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Exploring The *in vitro* Efficacy of *Psidium Guajava* Leaf Oil Against *Cutibacterium Granulosum*

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Abstract

One potential approach to treat acne vulgaris is by utilizing *Cutibacterium granulosum* (*C. granulosum*) to break down the biofilm formed by *Cutibacterium acnes* (*C. acnes*). When using cosmeceutical products containing certain essential oils, as they may have the unintended effect of eliminating *C. granulosum*, leading to the potential development of more severe acne vulgaris in the long term. No data for *Psidium guajava* (guava) leaves oil against *C. granulosum* has been reported. Therefore, this study aimed to investigate *in vitro* antimicrobial activity of guava leaves oil against *C. granulosum*. This oil was extracted from the guava leaves by hydrodistillation using a clevenger-type apparatus. Then, the chemical compositions of this crude oil were identified by gas chromatography-mass spectrometry. The percentage yield of this essential oil was 0.13 ± 0.2% (w/w) on fresh weight basis. The major compounds were β-caryophyllene (25.50%), limonene (11.58%), caryophyllene oxide (3.43%), α-copaene (2.92%), 1,8-cineole (2.08%), and α-pinene (1.28%). This essential oil exerted a potent antioxidant capacity with the half maximal inhibitory concentration of 32.7 ± 0.3 µg/mL from 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Additionally, the essential oil of guava leaves showed the susceptibility of *C. granulosum* by agar disc diffusion method with 22 ± 0.2 mm of clear inhibition zone. This essential oil also possessed against *C. granulosum* with the minimum inhibitory concentration (MIC) and minimum bacterial concentration (MBC) of 3.125 µg/mL and 6.25 µg/mL from the broth microdilution susceptibility test. From these findings, this essential oil exhibited notable synergistic antioxidant and antimicrobial activities against *C. granulosum*.

Keywords**:** *acne vulgaris; essential oil; Psidium gaujava leaves; Cutibacterium*

1. Introduction

Acne vulgaris is a usual inflammatory disorder of the pilosebaceous unit, it was the burden of adolescents and young adults in 204 countries worldwide from 1990 to 2019. *Cutibacterium acnes* (*C. acnes*) that is anaerobic pathogen induces a dominant key role of acne vulgaris pathogenesis by forming biofilm and influencing specific inflammatory mediators (Bronnec & Alexeyev, 2021). *C. acnes* and its biofilm play an associated factor in acne vulgaris development, the persistent opportunistic bacteria, and antibiotic treatment failure (Bronnec et al., 2022). As stated in a previous study, *C. acnes* and *Cutibacterium granulosum* (*C. granulosum*) are the dominant skin commensal microorganisms in the pilosebaceous unit, while their colonies commonly observed as a separate community (Jahns et al., 2015). From this observation, a previous study explored that *C. granulosum* was capable to destroy the biofilm of *C. acnes* by secreting an endogenous extracellular nuclease (BmdE) as a novel competitive

mechanism between *C. granulosum* and *C. acnes* (Bronnec et al., 2022). Therefore, one of the strategies used for acnes treatment is to destroy the biofilm of *C. acnes* by *C. granulosum*.

Some research groups have reported many essential oils from medicinal plants can be against *C. acnes*. Especially, *Psidium guajava* L. (guava) is one of Thai medicinal plants that has been researched and developed for effective products against *C. acnes* because this essential oil exhibits a good antibacterial activity against wide strain of *C. acnes* (Coenye et al., 2007; Dessinioti & Katsambas, 2017). Additionally, the neat essential oil of guava leaves is now accessible in marketplace worldwide, and the cosmeceutical products containing this oil as an active ingredient in some topical formulations are used to treat acne vulgaris (Chen et al., 2003).

While the antibacterial activity of this essential oil against *C. acnes* has been reported both *in vitro* and *in vivo*, there is currently no available data regarding the antimicrobial effects of guava leaf oil against *C. granulosum in vitro*. Given this information, our hypothesis is that if the essential oil derived from guava leaves does not exhibit antimicrobial properties against *C. granulosum*, it is possible that cosmetic products containing this oil could play a role in the treatment of acne vulgaris with extended usage.

2. Objectives

Therefore, the aim of this study was to explore the *in vitro* efficacy of guava leaf oil against *C. granulosum*.

