



Assessment of the Antibacterial, Antioxidant, and Phytochemical Potential of *Fernandoa Adenophylla*, *Curcuma Caesia*, and *Melaleuca Alternifolia*

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KEYWORDS

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ABSTRACT:

This study evaluates the antibacterial, antioxidant, and phytochemical profiles of three medicinal plants: Karenwood (*Fernandoa adenophylla*), Black Turmeric (*Curcuma caesia*), and Tea Tree (*Melaleuca alternifolia*) oil. Extracts were prepared using various solvents (aqueous, n-hexane, and ethanol) and subjected to preliminary phytochemical screening, quantitative estimation of total phenolics and flavonoids, and in vitro antioxidant assays including DPPH, ABTS, and hydrogen peroxide scavenging. Antibacterial activity was assessed against six bacterial strains, including *Staphylococcus aureus* and *Escherichia coli*, using the agar-cup plate method. Phytochemical analysis revealed diverse secondary metabolites across the species, including flavonoids, tannins, terpenoids, and alkaloids. Quantitative results indicated that the ethanolic extract of *C. caesia* (CCE3) contained the highest total phenolic content ($308.570 \pm 6.088 \mu\text{g/ml}$) and demonstrated superior antioxidant potency in DPPH and ABTS assays. While Tea Tree Oil (TTO) exhibited the highest total flavonoid content ($840 \pm 0.025 \mu\text{g/ml}$), it also emerged as the most potent antimicrobial agent, showing the largest Zones of Inhibition (ZOI) against *P. aeruginosa* (15 mm) and *E. coli* (14 mm). These findings reinforce the therapeutic potential of *C. caesia* and *M. alternifolia* as natural sources of antioxidants and antimicrobials, driven by their rich phenolic and terpenoid profiles.

1. Introduction

The increasing prevalence of antimicrobial resistance and the significant contribution of oxidative stress to chronic diseases have prompted intensified efforts to identify novel therapeutic agents from natural sources. Plants, which serve as abundant sources of secondary metabolites known as phytochemicals, have played a central role in traditional medicine for centuries and are currently the focus of renewed scientific investigation.¹ Phytochemicals, including flavonoids, tannins, alkaloids, and terpenoids, are synthesized by plants primarily for defense and contribute to various biological activities relevant to human health, particularly

antimicrobial and antioxidant effects.² Systematic assessment of crude extracts and isolated compounds from botanicals is essential for validating traditional medicinal uses, elucidating mechanisms of action, and supporting the development of new, safe, and effective natural therapeutics.³ This study evaluates the antibacterial, antioxidant, and phytochemical profiles of three medicinal plants: *Fernandoa adenophylla*, *Curcuma caesia*, and *Melaleuca alternifolia* oil. *Fernandoa adenophylla* (Karenwood) *Fernandoa adenophylla* (family *Bignoniaceae*) (Figure 1) is a woody medicinal plant traditionally utilized in South and Southeast Asia to treat skin disorders, urinary tract infections, diarrhea, and diabetes.^{4,5}



- **Phytochemicals:** *Fernandoa adenophylla* contains abundant secondary metabolites, including naphthoquinones such as lapachol and triterpenoids like ursolic acid. It also possesses flavonoids, tannins, and alkaloids, similar to the other selected plants. Preliminary screening indicates the presence of comparable phytochemical groups across these species, although the specific compounds vary.^{5,6}
- **Antimicrobial Potential:** Crude extracts from the leaves and heartwood of *Fernandoa adenophylla* exhibit significant antibacterial and antifungal activities, including efficacy against multidrug-resistant strains. Antioxidant activity is comparable to that of other two drugs but the primary active compounds are naphthoquinones in *F. adenophylla*, as compared to phenolics in other two herbs.^{5,7}
- **Antioxidant Potential:** Extracts of *F. adenophylla* demonstrate notable antioxidant properties. The predominant phytochemicals responsible for this activity are naphthoquinones and triterpenoids in Karenwood,^{6,7}



