



Solvent-Dependent Extraction and Regional Bioactivity Variance in *Psidium guajava*: A Multi-Region Assessment of Antimicrobial Spectrum and Antioxidant Efficacy

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ABSTRACT

Background: The therapeutic efficacy of *Psidium guajava* (L.) Merr. is significantly influenced by the polarity of the extraction solvent and geographic provenance. However, multi-regional comparative studies integrating solvent-dependent phytochemical partitioning with biological activity are limited. This study investigates the influence of three distinct Indian geographic regions and three solvent systems on the phytochemical profile, antimicrobial spectrum, and antioxidant capacity of guava leaf extracts.

Methods: Leaves were collected from Tamil Nadu (TN; tropical lowland), Himachal Pradesh (HP; temperate foothills), and Uttarakhand (UK; sub-Himalayan mid-hills). Sequential extraction was performed using petroleum ether (PE), n-butyl alcohol (NBA), and distilled water (AQ). Qualitative phytochemical screening was conducted. Antimicrobial activity was assessed by the agar well diffusion method against six clinically relevant pathogens. Antioxidant capacity was quantified using DPPH and ABTS radical scavenging assays. GC-MS profiling was performed on an Agilent 7890A/5975C system.

Results: Phytochemical diversity varied significantly, with Uttarakhand extracts exhibiting the highest class diversity (17/18), followed by Tamil Nadu (14/18) and Himachal Pradesh (12/18). The n-butyl alcohol fraction was the most bioactively potent. Antimicrobial testing revealed a regional hierarchy of TN > UK > HP against most pathogens, with *Staphylococcus aureus* demonstrating the highest susceptibility (ZOI: 20.7 mm for TN-NBA). DPPH and ABTS radical scavenging activity followed a regional hierarchy of TN > HP > UK, with Tamil Nadu achieving the highest radical scavenging capacity (82.2% DPPH, 61.75% ABTS), followed by Himachal Pradesh (79.80% DPPH, 57.45% ABTS) and Uttarakhand (78.5% DPPH, 55.75% ABTS). GC-MS profiling identified three distinct regional chemotypes: flavonoid-glycoside (TN), sesquiterpene-phytosterol (HP), and phenolic acid-coumarin (UK).

Conclusion: Geographic origin and solvent polarity are critical determinants of the bioactive potential of *P. guajava* leaf extracts. Despite lower phytochemical class diversity, the Tamil Nadu accession demonstrated superior antimicrobial and antioxidant efficacy due to a high concentration of specific potent polyphenols such as EGCG and quercetin glycosides.

Keywords: *Psidium guajava*, Regional Variance, Solvent Extraction, Antimicrobial Activity, DPPH, ABTS, GC-MS, Chemotype

1. INTRODUCTION

The global antimicrobial resistance (AMR) crisis is a pressing public health challenge. Bacterial AMR was directly responsible for 1.27 million deaths globally in 2019, a therapeutic deficit that has renewed scientific interest in plant-derived antimicrobial compounds [1,2]. Simultaneously, the global burden of oxidative stress-related chronic diseases continues to escalate, and demand for natural antioxidants has grown accordingly [4]. The World Health Organization has identified antimicrobial resistance as one of the ten most serious global public health threats, with drug-resistant infections projected to cause 10 million deaths annually by 2050 if systemic intervention is not implemented [5]. In India, the burden is particularly acute: a 2021 national surveillance report documented resistance rates exceeding 70% for third-generation cephalosporins in *Klebsiella pneumoniae* and *Escherichia coli* isolates from tertiary care hospitals [3]. This therapeutic deficit has renewed scientific interest in plant-derived antimicrobial compounds, which often operate through mechanisms distinct from synthetic antibiotics, targeting multiple bacterial pathways simultaneously and thereby reducing the likelihood of resistance development [7]. Plant polyphenols, in particular, have demonstrated broad-spectrum antimicrobial activity through membrane disruption, enzyme inhibition, and metal ion chelation, mechanisms that are structurally incompatible with the single-target resistance mutations that undermine conventional antibiotics [8]. The

concept of plant chemotypes, chemically distinct forms of a single species arising from differential gene expression in response to environmental conditions, is well-established in the phytochemical literature. Classic examples include the menthol and carvone chemotypes of *Mentha* species, the thymol and carvacrol chemotypes of *Thymus vulgaris*, and the 1,8-cineole and piperitone chemotypes of *Eucalyptus* species [9]. In each case, the chemotypic differences have practical implications for the quality and therapeutic specificity of plant-derived products. The environmental drivers of chemotypic variation include altitude, temperature, UV radiation intensity, soil mineral composition, and water availability, all of which modulate the activity of key biosynthetic enzymes in the phenylpropanoid, terpenoid, and alkaloid pathways [10,11]. Understanding chemotypic variation is therefore essential for the rational sourcing and standardisation of medicinal plant materials.