3. Materials and methods

3.1 Plant material

Fresh leaves of *Psidium guajava* L. (guava) was collected from the botanical garden of School of Pharmaceutical Sciences, University of Phayao, Phayao, Thailand. The voucher specimen was deposited in the herbarium of School of Pharmaceutical Sciences at University of Phayao, Phayao, Thailand.

3.2 Extraction and Identification of guava leaves oil

According to European Pharmacopoeia, fresh guava leaves were chopped into several small pieces. These small pieces were extracted in the Clevenger apparatus by hydrodistillation method for 4 hours. The obtained guava leaves oil was concentrated by a rotary evaporator, and the water in concentrated mixture was then eliminated by adding anhydrous sodium sulfate for 30 minutes. This crude oil was filtered, and it was stored in refrigerator at 2-4°C. The chemical composition of oil was analyzed by gas chromatography-mass spectrometry (GC-MS) on Agilent 6890 gas chromatography equipped with Hewlett Packard 5973 mass-selective detector in the electron impact mode (70 eV) a full-scan. The column was HP-5MS fused silica linked capillary column (30 m. x 0.25 mm. i.d.; 0.25 μM) of methyl silicon; The program of oven temperature was 70-280°C at 3°C/min; the inlet of injector and detector temperature was 260 and 280°C, respectively. 1 μL of diluted sample in dichloromethane 1:1000 (v/v) was injected in the splitless mode ratio, and helium was used as the carrier gas with the flow rate of 1 mL/min. The identification of individual compounds was analyzed by comparing the mass spectra to the Kováts retention indices (RI) related to *n*-alkanes mixture (C_9-C_{20}). All compounds were identified by matching their obtained mass spectra with Wiley & NIST database. The selection of peaks that were calculated from the peak area of gas chromatogram was above 90%.

3.3 Evaluation of antioxidant capacity

The antioxidant capacity (AOC) of guava leaves oil was analyzed by DPPH assay (Krongkeha & Pitaktim, 2022). Different concentrations of essential oil and Trolox (3.1, 6.2, 12.5, 25.0 and 50.0 μg/mL) were prepared. 1 mL of these were added to 2 mL of ethanolic DPPH solution (60 μM). The mixtures were shaken vigorously for 20 second at ambient temperature. A decrease in the absorbance of samples in a 1-cm disposable cuvette was measured at 517 nm by double beam UV-Vis spectrophotometer (JASCO V-630). The percentage of AOC was calculated by the equation as follows:

$$[(A_{blank} - A_{sample})/A_{blank}] \times 100$$

where A_{blank} is the absorbance of the control, and A_{sample} is the absorbance of different concentrations of guava leaves oil. The concentration of guava leaves oil that requires to scavenge 50% of the DPPH concentration was calculated by the linear equation from the linear graph. AOC of guava leaves oil was expressed as the half maximal

inhibitory concentration or IC_{50} compared to the standard, Trolox.

3.4 Identification of *C. granulosum*

C. granulosum was obtained from the disposable specimen collected at Medicinal Center of University of Phayao, Phayao, Thailand without disclosing specific details. The identification of the bacteria in the sample was then analyzed by 16S rRNA gene sequencing, and it was compared with the sequence of nucleotide from the GenBank database as accession number NR 118646. This isolation was not full-length of 16s RNA gene. Therefore, the conventional method, which involved examining the bacteria under a microscope, applying the Safranin staining protocol, conducting a catalase test, and performing an indole test, was used to identify this bacterial isolate. Then, the stock suspension of *C. granulosum* was cultured in the medium of brain heart infusion agar (BHIA) under anaerobic jar at 37°C for 48 hours.

3.5 Susceptibility test of *C. granulosum* by disc diffusion method

The susceptibility test of *C. granulosum* was achieved by the agar disc diffusion method. 100 µL of the suspension of *C. granulosum* inoculum containing 10^6 CFU/mL seeded on 20 mL of BHIA. Then, 6.0 mm in diameter of the discs were saturated with 10 µL of all sample and placed on the inoculated agar. To determine the sensitivity of *C.* granulosum to the guava leaves oil, the negative control was the empty disc, and clindamycin gel (50 µg/mL) was used as positive control. The inoculated plates were stored in an anaerobic jar, and all plates were incubated at 37°C for 48 hours. The susceptibility of *C. granulosum* to guava leaves oil was evaluated by the diameter of clear zone or inhibition zone.