Figure 1 Fresh Leaves of *Fernandoa adenophylla*

Curcuma Caesia

Curcuma caesia (family *Zingiberaceae*), (**Figure 2**) commonly known as Black Turmeric, is a perennial herb highly valued in Ayurveda for its bluish-black rhizome. It is traditionally used to treat asthma, cancer, and various metabolic and inflammatory disorders.⁸

- **Phytochemicals:** The distinctive color and medicinal properties of *C. caesia* are attributed to its rich phytochemical profile, which includes essential oils, curcuminoids, and anthocyanins. It is characterized by high levels of curcuminoids and anthocyanins, contributing

to its unique coloration and potential health benefits.^{9,10}

- **Antimicrobial Potential:** The essential oil and extracts of rhizome exhibit strong antimicrobial activity against bacteria such as *Staphylococcus aureus* and *Escherichia coli*, as well as fungi including *Aspergillus* species. This activity supports its traditional application in the treatment of wounds and infections.¹¹



Figure 2 Sliced *Curcuma caesia* Rhizomes. **Antioxidant Potential:** *C. caesia* demonstrates notable antioxidant capacity, primarily due to its curcuminoid and phenolic content. These compounds act as potent free-radical scavengers, indicating the plant's potential for managing oxidative damage.^{10,11}

Melaleuca alternifolia Oil (Tea Tree Oil)

Melaleuca alternifolia, native to Australia, is the source of Tea Tree Oil (TTO), a widely studied essential oil. Traditionally, TTO is valued for its antiseptic and anti-inflammatory properties.¹¹

- **Phytochemicals:** Tea Tree Oil comprises approximately 100 compounds, predominantly monoterpenes and sesquiterpenes, with terpinen-4-ol as the principal active component. Unlike *F. adenophylla* and *C. caesia*, which are characterized by naphthoquinones and curcuminoids respectively, the therapeutic effects of TTO are primarily attributed to its distinctive terpene composition.^{12,13}
- **Antimicrobial Potential:** Tea Tree Oil demonstrates broad-spectrum antimicrobial activity, primarily due to terpinen-4-ol.¹¹
- **Antioxidant Potential:** Although Tea Tree Oil is primarily recognized for its antimicrobial properties,



it also exhibits antioxidant activity attributable to its unique terpenic composition.¹¹

2. Material and Methods

Authentication: Both drugs *Fernandoa adenophylla* and *Curcuma caesia* were collected from Local market

in June/ 2023 and authenticated at PG Department of Dravya Guna, Uttarakhand Ayurvedic University, Rishikul Campus, Haridwar (No: DG/RAC/ UAU-342 dated 25/09/2023) by Dr DC Singh. *Melaleuca alternifolia* oil was purchased from local market.

Table 1: Plants Used In the Study

Name	Scientific Name	Part used
Karen wood	<i>Fernandoa adenophylla</i> (Bignoniaceae)	Leaves
Kali Haldi	<i>Curcuma caesia</i> (Zingiberaceae)	Rhizome
Tea Tree	<i>Melaleuca alternifolia</i> (Myrtaceae)	Oil

Extraction: The preparation and extraction of three plant materials: *F adenophylla* leaves, *C caesia* rhizomes, and *M alternifolia* oil.

F. adenophylla leaves were dried at for six days, ground to a powder (150 g per solvent), and extracted via maceration in three solvents: aqueous, n-hexane, and ethanol. The mixtures were soaked for 48 hours with intermittent shaking, filtered through coarse cloth and a Buchner funnel, and the solvent was recollected using a rotary evaporator at 20°C.⁷

C. caesia rhizomes were shade-dried for three weeks and powdered. Extraction was performed using different ratios of ethanol/water (of 100/0, 50/50, and 0/100) by immersing of powder in of solvent. The mixture was agitated for 72 hours in a rotary shaker at 25 °C . Filtration was done thrice over 24-hour intervals using Whatman filter paper No. 1. Solvents were removed at using a rotary evaporator, and subsequent freeze-drying to remove excess solvent.¹¹

In contrast, the *Melaleuca alternifolia* (Tea Tree Oil or TTO) leaves oil was simply purchased and stored, requiring no *in-house* extraction procedure.¹²

All resulting extracts FAE, FAH, FAEE, CCE1, CCE2, CCE3, and TTO of *F adenophylla* leaves, *C caesia* rhizomes, and *M alternifolia* oil respectively were stored at 4° C in amber coloured glass vials.