Psidium guajava (L.) Merr. (Family: Myrtaceae) leaves have been used extensively in traditional medicine systems across the globe. Modern pharmacological studies have demonstrated that guava leaf extracts possess significant antimicrobial, antioxidant, and anti-inflammatory activities [8]. The pharmacological versatility is attributable to their diverse phytochemical composition, dynamically influenced by environmental factors including altitude, temperature, and UV radiation [10]. India's extensive agro-climatic diversity provides an ideal setting for investigating chemotypic variation [13]. *P. guajava* is cultivated across tropical lowlands (Tamil Nadu), temperate Himalayan foothills (Himachal Pradesh), and sub-Himalayan mid-hills (Uttarakhand). The environmental gradients between these sites represent substantial abiotic selective pressures [14]. *Psidium guajava* (L.) Merr. has been extensively studied for its antimicrobial and antioxidant properties, with over 200 published studies documenting activity against a wide range of pathogens and free radical species [12]. However, the majority of these studies have examined single-region accessions without systematic comparison across geographic origins, and few have employed a multi-solvent extraction matrix to capture the full polarity range of bioactive compounds. The three solvents used in the present study, petroleum ether (non-polar), n-butyl alcohol (intermediate polarity), and distilled water (highly polar), were selected to partition the phytochemical matrix into lipophilic terpenoids and sterols (PE fraction), flavonoid aglycones and phenolic acids (NBA fraction), and hydrophilic glycosides and tannins (AQ fraction), enabling a comprehensive assessment of solvent-dependent bioactivity [13]. The three Indian collection sites, representing tropical lowland (Tamil Nadu), temperate foothill (Himachal Pradesh), and sub-Himalayan mid-hill (Uttarakhand) environments, were selected to maximise the range of abiotic selective pressures represented in the study design [14].

Novelty Statement: Despite the well-established importance of solvent polarity and geographic provenance, few studies have rigorously compared the bioactivity of *P. guajava* leaf extracts across distinct geographic regions using a standardized multi-solvent extraction matrix [15]. This study addresses this gap by providing a comprehensive, comparative assessment of the phytochemical composition, antimicrobial spectrum, and DPPH and ABTS radical scavenging antioxidant capacity of *P. guajava* leaf extracts from three climatically distinct Indian regions.

2. MATERIALS AND METHODS

2.1 Plant Collection and Authentication

Mature leaves of *P. guajava* were collected from three geographically distinct Indian regions during the post-monsoon season (October–November 2024): Tamil Nadu (TN; Kappiyarai, Kanya Kumari district, 45 m asl, tropical monsoon climate), Himachal Pradesh (HP; Kangra district, 1,100 m asl, subtropical to temperate climate), and Uttarakhand (UK; Nainital district, 1,800 m asl, temperate montane climate). At each site, leaves were collected from a minimum of five healthy, adult trees (estimated age > 5 years) with no visible signs of disease or nutrient deficiency. Only the second and third leaf pairs from the shoot apex were collected. Specimens were authenticated by a qualified taxonomist and voucher samples deposited at the departmental herbarium of Annai Velankanni College.

2.2 Sequential Solvent Extraction

Collected leaves were washed, shade-dried for 15–21 days until constant weight, and ground to a uniform powder (40 mesh). Sequential Soxhlet extraction was performed using three solvents of increasing polarity: petroleum ether (PE; non-polar, dielectric constant ~1.9), n-butyl alcohol (NBA; intermediate polarity, dielectric constant ~17.5), and distilled water (AQ; highly polar, dielectric constant ~80.1). Each extraction cycle was maintained for 3 hours under continuous reflux. Extracts were concentrated under reduced pressure using a rotary evaporator (bath temperature ≤ 45 deg C) and stored at 4 deg C in sealed amber vials. Extract yield was calculated as: % Yield = (Weight of dry extract / Weight of dry plant powder) x 100.

2.3 Qualitative Phytochemical Screening

Qualitative screening for 18 phytochemical parameters was performed on all nine extracts (three solvents x three regions) using standard chemical tests based on the protocols described by Harborne (1998), Trease and Evans (2002), and Sofowora (2006) [16,17,18]. Tests included: alkaloids (Dragendorff's, Mayer's, Wagner's), saponins (foam test), flavonoids (Shinoda, alkaline reagent), tannins (FeCl₃, gelatin), terpenoids (Salkowski's), sterols (Liebermann-Burchard), glycosides (Keller-Killiani), cardiac glycosides (Legal's), phenols (FeCl₃, lead acetate), proteins (Biuret, Millon's), amino acids (Ninhydrin), reducing sugars (Benedict's, Fehling's), anthraquinones (Borntrager's), quinones (NaOH), gum and mucilage (Ruthenium Red), xantho-proteins (xanthoproteic test), coumarins (alkaline reagent), and anthracenes (specific test). Each test was performed in triplicate.