3.6 Minimum inhibitory concentration and minimum bactericidal concentration tests by broth microdilution method

The minimum inhibitory concentration (MIC) value was studied for determining the lowest concentration of guava leaves oil that inhibits the visible growth of *C. granulosum* in BHI broth (Kumar, et al., 2003). The inoculation of *C. granulosum* was cultured in BHI broth with 1% glucose for 72 hours. The turbidity of this *C. granulosum* suspension was adjusted to McFarland standard number 0.5. The guava leaves oil was diluted in two-fold serial dilution. The first measured concentration of guava leaves oil was 50 µg/mL. Then, 90 µL of BHI broth was addressed into each well in 96-well plates followed by 5 µL of 2,3,5-triphenytetrazolium chloride (TTC) solution and 5 µL of the *C. granulosum* inoculum. After that 100 µL of the initial concentration was added on the first well to get the final volume of 200 µL. Afterwards, 100 µL of the final volume was transferred into 4 consecutive wells by two-fold serial dilution. Then, the 96-well plates were covered by a sterile plate sealing, and they were mixed on the plate shaker at 200 round per minute (rpm) for 1 minute. Subsequently, the plate was incubated at the proper condition for 72 hours. The diluted solution without the guava leaves oil and clindamycin gel were used as negative and positive controls. The MIC value of guava leaves oil is the first concentration that is not turn to pink color. Then, 10 µL of this solution was spread on BHIA plates. All samples were incubated in anaerobic condition at 37°C for 72 hours. The lowest concentration of guava leaves oil that did not show growth of C. *granulosum* was defined as the minimum bactericidal concentration (MBC) value.

3.7 Statistical analysis

The statistical analysis of two different samples was performed by using independent *t*-test at a significance level of 0.05.

4. Results

4.1 Extraction and identification of guava leaves oil

The guava leaves oil was a light-yellow color with a distinctive odor. From the hydrodistillation extraction method, the percentage yield of the crude oil was 0.13% (w/w) on fresh weight basis. Based on the order of elution on the HP-5MS column, the total of 100 compositions of 99.61% were identified from GC-MS analysis (data not shown). In our study, the major compounds of the guava leaves oil were terpenoids such as monoterpenes, sesquiterpenes, and oxygenated sesquiterpene as presented in Table 1. It was shown that the main compound in this essential oil was β-caryophyllene (25.50%). The monoterpene compounds were α-pinene (1.28%), limonene (11.58%), and 1,8-cineole (2.08%), respectively. The sesquiterpenes and oxygenated sesquiterpene were

α-copaene (2.92%), β-caryophyllene (25.50%), and caryophyllene oxide (3.43%), respectively.

4.2 Evaluation of antioxidant capacity

To evaluate the free radical scavenging activities against guava leaves oil, DPPH method has been used in this study. From the results, the essential oil of guava leaves that composed of terpenoids as a major lipophilic compound exhibited potentially scavenging DPPH radicals. The antioxidant capacity increased with increasing the concentration of the guava leave oil. The inhibition of antioxidant capacity at 50% (IC_{50}) of the essential oil of guava leaves was 32.7 ± 0.3 μg/mL that was calculated from the linear equation of y = 1.2614x + 8.7254 with 0.9923 of the square of correlation as shown in Figure 1.

4.3 Identification of *C. granulosum*

The identification of the microorganism in the isolated specimen involved sequencing the 16S ribosomal RNA gene and comparing it with the corresponding sequence in the GenBank database (accession number NR 118646). Analysis revealed a DNA homology value of 99%, with no gaps observed in the nucleotide sequences between positions 93 and 828 when compared to the reference (query) sequence as shown in Table 2. As a result, the isolated specimen was conclusively identified as *C. granulosum* strain DSM 20700.

Table 1 Sample of major compounds of guava leaves oil from GC-MS analysis

Chemical compounds	% Peak area	Retention time	Kováts retention indices (RI)
Monoterpenes			
α-Pinene	1.28	4.15	982
Limonene	11.58	5.81	1029
1,8-Cineole	2.08	6.59	1039
Sesquiterpenes			
α-Copaene	2.92	19.83	1359
β-Caryophyllene	25.50	21.76	1419
Oxygenated Sesquiterpene			
Caryophyllene oxide	3.43	28.1	1583