The extracts were subjected to preliminary phytochemicals testing and in-vitro antioxidant activity to select the most active fraction for subsequent antimicrobial activity based on these results

Preliminary Phytochemical Testing

Qualitative analysis was conducted to determine the presence of major secondary metabolites, including phenols, flavonoids, saponins, tannins, alkaloids, terpenoids, coumarins, anthocyanins, and anthraquinones, using standard chemical testing protocols as per Harborne.¹⁶

Calculating the Total Phenolic Content

Singleton and Rossi's (1965) method was used to figure out the total phenolic content. We used tannic acid as a standard to figure out how much phenolic acid was in FAEE (*Fernandoa adenophylla* ethanolic extract), CCE2 (*Curcuma caesia* 50/50 ethanol/water extract), CCE3 (*Curcuma caesia* ethanolic extract), and TTO (*Melaleuca alternifolia* oil). We used the Folin-Ciocalteu reagent method and spectrophotometric analysis to determine the amount of phenolic compounds in plant extracts. We used different amounts of tannic acid, mixed 5 mL of Folin-Ciocalteu reagent, added 4 mL of sodium carbonate, and then vortexed all the test tubes for 50 seconds. A water bath at 40°C was used for 30 minutes to hold all of the test tubes. We used different amounts of tannic acid as a reference to determine the total phenolic content of the plant extract. Using a UV-VIS spectrophotometer (Elico, India), we found that the color developed at 680 nm. We used absorbance measurements to measure phenols in some plant extracts. We then compared them to a standard curve for tannic acid and presented the results as milligrams of tannic acid per gram of extract.¹⁷



Total Amount of Flavonoids

Using the aluminium chloride method (Zhang et al., 2011), we determined the amount of flavonoid in the sample. To start, 500 μ l of plant extract (FAEE, CCE2, CCE3, and TTO) and 300 μ l of 5% NaNO₂ were mixed together for 10 seconds and then left at room temperature for 5 minutes. After that, 300 μ L of 10% AlCl₃, 2 mL of 1 M NaOH, and 1.9 mL of distilled water were added. Before measuring absorbance at 510 nm, the reaction mixture was vortexed for 10 seconds. Quercitrin hydrate (1 mg/mL) was used as the standard, and the concentrations ranged from 20 to 100 μ g/mL. We used the Quercitrin hydrate standard curve to figure out the total flavonoid concentration in the test sample (QE mg/g).¹⁸

In vitro antioxidant tests

Several techniques are used to assess antioxidant activity, including in vitro antioxidant screening. Since oxidative reactions limit accurate measurement of an antioxidant's total activity, this study employed three methods to evaluate total antioxidant activity: DPPH free radical scavenging, ABTS free radical scavenging, and hydrogen peroxide scavenging assays.¹⁹

DPPH Radical Scavenging Assay (Gyamfi & Aniya, 2002)

We used Gyamfi and Aniya's (2002) method to determine how effectively the plant extracts (FAEE, CCE2, CCE3, and TTO) could eliminate free radicals. We mixed DPPH, a stable free radical, with ethanol to make a 0.1 mM solution. We added the extract to 3.0 mL of a 0.1 mM ethanolic DPPH solution. After 10 minutes, the DPPH absorbance at 517 nm went down. We used BHT (Butylated hydroxy toluene) (1–5 mM) as a positive control and pure ethanol as a blank.

We used the following formula to figure out the DPPH radical scavenging activity.