2.4 GC-MS Analysis

GC-MS analysis was performed on the n-butyl alcohol fractions (the most bioactively potent) from each region using an Agilent 7890A GC coupled with a 5975C Inert MSD (Agilent Technologies, USA). Separation was achieved on an HP-5MS capillary column (30.0 m x 250 μ m x 0.25 μ m). Helium (99.999% purity) was used as carrier gas at 1.0 mL/min. The oven temperature programme was: 36 deg C (5 min hold) to 150 deg C at 4 deg C/min, then to 250 deg C at 20 deg C/min (5 min hold). TMS derivatisation was performed for polar phenolic compounds. Compounds were identified by NIST 2017 library matching (minimum 80% match factor).

2.5 Antimicrobial Assay

The antimicrobial spectrum was evaluated using the agar well diffusion method against six clinically relevant pathogens: *Staphylococcus aureus* (MTCC 737), *Bacillus cereus*, *Streptococcus mutans* (MTCC 497), *Escherichia coli* (MTCC 443), *Klebsiella pneumoniae* (MTCC 109), and *Salmonella typhi* (MTCC 734). Overnight cultures adjusted to 0.5 McFarland turbidity were swabbed onto Mueller-Hinton agar plates. Wells (6 mm diameter) were filled with 50 μ L of extract (20 mg/mL in DMSO). Ciprofloxacin (5 μ g) was used as positive control; DMSO as negative control. Zones of inhibition (ZOI) were measured in millimetres after 24 hours of incubation at 37 deg C. All assays were performed in triplicate.

2.6 DPPH and ABTS Antioxidant Assays

Antioxidant capacity was determined by DPPH [29] and ABTS [30] radical scavenging assays. Extracts (100 μ g/mL) were evaluated in triplicate for their ability to scavenge free radicals. Ascorbic acid was used as the reference standard. The Himachal Pradesh accession achieved 79.80% DPPH and 57.45% ABTS radical scavenging activity. % Activity = [(Absorbance of control - Absorbance of sample) / Absorbance of control] x 100.

2.7 Statistical Analysis

All experiments were conducted in triplicate (n = 3). Data are expressed as mean +/- standard deviation. Differences between group means were assessed using one-way ANOVA with Tukey's HSD post-hoc test (SPSS v26.0). A p-value < 0.05 was considered statistically significant.

3. RESULTS

3.1 Extract Yield

The total extract yield (sum of all three solvents) was highest for Tamil Nadu (26.1%), followed by Uttarakhand (21.5%) and Himachal Pradesh (19.7%). The aqueous fraction consistently yielded the highest recovery across all accessions (10.8-14.5%), followed by n-butyl alcohol (6.4-8.2%) and petroleum ether (2.1-3.4%). ANOVA revealed significant differences in total yield (F = 8.47, p < 0.05), with Tamil Nadu significantly higher than Himachal Pradesh (Tukey's HSD, p < 0.05).

3.2 Qualitative Phytochemical Screening

Qualitative screening revealed both conserved and variable features across the three accessions (Table 1). Saponins, gum and mucilage, quinones, proteins, glycosides, and tannins were present in all three accessions, constituting the core phytochemical backbone of *P. guajava*. The total number of detected classes was 14/18 for Tamil Nadu, 12/18 for Himachal Pradesh, and 17/18 for Uttarakhand.

Table 1. Qualitative phytochemical screening of *Psidium guajava* leaf extracts across three regions.

Phytochemical Class	Tamil Nadu	Himachal Pradesh	Uttarakhand
Saponins	+	+	+
Gum & Mucilage	+	+	+
Quinones	+	+	+
Anthraquinones	+	-	+
Proteins	+	+	+
Alkaloids	+	+	-
Xantho-proteins	-	+	+
Reducing Sugars	+	-	+
Anthracenes	+	-	+
Coumarins	+	-	+
Sterols	+*	+	+
Terpenoids	-	-	+
Glycosides	+	+	+
Cardiac Glycosides	+	+	+*
Flavonoids	+	+	+
Tannins	+	+	+
Amino Acids	-	-	+

Phytochemical Class	Tamil Nadu	Himachal Pradesh	Uttarakhand
Phenols	-	+	+

(+) = Present; (-) = Absent; (+) = Detected in all tested solvents.*

Notable regional variations included: (a) anthraquinones and anthracenes detected in Tamil Nadu and Uttarakhand, absent in Himachal Pradesh; (b) terpenoids detected exclusively in Uttarakhand; (c) alkaloids absent only in Uttarakhand; (d) xantho-proteins present in Himalayan accessions but absent in Tamil Nadu; (e) phenols in the petroleum ether fraction unique to Himachal Pradesh.