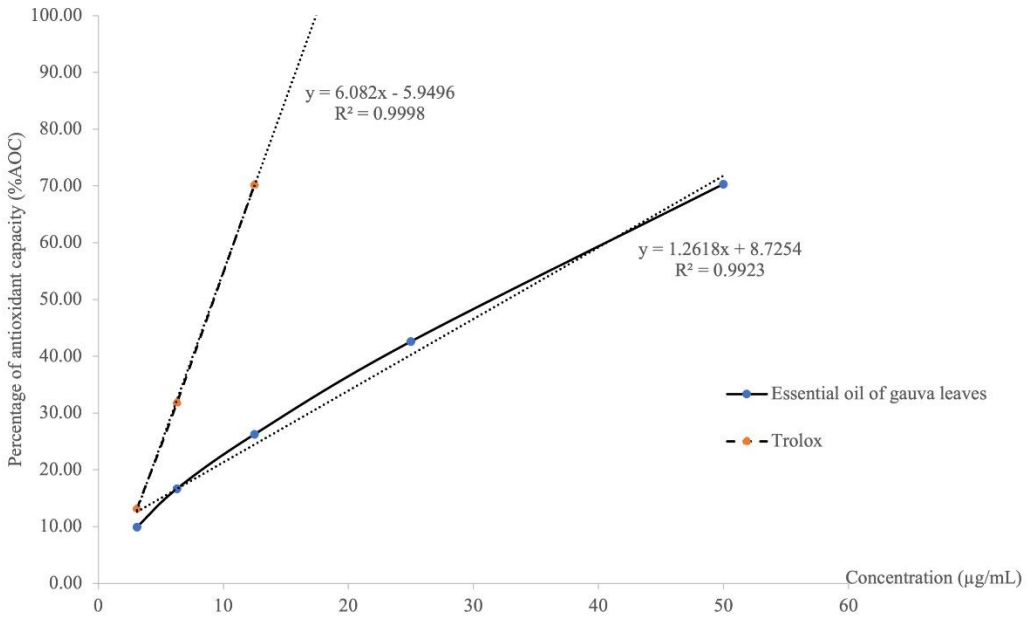

Figure 1 The percentage of antioxidant capacity of the essential oil from guava leaves and Trolox

Table 2 Sequencing of the 16S ribosomal RNA gene of *C. granulosum*

Score 1354 bits (733)	**Expect 0.0**	**Identities 735/736 (99%)**	**Gaps 0/736 (0%)**	**Strand Plus/Plus**
Query 1	AACACGTGAGTAACCTGCCCACAACTTTGGGATAACGCTAGGAAACTGGTGCTAATACTG			60
Subject 93	AACACGTGAGTAACCTGCCCACAACTTTGGGATAACGCTAGGAAACTGGTGCTAATACTG			152
Query 61	GATATGTGCTCCTGCTGCTAGGTGGGGGTTGGAAAGCTCCGGCGGTTGTGGATGGACTCG			120
Subject 153	GATATGTGCTCCTGCTGCTAGGTGGGGGTTGGAAAGCTCCGGCGGTTGTGGATGGACTCG			212
Query 121	CGGCCTATCAGCTTGTTGGTGGGGTAGTGGCCTACCAAGGCGGCGACGGGTAGCCGGCCT			180
Subject 213	CGGCCTATCAGCTTGTTGGTGGGGTAGTGGCCTACCAAGGCGGCGACGGGTAGCCGGCCT			272
Query 181	GAGAGGGTGACCGGCCACATTGGGACTGAGATACGGCCCAGACTCCTACGGGAGGCAGCA			240
Subject 273	GAGAGGGTGACCGGCCACATTGGGACTGAGATACGGCCCAGACTCCTACGGGAGGCAGCA			332
Query 241	GTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCAACGCCGCGTGCGGGATGACG			300
Subject 333	GTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCAACGCCGCGTGCGGGATGACG			392
Query 301	GCCTTCGGGTTGTAAACCGCTTTCAGCAGGGACGAAGCTTTTTGTGACGGTACCTGCAGA			360
Subject 393	GCCTTCGGGTTGTAAACCGCTTTCAGCAGGGACGAAGCTTTTTGTGACGGTACCTGCAGA			452
Query 361	AGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTGATACGTAGGGTGCGAGCGTTGTC			420
Subject 453	AGAAGCACCGGCTAACTACGTGCCAGCAGCCGCGGTGATACGTAGGGTGCGAGCGTTGTC			512
Query 421	CGGATTTATTGGGCGTAAAGGGCTCGTAGGCGGTTGATCGCGTCGGAAGTGGAAACTTGA			480
Subject 513	CGGATTTATTGGGCGTAAAGGGCTCGTAGGCGGTTGATCGCGTCGGAAGTGGAAACTTGA			572
Query 481	TGCTTAACGTTGAGCGTGCTTTCGATACGGGTTGACTTGAGGAAGGTAGGGGAGAATGGA			540
Subject 573	TGCTTAACGTTGAGCGTGCTTTCGATACGGGTTGACTTGAGGAAGGTAGGGGAGAATGGA			632
Query 541	ATTCTTGGTGGAGCGGTGGAATGCGCAGATATCAGGAGGAACACCAGTGGCGAAGGCGGT			600
Subject 633	ATTCTTGGTGGAGCGGTGGAATGCGCAGATATCAGGAGGAACACCAGTGGCGAAGGCGGT			692
Query 601	TCTCTGGATCTTTCCTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGCTTAGATAC			660
Subject 693	TCTCTGGATCTTTCCTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGCTTAGATAC			752
Query 661	CCTGGTAGTCCACGCTGTAAACGGTGGGTACTAGGTGTGGGGTCCATTCCACGGATTCCTG			720
Subject 753	CCTGGTAGTCCACGCTGTAAACGGTGGGTACTAGGTGTGGGGTCCATTCCACGGATTCCTG			812
Query 721	TGCCGTAGCTAACGCA			736
Subject 813	TGCCGTAGCTAACGCA			828