To find the radical scavenging activity, use the formula $(A_0 - A_1) \times 100 / A_0$, where A₀ is the absorbance of the control sample and A₁ is the absorbance of the test sample.²⁰

ABTS Radical Scavenging Assay (Re Et AL, 1999)

The ABTS assay reliably evaluates antioxidant activity for both hydrogen-donating and chain-breaking antioxidants. To prepare ABTS solution, 5 mL of 14 mM ABTS was combined with 5 mL of 4.9 mM potassium

persulfate (K₂S₂O₈) and stored in the dark at room temperature (25 \pm 1 $^{\circ}$ C) for 16 hours. The addition of methanol adjusted the solution's absorbance to 0.700 \pm 0.02 at 734 nm. The test used this solution as the medium for the antioxidant assay. Each reaction mixture consisted of 950 μ L of ABTS solution and 50 μ L of either the standard or extract sample. After vortexing for 10 seconds, absorbance at 734 nm was measured at 6 minutes using a UV-Visible Spectrophotometer (Shimadzu UV-1800, Kyoto, Japan) and compared to the control. A calibration curve for vitamin C was created by plotting the percentage inhibition at concentrations ranging from 5 to 100 μ g mL⁻¹. The ABTS radical inhibition was calculated using the same formula as the DPPH assay. Results were expressed as milligrams of Vitamin C Equivalent Antioxidant Capacity (VCEAC) per gram of extract.²¹

H₂O₂ Exhibited Radical Scavenging Activity (Kesor Et AL, (2012)

A phosphate buffer (pH 7.4) with H₂O₂ (40 mM) was prepared. Plant extracts (FAEE, CCE2, CCE3, and TTO, 100 μ g/ml) were introduced into 3.4 ml of phosphate buffer and 0.6 ml of H₂O₂. Reaction mixture absorbance was read at 230 nm. The blank solution contained phosphate buffer without H₂O₂. The DPPH assay formula was used to quantify the scavenging efficiency of the water extract and standard compound against hydrogen peroxide.²²

Antimicrobial Studies

Preparation of Extract solution, positive and negative control

The preparation of extract solution refers to Benhabiles et al. (2012). Crude extracts were dissolved in (Dimethyl sulfoxide) DMSO to obtain a solution of 25 μ g/ml concentrations. DMSO 25 mL. The positive control antibiotic used is ampicillin. A total of 10 mg of ampicillin powder was weighed and then dissolved with 1 mL of distilled water. The negative control used the same solvent as extract, 1% DMSO.²³

Antimicrobial activity

The antibacterial activity of extracts was determined by agar-cup plate method. Bacteria (*Micrococcus luteus* (MTCC 106), *Brevibacterium epidermidis* (MTCC9884), *Propionibacterium acnes* (MTCC 1951),



Escherichia coli (MTCC2401), *Pseudomonas aeruginosa* (MTCC2453) and *Staphylococcus aureus* (MTCC902) and incubated overnight at 37°C to adjust the turbidity to 0.5 McFarland standards giving a final inoculum of 1.5×10^8 CFU/ml.

The standardized cell suspensions were spread on an agar media (Merck). Sterile 6mm diameter of corn borer was used to bore wells into the agar plate. Tetracycline (10mg/ml) was used as a positive control and DMSO as a negative control.

Anaerobic bacteria will be cultured in Robertson cooked meat (RCM) broth. MHA plate was

lawn cultured with standardized microbial culture broth. The plates were then hatched at 37°C for 48 hrs under anaerobic conditions in anaerobic container (Hi-Media) with gas pack and pointer tablet and jug was kept in hatchery for 48 h at $35.5 \pm 1^\circ\text{C}$. Anaerobic gas pack is used in anaerobic jug (Figure 3) was utilized to keep up and check condition, also anaerobic tablet having pink shading is added, to visually check anaerobic conditions (but in presence of oxygen in the jug the unique pink shading changes to purplish-blue).²⁴



Figure 3 Anaerobic chamber

Standard drug, solvent and extracts solution was allowed to diffuse for about 30 minutes at room temperature and incubated for 18-24 hours at 37°C. After incubation, plates were observed for the formation of a clear zone around the well which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition (ZOI) was observed and measured in mm. The experiments repeated three times.²⁵

Statistical Analyses

Results for each experiment were determined using data obtained in triplicate with three independent assay repeats.²⁴

3. Results

Plant Extracts

The extracts of all the selected plants were prepared and evaluated for percentage yield of each of the fractions. The details are shared in Table 2.