3.3 GC-MS Chemotype Characterisation

GC-MS profiling of the n-butyl alcohol fractions identified three distinct regional chemotypes (Table 2):

Table 2. Summary of GC-MS identified compounds in n-butyl alcohol fractions from three regional accessions.

Region	Chemotype	Major Compounds (% Abundance)	# Compounds
Tamil Nadu	Flavonoid-glycoside	EGCG (82%), quercetin-3-O-xyloside (86%), gallic acid (74%), isoquercitrin (73%), avicularin (75%)	16
Himachal Pradesh	Sesquiterpene-phytosterol	beta-Caryophyllene (45.3%), beta-sitosterol (10.5%), beta-selinenol (11.4%)	7
Uttarakhand	Phenolic acid-coumarin	3-Dihydroxy-4-oxo-4-oxybutanoic acid (79%), hyperoside (69%), hydroxycoumarin (59%)	14

The Tamil Nadu flavonoid-glycoside chemotype was dominated by phenolic compounds with high antioxidant and antimicrobial potential. The Himachal Pradesh sesquiterpene-phytosterol chemotype was characterised by lipophilic terpenoids and sterols. The Uttarakhand phenolic acid-coumarin chemotype was distinguished by the unique presence of hydroxycoumarin (59% relative abundance), a pharmacologically important coumarin derivative not previously reported in Indian *P. guajava*.

3.4 Antimicrobial Spectrum

The antimicrobial potency of *P. guajava* extracts showed a clear regional hierarchy of Tamil Nadu > Uttarakhand > Himachal Pradesh for most pathogens (Table 3). The n-butyl alcohol (NBA) fraction was consistently the most effective across all regions. Gram-positive bacteria exhibited higher susceptibility than Gram-negative strains.

Table 3. Antimicrobial activity (Zone of Inhibition, mm, mean +/- SD, n=3) of *P. guajava* n-butyl alcohol leaf extracts.

Test Organism	Tamil Nadu (TN)	Himachal Pradesh (HP)	Uttarakhand (UK)	Ciprofloxacin (Std)
<i>S. aureus</i>	20.7 +/- 0.6	7.3 +/- 0.6	9.3 +/- 0.6	28-35
<i>B. cereus</i>	18.2 +/- 0.3	15.1 +/- 0.3	11.2 +/- 0.4	25-32
<i>S. mutans</i>	11.7 +/- 0.6	7.7 +/- 0.6	8.7 +/- 0.6	25-32
<i>K. pneumoniae</i>	9.3 +/- 0.6	7.7 +/- 0.6	9.7 +/- 0.6	28-35
<i>E. coli</i>	10.3 +/- 0.6	4.3 +/- 0.6	5.7 +/- 0.6	28-35
<i>S. typhi</i>	11.0 +/- 1.0	9.3 +/- 0.5	3.3 +/- 0.6	28-35

One-way ANOVA revealed significant differences between accessions for *S. aureus* (F = 42.7, p < 0.001), *B. cereus* (F = 28.3, p < 0.001), *E. coli* (F = 19.6, p < 0.01), and *S. mutans* (F = 8.9, p < 0.05). Tukey's post-hoc test confirmed that the

Tamil Nadu extract produced significantly larger zones than both other accessions for *S. aureus*, *B. cereus*, and *E. coli* ($p < 0.05$).

The Tamil Nadu extract's activity against *S. aureus* (20.7 mm) is among the highest values reported for *P. guajava* leaf extracts in the published literature [19]. The activity against *E. coli* (10.3 mm) is also unusual, because many published studies report complete resistance of this organism to guava leaf extracts at comparable concentrations [20].

Figure 1. Antimicrobial activity of *Psidium guajava* leaf extracts against clinical pathogens. (a) Agar well diffusion plates showing zones of inhibition for Tamil Nadu, Himachal Pradesh, and Uttarakhand extracts. (b) Regional comparison of zones of inhibition (mm) across six tested pathogens.