Due to the partial length of the 16S RNA gene isolation, the conventional method was utilized for identification purposes, ensuring originality. Then, the identification of the isolated bacteria from the disposable specimens involved morphological observations and biochemical tests. The examination revealed that the bacteria in the disposable specimen were gram-positive rods, as evidenced by the pink counter-staining with Safranin under light microscopy. However, neither the sample nor the control exhibited a cherry-red ring in the indole test. Additionally, the specimen demonstrated a positive result in the catalase test, where the addition of hydrogen peroxide resulted in observable reactions. These findings were conclusively supported as *C. granulosum.* Then, *C. granulosum* culture in BHI agar was incubated in an anaerobic jar at 37°C for 48-72 hours, and the susceptibility testing of the essential oil from guava leaves was further studied.

4.4 Susceptibility test of *C. granulosum* by the agar disc diffusion method

To evaluate the susceptibility of *C. granulosum* to the essential oil from guava leaves, the agar disc diffusion method was used in this study. From the results, the clear zone was not observed from the empty disc, while the mean diameter of the clear zone was 22.0 ± 0.2 mm for the essential oil and 53.5 ± 0.2 mm for clindamycin gel. From this finding, it was noticed that the essential oil from guava leaves showed the susceptibility of *C. granulosum* by agar disc diffusion method. Therefore, MIC and MBC values were further studied in the next step.

4.5 Minimum inhibitory concentration and minimum bactericidal concentration tests by broth dilution method

The value of MIC and MBC of guava leaves oil against C. granulosum were determined by broth dilution method. The results showed that the MIC and MBC values of the essential oil from guava leaves were higher than those of clindamycin gel. MIC value of the essential oil from guava leaves was 3.125 μg/mL, and MBC value was 6.25 μg/mL. The MIC and MBC values of positive control, clindamycin gel, were 3.125 μg/mL and 1.5625 μg/mL.