Table 2 Percentage Yield of Each of the Fractions

Plant	Extract Code	Yield (%)
<i>F. adenophylla</i>	FAE	10
	FAH	8
	FAEE	11
<i>C. caesia</i>	CCE1	7



	CCE2	10
	CCE3	12
<i>M. alternifolia</i>	TTO	100

Preliminary Phytochemical Evaluation of the Extracts

Phytochemical analysis of extract(s) of *F. adenophylla*, *C. caesia*, and *M. alternifolia* (TTO) reveals the presence

of different phytoconstituents. The results tabulated in Table 3 was the basis for selection of FAEE, CCE2, CCE3 and TTO.

Table 3: Preliminary Phytochemical Evaluation of Extracts

Phytoconstituent	Extracts						
	F. adenophylla			C caesia			M. alternifolia
	FAE	FAH	FAEE	CCE1	CCE2	CCE	TTO
Alkaloids	--	--	+	--	--	+	--
Anthraquinones	+	+	++	-	+	++	-
Anthocyanin	+	+	++	+	++	+	+
Coumarins	+	+	+	+	+	++	+
Flavonoids	+	++	++	+	+	++	+
Tannins	-	++	+	+	+	++	-
Steroids	-	++	+	+	++	+	++
Saponins	-	++	++	+	+	++	++
Terpenoids	-	+	+	+	++	+	+
Phenols	-	++	++	+	+	+	++
vitamin C	+	+	++	+	+	+	++
Quinones	-	+	+	+	+	+	++
phytosterol	-	++	++	+	+	++	++
Proteins	-+	+	+	+	+	+	-

The results (Table 4) show CCE3 contains the highest total phenolic content ($308.570 \pm 6.088 \mu\text{g/ml}$), while TTO exhibits the highest total flavonoid content 308.570

$\pm 6.088 \mu\text{g/ml}$). There is a general trend where extracts with higher phenolic content (CCE3 and TTO) also show robust results in biological activity assays.

Table 4 Estimation of Total Phenolic Content and Flavonoid Content

Extract	PHENOL ($\mu\text{g/ml}$)s	FLAVONOID ($\mu\text{g/ml}$)
FAEE	237.827 ± 10.130	644 ± 0.011
CCE2	149.380 ± 6.088	521 ± 0.032
CCE3	308.570 ± 6.088	750 ± 0.061
TTO	296.860 ± 6.088	840 ± 0.025

Free Radical Scavenging: Antioxidant results (Table 5) indicated CCE3 and TTO demonstrated superior scavenging abilities. Lower numerical values in these assays (when compared to standard equivalents) often indicate higher potency; CCE3 showed the most competitive DPPH and ABTS values (14.00 ± 3 and

13.00 ± 5 respectively). CCE3 significantly outperformed the others with a value of 13.00 ± 5 , indicating a strong ability to neutralize hydroxyl radicals. CCE2 showed the least antioxidant potential across all three assays, correlating with its low phenolic profile.



Table 5 Dpph, Abts and H2o2 Anti-Oxidant Activity Results

Extract	DPPH (BHT equivalent)	ABTS (VCEAC)	H ₂ O ₂ (BHT equivalent)
FAEE	17.00±2	14.00±1	20.38 ±0.41
CCE2	20.00±3	19.00±4	8.85±0.32
CCE3	14.00 ±3	13.00 ±5	13.00 ±5
TTO	16.00 ±3	10.00 ±1	20.38±0.62

Antimicrobial activity of the selected extracts: The extracts were tested against a variety of Gram-positive and Gram-negative bacteria.

TTO (Tea Tree Oil) emerged as the most potent antimicrobial agent among the test extracts, showing the largest Zones of Inhibition (ZOI) across all strains, particularly against *P. aeruginosa* (15 mm) and *E. coli* (14 mm).

CCE3 (*Curcuma Cassia* Extract) followed closely, showing consistent activity (11–13 mm) against all tested pathogens, including skin-related bacteria like *P. acnes* and *B. epidermidis*.

While all extracts showed measurable activity, they remained less potent than the standard drug, which maintained higher ZOI (15–19 mm) and DMSO (the negative control) showed no activity, confirming that the results are due to the extracts themselves.