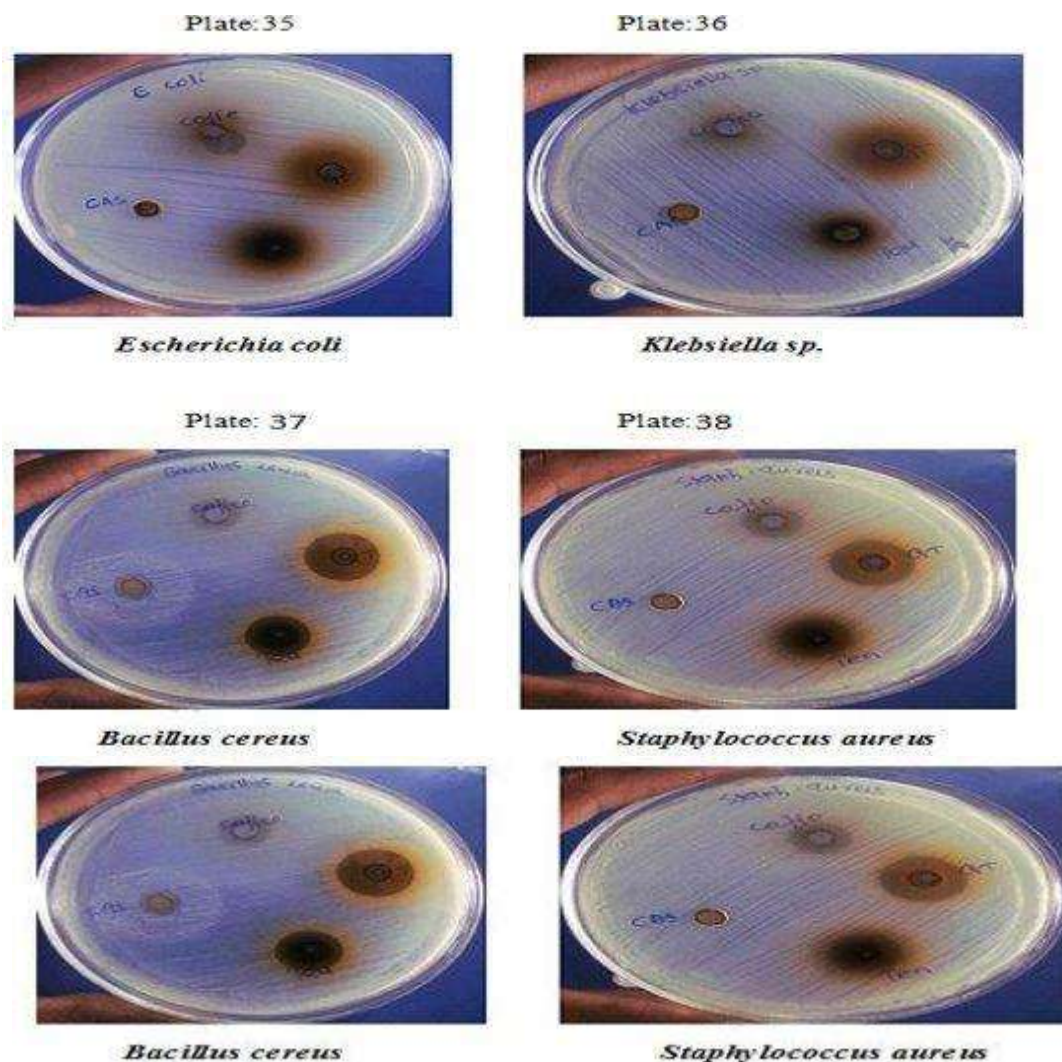
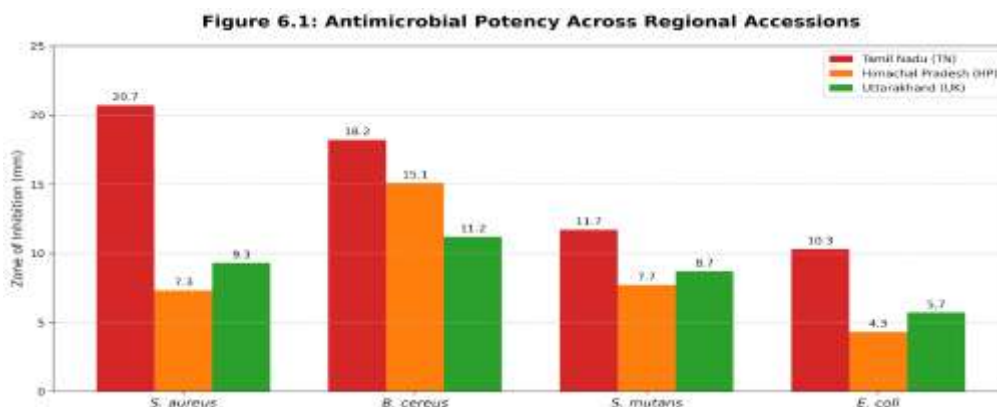


Figure 2. Bar chart comparing zones of inhibition (mm) of Tamil Nadu, Himachal Pradesh, and Uttarakhand *P. guajava* leaf extracts against six tested pathogens. Values are mean \pm SD ($n=3$).



