biology (mainly the types of bacteria which are most prevalent in AD and identifying S. aureus as a research topic of interest) which may come up during the experiment, along with beginning to research a few variables of the investigation (looking at traditional Thai herbs) I hope to present my idea and concept to my science teachers during our next meeting next Friday and receive their approval, along with receiving advice on how I could conduct and analyse the data. I will continue working on this in the meantime whenever I have free time after school, and I hope I can create an Oliphant science awards project I am proud of this time around.
Log 2 02/05/26	I just finished athletics training in the morning with my friends and am quite exhausted, but since I had to pass through the city on the way back home anyways and I'd caught up on my schoolwork, I decided to grab some lunch at Rundle Mall and stop by at the city library (the state library is closed for renovation) for the rest of the afternoon to continue working on this investigation. Today ended up being an incredibly productive session. During this session, building from the preliminary research I'd done last month, I mainly focused on the mechanism of S. aureus colonization and identified the key variables for my experiment: **LINK TO DRAFT DOCUMENT:** https://docs.google.com/document/d/1eYTaRFwbHohXbCYgIeeiJU3sZWv-Ofn3BLFKYHZFsSo/edit?usp=sharing

	I have also drafted a list of necessary equipment to request from the science lab, including items I am not familiar with, such as the Mueller-Hinton Agar plates and the bacteria culture solution. After all the base information was obtained, I made a brief draft for my experiment, including the method, equipment, timeline of the investigation and a start on the risk assessment . Along with this, I included some questions I had about the logistics of the methods and the available equipment the school lab is able to provide.
Log 3 05/05/26	During the Oliphants Science Awards meeting with our responsible science teachers last Friday (1st of May) where I presented my concept, I was told that I should email our school lab tech (Ms. C) with the method, equipment and timeline of my experiment in order to receive approval and confirmation that I am able to conduct it. I ended up finalising my draft and emailing it to Ms. C the following Monday **LINK TO DRAFT DOCUMENT:** https://docs.google.com/document/d/1eYTaRFwbHohXbCYgIeeiJU3sZWv-Ofn3BLFKYHZFsSo/edit?usp=sharing  Materials & Methods check for Oliphant Science Investigation RB Rodjanathum, Beacon (School SA) To: +1 other Cc: +1 other Mon 5/4/2026 2:03 PM Oliphant prep draft - Beacon.... 2 MB I also have some questions about the experiment's feasibility & equipment written in red towards the bottom of the document Thank you, Napon (Beacon) Rodjanathum I'm now waiting for confirmation that I can conduct the experiment, and additional information/advice I would need in order to refine my method and ensure it is feasible
Log 4 10/05/26	I received the response from the Lab Technician. We cannot use McFarland standards or culture our own broth in tubes due to safety protocols; instead, I have to use a direct-to-plate 10µl inoculation from their stock solution. Also, we are restricted to Nutrient Agar and an incubation temperature of 30C for 24 hours.I spent this afternoon rewriting my methodology to account for these constraints. While it's not the method I initially planned at the library, it's something I'll have to adapt to my report. My Biology teacher also suggested an extension to my research: once I find the most effective individual herbs, I should test a combination of them. This adds a layer of synergy testing to the project which I will include as my secondary round of testing. If Lemon Myrtle and Turmeric are both strong, does mixing them create an even larger Zone of Inhibition?
Log 5 14/05/26	I received the official go ahead from Ms. C today. The school laboratory has processed and approved my revised methodology, and I now need to wait for supplies to come in and get a response. While I am excited to do the experiment, I am currently completely buried in my schoolwork and assessment deadlines. I still have to look at potential suppliers for my herbal samples, but for the next week or so, I will be prioritizing my classes
Log 6 22/05/26	Going forward will likely be using short dot notes rather than writing because of time constriants - Spent my free time doing a massive search online to find local suppliers for my plant samples in Adelaide. - Compiled a directory of organic shops, spice houses, and indigenous nurseries around the CBD to visit or call during late-night trading hours. Supplier list after end of search session:

	0882118526 (Tumeric) LIKELY Central Organic, 72/44-60 Gouger St, Adelaide SA 5000 -5:30pm THU 0870711626 (Lemon Myrtle) LIKELY Gewürzhaus Spice House - Adelaide Central Market, Stall 43, Adelaide Central Market, 44-60 Gouger St, Adelaide SA 5 -5:30pm THU 0451275213 (Sappan wood) LIKELY City Thuan Phat Supermarket - 75 Grote St, Adelaide SA 5000 Thursday 9 am–6 pm 0409675477 UNSURE Provenance Indigenous plants (Emu bush?) - 40 Sandy Cres, Salisbury Park SA 5109 Saturday 10 am–3 pm ** BRIGHT INDIAN GROCERY Shop no 6-7/418 Payneham Rd, Glynde SA 5070 0870738250 9PM TEA TREE UNSOLVED CONTACT (Bush/Tea tree??) 0489015747 Biophilia health???? - 143 Sturt St, Adelaide SA 5000. **********Emu bush - Tea tree??????????/ Thursday 10 am–6 pm 08 8271 1827 Botanica Medica - 97/99 Glen Osmond Rd, Eastwood SA 5063 ******** Thursday 10 am–7 pm 0451972400 Exotic Botanic Royston Park 1/291 Payneham Rd, Royston Park SA 5070 Thursday 10 am–5 pm 0882787777 State Flora Nursery Queens Jubilee Dr, Belair SA 5052 Thursday 9 am–5 pm
Log 7 27/05/26	Right after my last class today, I sat down and called every single supplier on my shortlist - Identified Gewürzhaus for Lemon Myrtle, though learned a raw fresh sample is unobtainable in Adelaide (noting as a methodology limitation). - Found out Tea Tree is available at Belair as tube stock, and identified a local Chinese herbal shop near the Central Markets for raw Turmeric and Sappan wood.
Log 8 31/05/26	- Massive run in the Adelaide CBD. Collected the Lemon Myrtle from Gewürzhaus, along with raw Turmeric and Sappan Wood specimens from Grote Street grocers. - Ran out of time to go up to the hills for the Tea Tree as I had to rush back to my part-time job.

	 Sappan wood samples! Dried, will note in botanical variance similar to lem myrtle
Log 9 06/06/26	- Finally had time to travel down to Belair and bought two Tea Tree tube stocks from the State Flora Nursery, which took almost the entire day. - All materials officially gathered; ready to start laboratory trials next week. 
Log 10 09/06/26	Night before the trial. Went out to the backyard with a flashlight to pluck and weigh leaves from the fresh Tea Tree tube stocks so they are ready for maceration tomorrow. Everything is packed into my school bag and I'm ready to conduct the trials, confirmed lab and equipment availability with Ms. C yesterday in person  Tea tree leaves collected
Log 11 13/06/26	- First round of trials successful (started extraction on Wednesday, June 10th). - Took photographic evidence and recorded measurements with digital calipers. Initial hypothesis was proven wrong; Australian monoterpenes

	had almost no individual clear zone, likely due to hydrophobic diffusion limits in agar, while Thai Sappan wood had much greater effect. - might be a limitation of the agar diffusion method itself, and some of the essential oils might be getting stuck in the paper disc and failing to diffuse through the water-based agar gel. Interested to see how the results of the secondary trial appear 
Log 12 17/06/26	- Finally finished the secondary round of trials to ensure data reliability. - Extremely busy with schoolwork and the end of the IB term, but managing data entry. The data is highly consistent and the tree-extract blend significantly outperformed all the other trials 
Log 13 28/06/26	- Spent the last 3 days finishing writing, editing, and calculating statistical standard deviations. Now uploading my submission to the site. Logbook closed.

caliper: x.xx | Disk Diameter (Measured from hole punch) - 6.5mm

Species	Observations	Disk 1 (mm)	Disk 2 (mm)	Disk 3 (mm)	Disk 4 (mm)	Disk 5 (mm)	Disk 6 (mm)	Calculation	Mean diamter (mm)	Difference from disk diameter (mm)	Standard deviation/percentage uncertainty??
Ethanol (Negative control)	Zero observable ZOI beyond disc across both trials, negative control successful	6.5, 6.5	6.5, 6.5	6.5, 6.5	6.5, 6.5	6.5, 6.5	6.5, 6.5		6.5	0	
Potassium Triiodide (Positive control)	Clear and large ZOI around every disk, consistent across both trials, positive control successful	21.31, 18.33	15.76, 18.44	19.68, 17.4	13.75, 18.7	19.73, 18.7	15.86, 18.01				
Sappan wood	Small to moderately sized ZOI observed, colour stains from maceration extract	9.92, 14.8	12.43, 14.24	10.71, 11.79	15.02, 11.55	12.51, 9.94	11.96, 9.77				
Turmeric	Very small ZOI in some disks to no effect, overall ineffective. Notably stains of turmeric outside of disks seemed to inhibit growth	6.95, 6.5	6.5, 6.54	6.5, 8.98	6.5, 6.5	6.5, 6.65	6.5, 6.5				
Tumeric stains	See turmeric observations, four of the six discs were identified as having an observable ZOI as a results of the turmeric stains	13.07, 4.33	N/A	12.86, 7.6	7.97, 19.75	5.64, 4.99	N/A				
Lemon myrtle	Small ZOI in some disks to no effect, slightly better than turmeric but overall ineffective	7.79, 7.78	7.7, 6.5	7.74, 7.22	6.57, 7.3	7.83, 6.83	7.36, 6.93				
Tea tree	Slight greenish tint likely from leaf maceration, roughly equal ZOI to lemon myrtle and overall ineffective	6.79, 6.8	6.5, 7.2	6.5, 6.9	7.24, 6.53	6.54, 6.5	7.08, 6.5				