Table 6 In-Vitro Antimicrobial Activity of the Lant Extracts

Drug	Zone of Inhibition (in mm)*					
	M. luteus	B. epidermidis	P. acnes	E. coli	P. aeruginosa	S. Aureus
FAEE	10	11	12	10	11	11
CCE2	9	6	7	8	10	6
CCE3	12	13	12	11	12	12
TTO	13	12	12	14	15	14
Standard	16	15	18	19	17	18
DMSO	--	--	--	--	--	--

4. Discussion and Conclusion

The present study's findings on the plant extracts from *F. adenophylla* (FAEE), *C. caesia* (CCE2 and CCE3), and *M. alternifolia* (TTO) align well with existing literature on their phytochemical profiles, antioxidant capacities, and antimicrobial activities, while also highlighting some notable variations likely attributable to extraction methods, plant parts, geographical origins, and chemotype differences.¹

Phytochemical Composition and Phenolic/Flavonoid Content

The high total phenolic content in CCE3 (308.570 ± 6.088 µg/ml) and equivalent flavonoid levels in TTO are consistent with reports on *C. caesia* (black turmeric) and TTO. Literature indicates that *C. caesia* rhizome and leaf extracts often yield phenolic contents ranging from approximately 2–109 mg GAE/g extract (or equivalents

like 22.5 µg GAE/µL in essential oils), with higher values in polar solvent extracts (e.g., methanol or ethanol). Flavonoid contents in *C. caesia* leaf oil have been reported around 11.36 mg/mL, supporting the robust phenolic/flavonoid profiles observed here.¹⁰ TTO, primarily composed of terpenoids like terpinen-4-ol (30–40%), typically shows lower measurable phenolics compared to phenolic-rich extracts but contributes flavonoids and other antioxidants.¹⁴ Limited data on *F. adenophylla* suggest steroidal alkaloids and other metabolites, which may explain FAEE's comparatively lower phenolic emphasis in the study.⁷

Antioxidant Activity The superior free radical scavenging of CCE3 and TTO, with competitive DPPH (14.00 ± 3 µg/ml) and ABTS (13.00 ± 5 µg/ml) values for CCE3, corroborates literature trends. For *C. caesia*, DPPH IC₅₀ values often range from 1–48 µg/ml in essential oils or extracts, frequently outperforming



standards like ascorbic acid due to high phenolics and compounds like camphor and eucalyptol. TTO exhibits DPPH IC₅₀ values around 12.5–48 µg/ml, attributed to terpinen-4-ol and monoterpenes, aligning closely with the current results. The correlation between elevated phenolics/flavonoids and enhanced antioxidant potency is well-established in both species, with polar extracts (like CCE3) yielding stronger activity than less polar ones (e.g., CCE2). This supports the observed trend and suggests CCE3 and TTO as promising natural antioxidants comparable to or exceeding some standards.¹⁷

Antimicrobial Activity TTO's broad-spectrum potency, with large zones of inhibition (e.g., 15 mm against *P. aeruginosa* and 14 mm against *E. coli*), matches extensive literature documenting its efficacy against Gram-positive and Gram-negative bacteria, including *P. aeruginosa*, *E. coli*, and skin pathogens like *P. acnes* (often with MICs of 0.05–1.25% v/v and zones of 10–30 mm). Its mechanism involves membrane disruption by terpinen-4-ol. CCE3's consistent activity (11–13 mm ZOI) against tested strains, including *P. acnes* and *B. epidermidis*, aligns with *C. caesia* reports of antimicrobial effects against *S. aureus*, *E. coli*, and others, driven by phenolics, terpenoids, and curcuminoids.²⁴ While both extracts were less potent than synthetic standards (ZOI 15–19 mm), their measurable activity and lack of DMSO interference confirm intrinsic efficacy, consistent with prior studies on essential oils and rhizome extracts.

Overall, the results reinforce the therapeutic potential of CCE3 and TTO as natural sources of antioxidants and antimicrobials, in line with literature emphasizing their phenolic/terpenoid-driven bioactivities. Variations in potency may stem from specific extraction conditions or regional chemotypes. These extracts could complement conventional therapies, particularly against resistant pathogens and oxidative stress-related conditions. Further *in vivo* studies and standardization are recommended to advance their pharmaceutical applications.

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