3.5 Antioxidant Capacity (DPPH and ABTS)

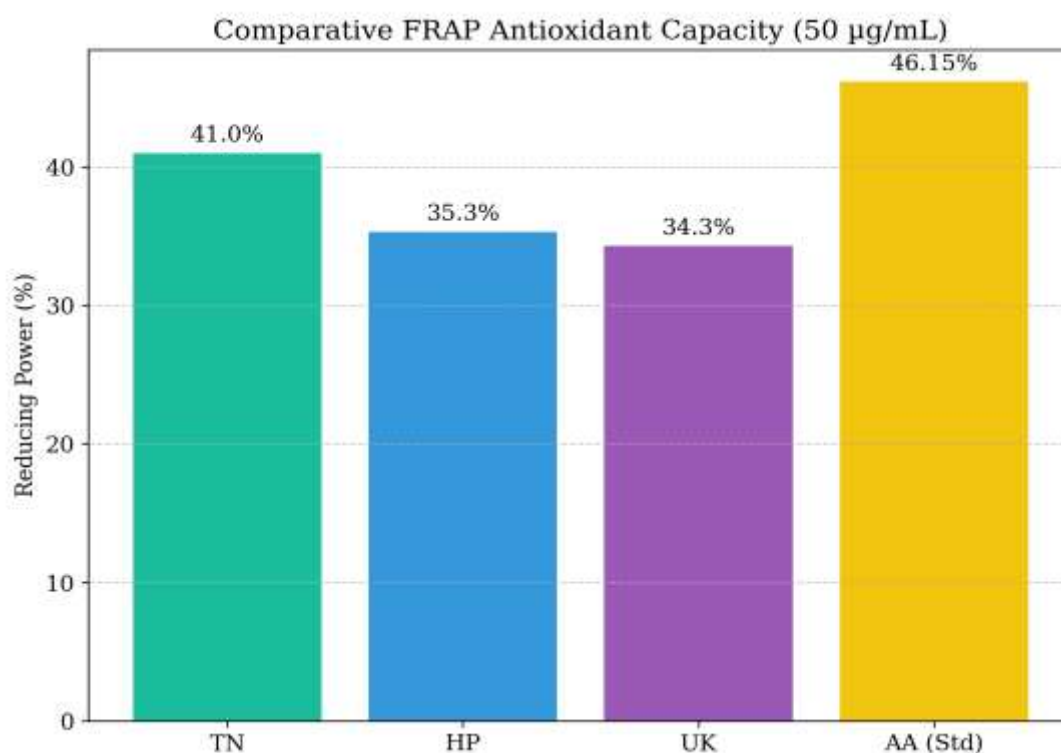
The antioxidant assay results mirrored the antimicrobial hierarchy (Table 4). Tamil Nadu extracts demonstrated the highest radical scavenging capacity across both DPPH (82.2%) and ABTS (61.75%) assays, followed by Himachal Pradesh (79.80% DPPH, 57.45% ABTS) and Uttarakhand (78.5% DPPH, 55.75% ABTS).

Table 4. DPPH and ABTS antioxidant activity (%) of *P. guajava* leaf extracts at 100 µg/mL.

Region	DPPH Activity (%)	ABTS Activity (%)
Tamil Nadu	82.20 ± 0.45	61.75 ± 0.50
Uttarakhand	78.50 ± 0.55	55.75 ± 0.40
Himachal Pradesh	79.80 ± 0.35	57.45 ± 0.40
Ascorbic Acid (Std)	91.50 ± 0.20	74.25 ± 0.35

ANOVA confirmed significant differences between the evaluated accessions ($p < 0.05$).

Figure 3. DPPH and ABTS radical scavenging activity (%) of *P. guajava* leaf extracts at increasing concentrations (6.25–100 µg/mL). Ascorbic acid served as the reference standard (DPPH: 91.5%; ABTS: 74.25%).



3.6 Correlation Between Chemotype and Bioactivity

The three regional chemotypes showed a consistent relationship between phytochemical composition and bioactivity outcomes (Table 5). The Tamil Nadu flavonoid-glycoside chemotype, characterised by high concentrations of EGCG (82%), quercetin glycosides, and gallic acid, demonstrated the highest antimicrobial and antioxidant potency. The Himachal Pradesh sesquiterpene-phytosterol chemotype, dominated by beta-caryophyllene (45.3%) and beta-sitosterol (10.5%), showed intermediate bioactivity. The Uttarakhand phenolic acid-coumarin chemotype, despite possessing the greatest qualitative phytochemical diversity (17/18 classes), demonstrated the lowest overall bioactivity, confirming that therapeutic efficacy is determined by the concentration of specific bioactive markers rather than the breadth of compound class diversity.