5. Discussion

The percentage yield of the obtained essential oil of guava leaves was lower than previous reports (Satyal et al., 2016; Ji et al., 1991; Ogunwande et al., 2003; da Silva et al., 2003; Adam et al., 2011). It may be concerned to growing habitats and seasonal variation (Matias et al., 2016; Dhouioui et al., 2016), while the GC/MS result was similarly to previous study that found terpenoids as the major chemical classes of essential oil from guava leaves (Ogunwande et al., 2003; da Silva et al., 2003; Pino et al., 1999). Particularly, β-caryophyllene (25.50%) in this essential oil of guava leaves showed higher concentration than previous studies that found 20-22% of β-caryophyllene (Ogunwande et al., 2003; Pino et al., 1999). On the other hand, monoterpenes in this essential oil such as limonene, α-Pinene, and 1,8-Cineole showed lower concentration than previous studies (Ogunwande et al., 2003; da Silva et al., 2003). The different quantitative variations between these results and previous studies could be attributed to geographic or environmental conditions, genetic variability, analytical procedures, and procedures used for the extraction of guava leaves. Additionally, the result from the evaluation of antioxidant capacity of guava leaves oil was similarly to a previous study that reported IC_{50} of the essential oil of guava leaves 29.3 ± 0.7 μg/mL (Mandal et al., 2022). Although IC_{50} of this guava leaves oil (32.7 ± 0.3 μg/mL) was significantly higher ($p < 0.05$) than IC_{50} of Trolox (9.4 ± 0.1 μg/mL), but this essential oil with this IC_{50} value was also capable of scavenging DPPH free radicals as a potent antioxidant. The different values of IC_{50} between this essential oil and Trolox may be attributed to their different solubilities and oxidation properties in DPPH solutions. In addition to showing a good antioxidation potency, this essential oil of guava leaves showed the susceptibility of *C. granulosum* by agar disc diffusion method, and this essential oil was also possessed against *C. granulosum* with low values of the MIC and MBC. Although the MIC and MBC values of the essential oil from guava leaves were higher than those of clindamycin gel, the essential oil still showed potent antimicrobial activity against *C. granulosum*. From these findings, it is possible that the combined action of different bioactive compounds present in guava leaves oil contributes to both its antioxidant capacity and antimicrobial activity. These compounds may work synergistically, enhancing each other's effects. For instance, certain antioxidants can enhance the efficacy of antimicrobial agents by promoting the penetration of antimicrobials into bacterial cells or by inhibiting microbial enzyme activity. To explore the antibacterial activity of guava leaves oil against *C. granulosum* and investigate its underlying mechanism. Further experiments should be conducted to elucidate the specific mechanism by which the guava leaf oil exerts its antibacterial effects. This will involve analyzing factors such as disruption of bacterial cell membranes, inhibition of essential metabolic pathways, or modulation of bacterial gene expression to gain a comprehensive understanding of the antibacterial properties and the specific mechanism of action of guava leaves oil against *C. granulosum*. However, there is currently no available literature regarding the antimicrobial activity of guava leaf essential oil against *C. granulosum* although prior studies have demonstrated the effective inhibition of *C. acne* growth by guava leaf essential oil (Athikomkulchai et al., 2008). Therefore, the use of guava leaf oil might inadvertently result in the elimination of *C. granulosum*, which could potentially contribute to the long-term development of more severe acne vulgaris. This finding could be significant in the context of acne vulgaris treatment, particularly considering the persistent presence of biofilm associated with *C. acnes*, as mentioned previously.

6. Conclusion

The essential oil of guava leaves was extracted by the hydrodistillation method. The major compound of this essential oil was terpenoids, and this essential oil showed strong DPPH radical scavenging activity and was possessed against *C. granulosum*. The application

of guava leaf oil may unintentionally lead to the elimination of *C. granulosum*, potentially exacerbating the long-term progression of acne vulgaris and its severity. However, the cosmeceutical products which compose of the guava leaves oil should further study *in vivo* to confirm the effect of the essential oil to *C. acne* and *C. granulosum*. It will get valuable data for the treatment guideline of acne vulgaris.

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8. References

Adam, F., Vahirua-Lechat, I., Deslandes, E., & Menut, C. (2011). Aromatic plants of French Polynesia. V. Chemical composition of essential oils of leaves of Psidium guajava L. and Psidium cattleyanum Sabine. *Journal of Essential Oil Research*, *23*(1), 98-101. https://doi.org/10.1080/10412905.2011.9700433

Athikomkulchai, S., Watthanachaiyingcharoen, R., Tunvichien, S., Vayumhasuwan, P., Karnsomkiet, P., Sae-Jong, P., Ruangrungsi, N. (2008). The development of anti-acne products from *Eucalyptus globulus* and *Psidium guajava* oil. *Journal of Health Research*, *22*(3), 109-113.

Bronnec, V., & Alexeyev, O. A. (2021). In vivo model of *propionibacterium (cutibacterium)* spp. biofilm in drosophila melanogaster. *Anaerobe*, 72, Article 102450. https://doi.org/10.1016/j.anaerobe.2021.102450

Bronnec, V., Eilers, H., Jahns, A. C., Omer, H., & Alexeyev, O. A. (2022). Propionibacterium (Cutibacterium) granulosum extracellular DNase BmdE targeting propionibacterium (Cutibacterium) acnes biofilm matrix, a novel inter-species competition mechanism. *Frontiers in Cellular and Infection Microbiology*, *11*, Article 1337. https://doi.org/10.3389/fcimb.2021.809792