Betadine	21.31	18.33	15.76	18.44	19.68	17.4	13.75	18.7	19.73	18.7	15.86	18
Sappan	9.92	14.8	12.43	14.24	10.71	11.79	15.02	11.55	12.51	9.94	11.96	9
Turme	6.95	6.5	6.5	6.5	6.5	8.98	6.5	6.5	6.5	6.65	6.5	
Lem	7.79	7.78	7.7	6.5	7.74	7.22	6.57	7.3	7.83	6.83	7.36	6
tea	6.79	6.8	6.5	7.2	6.5	6.9	7.24	6.53	6.54	6.5	7.08	
		1	2	3	4	5	6	7	8	9		
Sappan + Turmeric	10.47, 12.08	10.13, 10.42	8.79, 9.52	12.55, 7.76	16.65, 10.9	14.33, 12.03	14.9, 7.9	9.14, 9.44	9, 6.8			
Sappan + Turmeric + Lemon myrtle	12.6, 16.57	18.95, 12.75	11.6, 26.26	14.28, 10.13	14.27, 17.13	16.61, 12.73	12.61, 18.97	22.26, 14.9	12.86, 12.58			

OSA RISK ASSESSMENT FORM

for all entries in (✓) ☐ Models & Inventions and ☐ Science Investigations

This must be included with your report, logbook or entry. One form per entry.

STUDENT(S) NAME: Napon (Beacon) Rodjanathum ID:

SCHOOL: Glenunga International High School

Activity: Give a brief outline of what you are planning to do.

Explore & compare the effectiveness of differeat Thai and Australian herbs at inhibiting the growth of Staphylococcus aureas This study will include the extraction of active compounds from samples into an ethanol solution. Extract efficacy will be measured the mean diameter of Zone of Inhibition (measured in mm) for each herb

Are there possible risks? Consider the following:

- Chemical risks: Are you using chemicals? If so, check with your teacher that any chemicals to be used are on the approved list for schools. Check the safety requirements for their use, such as eye protection and eyewash facilities, availability of running water, use of gloves, a well-ventilated area or fume cupboard.
- Thermal risks: Are you heating things? Could you be burnt?
- Biological risks: Are you working with micro-organisms such as mould and bacteria?
- Sharps risks: Are you cutting things, and is there a risk of injury from sharp objects?
- Electrical risks: Are you using mains (240 volt) electricity? How will you make sure that this is safe? Could you use a battery instead? **Only batteries can be used for Models & Inventions entries*
- Radiation risks: Does your entry use potentially harmful radiation such as UV or lasers?
- Other hazards.

Also, if you are using other people as subjects in an investigation you must get them to sign a note consenting to be part of your experiment.

Risks	How I will control / manage the risk
Infection from S. aureas	Work environment cleaned before & after experiment. Gloves, goggles, face mask and lab apron worn. Bunsen burner used to sterilise environment
Flammability of 70% ethanol solution Please turn over	ethanol stocked beakers kept a minimum of 2 meters away from open flame, fire extinguisher kept nearby. All PPE listed above worn

(Attach another sheet if needed.)

Risk Assessment indicates that this activity can be safely carried out

RISK ASSESSMENT COMPLETED BY (student name(s)): Napon Rodjanathum

SIGNATURE(S): Napon R.

☑ By ticking this box, I/we state that my/our project adheres to the listed criteria for this Category.

TEACHER'S NAME: K. TOLIJ

SIGNATURE: DATE: 15/06/2026

Risk	Management
Chemical irritation	Listed PPE worn, forceps used to handle discs & avoid skin contact with liquid
Use of Bunsen burner (fire burns)	Leave burner on safety flame when not being used for sterilisation purposes
Cuts from glassware	Inspect equipment for cracks before use, use a dustpan & brush to clear shards in case of breakage
Biohazardous waste	Used contaminated equipment will be separated, put in a biohazard bag & handed to school lab staff for cleaning/disposal. Throughly clean work environment afterwards