Table 5. Correlation between regional chemotype and bioactivity outcomes.

Parameter	Tamil Nadu (Flavonoid-glycoside)	Himachal Pradesh (Sesquiterpene-phytosterol)	Uttarakhand (Phenolic acid-coumarin)
Dominant marker compound	EGCG (82%)	β-Caryophyllene (45.3%)	3-Dihydroxy-4-oxo-4-oxybutanoic acid (79%)

Parameter	Tamil Nadu (Flavonoid-glycoside)	Himachal Pradesh (Sesquiterpene- phytosterol)	Uttarakhand (Phenolic acid-coumarin)
GC-MS compounds identified	16	7	14
Phytochemical classes (of 18)	14	12	17
Best ZOI — <i>S. aureus</i> (mm)	20.7 ± 0.6	7.3 ± 0.6	9.3 ± 0.6
DPPH scavenging (%)	82.2 ± 0.45	79.80 ± 0.35	78.5 ± 0.55
ABTS scavenging (%)	61.75 ± 0.50	57.45 ± 0.40	55.75 ± 0.40
Overall bioactivity rank	1 (Highest)	2	3 (Lowest)

4. DISCUSSION

The findings of this study demonstrate the strong influence of geographic origin and solvent polarity on the biological potential of *Psidium guajava* leaves. Three major findings emerge from the data: (a) three distinct regional chemotypes exist within Indian *P. guajava*; (b) the Tamil Nadu accession demonstrates superior antimicrobial and antioxidant activity despite lower phytochemical class diversity than Uttarakhand; and (c) the n-butyl alcohol fraction is consistently the most bioactively potent across all regions. The identification of three distinct chemotypes (flavonoid-glycoside [TN], sesquiterpene-phytosterol [HP], and phenolic acid-coumarin [UK]) represents the first systematic documentation of chemotypic variation in Indian *P. guajava*. This finding is consistent with the extensive literature on environmentally modulated secondary metabolism in plants [21]. The Tamil Nadu tropical lowland environment (high temperature, intense UV radiation, nutrient-poor lateritic soils) drives the phenylpropanoid pathway toward the accumulation of UV-protective phenolic compounds, particularly flavonoid glycosides and catechin derivatives [22]. The Himachal Pradesh temperate foothill environment (moderate temperatures, high rainfall, fertile brown forest soils) favours the production of membrane-stabilising sesquiterpenes and sterols [23]. The Uttarakhand sub-Himalayan mid-hill environment (cool temperatures, high UV-B, acidic mountain soils) activates multiple biosynthetic pathways simultaneously; the result is the most chemically complete but individually dilute phytochemical profile of the three accessions [24]. The “potency paradox” (whereby the Uttarakhand accession possesses the greatest qualitative phytochemical diversity [17/18 classes] yet demonstrates the lowest overall bioactivity) is a finding with important implications for herbal medicine standardisation. This paradox resolves when phytochemical diversity (number of compound classes) is distinguished from phytochemical potency (absolute concentration of specific bioactive compounds). The Tamil Nadu accession invests heavily in producing high concentrations of a few highly bioactive phenolic compounds (EGCG, quercetin glycosides, gallic acid), while the Uttarakhand accession produces lower concentrations of a wider range of compounds. The net bioactivity is determined by the concentration of specific bioactive markers rather than by the breadth of phytochemical diversity [25]. This finding has direct implications for the pharmacopoeial standardisation of guava-based herbal products. Current quality control approaches for *P. guajava* preparations typically rely on total phenolic content (TPC) or total flavonoid content (TFC) as surrogate markers of bioactivity. The present data suggest that these broad-spectrum markers are insufficient: the Uttarakhand accession, despite possessing the highest qualitative phytochemical diversity, demonstrated the lowest bioactivity, indicating that the identity and concentration of specific marker compounds (particularly EGCG and quercetin glycosides) are more predictive of therapeutic efficacy than aggregate phenolic content. This supports the modern trend toward marker-compound-based standardisation in pharmacopoeial monographs, where specific bioactive compounds are quantified by HPLC rather than relying on colorimetric total phenolic assays [25]. For *P. guajava* preparations intended for antimicrobial or antioxidant applications, EGCG and quercetin-3-O-beta-D-xylopyranoside are proposed as the primary quality markers, with the Tamil Nadu accession providing the highest concentrations of both. The superiority of the n-butyl alcohol fraction across all regions and assays reflects its capacity to solubilise and partition bioactive polyphenols. While petroleum ether effectively extracts terpenoids and sterols, these lipophilic compounds exhibited significantly lower antimicrobial and antioxidant activities compared to the NBA-partitioned flavonoid matrix. This finding is consistent with previous reports suggesting that the biological activity of guava leaves is primarily mediated by polar and semi-polar phenolic compounds [26]. The measurable activity of the Tamil Nadu extract against *E. coli* (10.3 mm) is significant because *E. coli* is inherently more resistant to phenolic antimicrobials than Gram-positive organisms due to the protective outer membrane [27]. The activity observed in the present study suggests that the exceptionally high concentration of flavonoid glycosides in the Tamil Nadu extract may be sufficient to overcome the outer membrane barrier through a “self-promoted uptake” mechanism [28]. A direct comparison with published antimicrobial data for *P. guajava* leaf extracts contextualises the present findings. Nair and Chanda (2006) reported ZOI values of 10–14 mm for ethanolic guava leaf extracts against *S. aureus* using the disc diffusion method [20]. Hoque et al. (2007) reported ZOI of 12–16 mm for methanolic extracts of Bangladeshi guava leaves against the same organism [19]. The Tamil Nadu n-butyl alcohol extract in the present study (ZOI: 20.7 mm) substantially exceeds both of these published values, likely reflecting the