Chen, C., Krishnan, V., Macon, K., Manne, K., Narayana, S. V., & Schneewind, O. (2013). Secreted proteases control autolysin-mediated biofilm growth of Staphylococcus aureus. *Journal of Biological Chemistry*, *288*(41), 29440-29452. https://doi.org/10.1074/jbc.M113.502039

Coenye, T., Peeters, E., & Nelis, H. J. (2007). Biofilm formation by Propionibacterium acnes is associated with increased resistance to antimicrobial agents and increased production of putative virulence factors. *Research in microbiology*, *158*(4), 386-392. https://doi.org/10.1016/j.resmic.2007.02.001

da Silva, J. D., Luz, A. I. R., da Silva, M. H. L., Andrade, E. H. A., Zoghbi, M. D. G. B., & Maia, J. G. S. (2003). Essential oils of the leaves and stems of four Psidium spp. *Flavour and Fragrance Journal*, *18*(3), 240-243. https://doi.org/10.1002/ffj.1219

Dessinioti, C., & Katsambas, A. (2017). Propionibacterium acnes and antimicrobial resistance in acne. *Clinics in dermatology*, *35*(2), 163-167. https://doi.org/10.1016/j.clindermatol.2016.10.008

Dhouioui, M., Boulila, A., Chaabane, H., Zina, M. S., & Casabianca, H. (2016). Seasonal changes in essential oil composition of Aristolochia longa L. ssp. paucinervis Batt. (Aristolochiaceae) roots and its antimicrobial activity. *Industrial Crops and Products*, *83*, 301-306. https://doi.org/10.1016/j.indcrop.2016.01.025

Jahns, A. C., Eilers, H., Ganceviciene, R., & Alexeyev, O. A. (2015). Propionibacterium species and follicular keratinocyte activation in acneic and normal skin. *British Journal of Dermatology*, *172*(4), 981-987. https://doi.org/10.1111/bjd.13436

Ji, X. D., Pu, Q. L., Garraffo, H. M., & Pannell, L. K. (1991). The essential oil of the leaves of Psidium guajava L. *Journal of Essential Oil Research*, *3*(3), 187-189. https://doi.org/10.1080/10412905.1991.9700501

Krongkeha, W., & Pitaktim, S. (2022). Growth inhibition of Escherichia coliand Pseudomonas aeruginosastrains by Piper betleLinn. extracts. *Journal of Current Science and Technology*, *12*(1), 141-150.

Kumar, A., Semwal, R., Chauhan, A., Semwal, R. B., Andola, H. C., Joshi, S. K., & Semwal, D. K. (2023). Microscopic identification, GC-MS analysis of essential oil and antioxidant activity of Artemisia

roxburghiana. *Journal of Current Science and Technology*, *13*(1), 36-45.

Mandal, A. K., Paudel, S., Pandey, A., Yadav, P., Pathak, P., Grishina, M., ... & Verma, A. (2022). Guava Leaf Essential Oil as a Potent Antioxidant and Anticancer Agent: Validated through Experimental and Computational Study. *Antioxidants*, *11*(11), Article 2204. https://doi.org/10.3390/antiox11112204

Matias, E. F., Alves, E. F., Silva, M. K., Carvalho, V. R., Figueredo, F. G., Ferreira, J. V., ... & Costa, J. G. (2016). Seasonal variation, chemical composition and biological activity of the essential oil of Cordia verbenacea DC (Boraginaceae) and the sabinene. *Industrial Crops and Products*, *87*, 45-53. https://doi.org/10.1016/j.indcrop.2016.04.028

Ogunwande, I. A., Olawore, N. O., Adeleke, K. A., Ekundayo, O., & Koenig, W. A. (2003). Chemical composition of the leaf volatile oil of Psidium guajava L. growing in Nigeria. *Flavour and Fragrance Journal*, *18*(2), 136-138. https://doi.org/10.1002/ffj.1175

Pino, J. A., Ortega, A., & Rosado, A. (1999). Volatile constituents of guava (Psidium guajava L.) fruits from Cuba. *Journal of Essential Oil Research*, *11*(5), 623-628. https://doi.org/10.1080/10412905.1999.9701227

Satyal, P., Paudel, P., Lamichhane, B., & Setzer, W. N. (2015). Leaf essential oil composition and bioactivity of Psidium guajava from Kathmandu, Nepal. *American Journal of Essential Oils and Natural Products*, *3*(2), 11-14.