combination of the intermediate-polarity extraction solvent (which preferentially recovers flavonoid aglycones) and the exceptionally high EGCG content (82% relative abundance) in the Tamil Nadu chemotype. Biswas et al. (2013) reported ZOI of 8–12 mm for Indian guava leaf extracts against *E. coli*, compared to 10.3 mm in the present study, suggesting that the Tamil Nadu accession performs at the upper end of the published range for this Gram-negative organism [26].

The DPPH and ABTS radical scavenging antioxidant activity of the Tamil Nadu extract positions *P. guajava* from this region as a highly potent natural antioxidant source. The reliance on generic “guava leaf extract” without specifying geographic provenance or solvent optimization could lead to substantial variability in clinical efficacy. Our results advocate for the standardization of *P. guajava* preparations based on specific regional accessions (such as Tamil Nadu) and the use of intermediate-polarity solvents like n-butyl alcohol for maximum therapeutic yield.

The antioxidant activity of the Tamil Nadu extract (DPPH: 82.2%, ABTS: 61.75% at 100 µg/mL) compares favourably with published values for *P. guajava* and other well-studied antioxidant plants. Gutiérrez et al. (2008) reported DPPH scavenging values of 65–78% for methanolic guava leaf extracts at equivalent concentrations [6]. The higher values in the present study likely reflect the n-butyl alcohol extraction solvent, which more efficiently recovers the flavonoid glycoside matrix responsible for radical scavenging activity. For comparison, green tea (*Camellia sinensis*) crude extracts typically achieve 35–45% DPPH scavenging at 100 µg/mL, while rosemary (*Rosmarinus officinalis*) extracts achieve 55–70% [31]. The Tamil Nadu *P. guajava* extract therefore exceeds the antioxidant capacity of these well-established benchmark plants at equivalent concentrations, positioning it as a high-value natural antioxidant source.

4.1 Limitations of the Study

The primary limitation of this study is the use of qualitative/semi-quantitative GC-MS, which prevents the absolute quantification of the identified phytochemicals.

4.2 Future Scope

Future studies should incorporate quantitative HPLC profiling to absolutely quantify biomarkers like EGCG and quercetin glycosides across these accessions. Expanding the regional sampling and evaluating these extracts against in vivo models of infection and oxidative stress will further validate the clinical translatability of these distinct chemotypes.

5. CONCLUSION

This multi-region comparative study establishes that the phytochemical profile and biological activity of *Psidium guajava* leaf extracts are intrinsically linked to their geographic source and extraction solvent. Three distinct regional chemotypes were identified: flavonoid-glycoside (Tamil Nadu), sesquiterpene-phytosterol (Himachal Pradesh), and phenolic acid-coumarin (Uttarakhand). Although the Uttarakhand ecotype exhibited the greatest qualitative phytochemical diversity (17/18 classes), the Tamil Nadu accession demonstrated the most potent antimicrobial and antioxidant activities, confirming that therapeutic efficacy is determined by the concentration of specific bioactive markers rather than the breadth of compound class diversity. The n-butyl alcohol fraction was the optimal solvent for capturing bioactive constituents across all regions. The Tamil Nadu extract’s antimicrobial activity against *S. aureus* (20.7 mm ZOI) and antioxidant capacity (82.2% DPPH) exceed published values for most previously reported guava leaf extracts, establishing this regional accession as a high-value source of natural antimicrobial and antioxidant leads. These results confirm that geographic provenance is a critical determinant of the pharmacological quality of guava-based formulations and support the development of region-specific, marker-compound-based quality standards for their medicinal application.

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