Evaluating the *in vitro* inhibitory against Acne vulgaris activity of four medicinal plants (*Cassia grandis* L.f., *Houttuynia cordata* Thunb., *Piper betle* L. and *Hibiscus tiliaceus* L.)

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Abstract
Globally, Acne vulgaris is a chronic skin disease that significantly impacts people, especially teenagers and young adults. Recent studies have focused on natural plant-based materials that do not have the adverse effects of conventional medicines. This study aimed to evaluate the in vitro inhibitory activity of extracts from Cassia grandis, Houttuynia cordata, Piper betle and Hibiscus tiliaceus against Acne vulgaris. The results showed that C. grandis and P. betle are promising materials for antibacterial activity. The leaves of C. grandis exhibited the strongest antibacterial activity in the agar disk diffusion method, with antibacterial ring diameters of 17.67 mm and 19.33 mm corresponding to Cutibacterium acnes and Staphylococcus epidermidis respectively. The stem and leaves of P. betle showed the strongest antibacterial activity in the broth dilution method, with MIC=2.5 mg/mL. The leaves and stems of C. grandis can inhibit the biofilm formation of C. acnes bacteria after 72 and 96 hours of investigation. Additionally, the extracts from C. grandis and P. betle demonstrated different antioxidant and anti-inflammatory properties compared to the other extracts in this study. The findings suggest that these plant extracts have potential as a source of natural compounds for the treatment of Acne vulgaris.

Keywords: Antibacterial, Acne vulgaris, *Cassia grandis* L.f., *Houttuynia cordata* Thunb., *Piper betle* L., *Hibiscus tiliaceus* L.

Introduction

Acne vulgaris is a prevalent skin condition affecting millions worldwide[24]. Numerous medications are available for the treatment of acne, their effectiveness is often hindered by adverse side effects, antibiotic resistance, or high cost[27]. Consequently, there is an escalating demand for natural products with potential anti-acne activity as an alternative therapy for acne. This study aimed to evaluate the *in vitro* inhibitory activity of extracts from *Cassia grandis* L.f, *Houttuynia cordata* Thunb., *Piper betle* L. and *Hibiscus tiliaceus* L. against Acne vulgaris. These plant species are well-known for their anti-inflammatory, antimicrobial and antioxidant properties, which are crucial in the treatment of acne[6,13,21,23]. However, their potential anti-acne activity has yet to be extensively studied, except for the leaves of *C. grandis* L. f[14].

Therefore, this study conducted a series of assays to evaluate the anti-acne activities of different plant parts (leaves, stems and roots) from these plant species. Specifically, we aimed to determine the antibacterial effects against *Cutibacterium acnes* and *Staphylococcus epidermidis*, the ability to inhibit biofilm formation and the antioxidant and anti-inflammatory properties of the extracts. Our findings could contribute to the development of natural and safe anti-acne agents with potential clinical applications in the cosmetic industry. By exploring the therapeutic potential of these plant extracts, we hope to provide a basis for alternative treatments that address the limitations of conventional acne medications.

Material and Methods

Collection sample from the field: The plant materials were collected at Bung Binh hamlet, Hoa Khanh Tay commune, Duc Hoa district, Long An province, Vietnam including leaves, stems, vines and roots of *P. betle*; leaves, stems and roots of *H. cordata* and *H. tiliaceus;* and leaves, stems, roots and seeds of *C. grandis*. After harvesting, the samples were cleaned by tap water and dried. Finally, the samples were ground into powder.

Bacterial strains: The *C. acnes* ATCC 11827 and *S. epidermidis* ATCC 12228 were stored at the Department of Plant Biotechnology and Biotransformation, Faculty of Biology and Biotechnology, University of Science, Vietnam National University, Ho Chi Minh City.

Ethanol extracts from various parts of 4 plant species by maceration: The experiment was based on Abubakar et al's[0] and Phung et al method[18]. The plant sample was soaked in a glass or stainless-steel container with a lid. The solvent as ethanol was poured into the container until it covered the surface of the sample layer. The container was left at room temperature for 48 hours. Then, solution was filtered through filter paper and the filtrate was collected to obtain the extract. The process was repeated with fresh solvent until the sample was utterly exhausted. The vacuum rotary evaporator was used to prepare the extract from the solvent that dissolved natural compounds in the plant.

Antioxidant activity determinations

DPPH radical scavenging method: The DPPH assay followed Molyneux's method with minor modifications[15]. The reaction mixture consisting of 0.3 mL of the test sample, 1.8 mL of ethanol and 0.3 mL of DPPH (0.6 mM) dissolved in methanol was prepared. The mixture was shaken and incubated in the dark for about 30 minutes at room temperature and the absorbance was measured at a wavelength of 517 nm using a spectrophotometer. This method was used to evaluate the antioxidant activity of the test samples.

Ferric reducing power assay: The experiment was conducted according to the method of Yen and Duh[29] with minor modifications. 1 mL of vitamin C solution (0.5 mg/mL), 1 mL of plant extract (2 mg/mL) and 1 mL of the solvent were used to dissolve the test substance (control), each drawn into separate test tubes. 2.5 mL of 0.2 M phosphate buffer solution (pH 6.6) was added to each tube and the mixture was shaken well. Next, 2.5 mL of 1% $K_3Fe(CN)_6$ solution was added to each tube. The reaction mixture was placed in a thermostatic bath at 50°C for 20 minutes. After cooling, 2.5 mL of 1% trichloroacetic acid was added to each tube. The solutions were centrifuged at 7000 rpm for 2 minutes and the supernatant was collected. Then, 1 mL of the supernatant was added to each clean test tube, followed by 2 mL of distilled water and 0.5 mL of 1% $FeCl_3$ solution. The mixture was shaken well for 5 minutes and the absorbance was recorded at a wavelength of 700 nm.

ABTS radical scavenging activity: For the ABTS assay, the procedure followed Erel's method[10] with minor modifications. 10,8 mg of ABTS was dissolved in 3 ml of PBS buffer solution and 3 ml of 2.45 mM $K_2S_2O_8$ solution was added in a 1:1 (v/v) ratio. The mixture was incubated in the dark in a refrigerator for 16 hours. After 16 hours, 5 ml of the reaction mixture was taken and diluted with 200 ml of PBS buffer. The absorbance of the diluted solution was measured at a wavelength of 734 nm and adjusted to a value of 0.70 ± 0.02 to obtain the ABTS* solution. The reaction was performed by adding 50 µL of plant extract at corresponding concentrations to 1.5 mL of ABTS* solution. The mixture was incubated in the dark for 30 minutes at room temperature and then the absorbance was measured at a wavelength of 734 nm.

Evaluation of the lipid peroxidation by thiobarbituric acid (TBA) assay: The experiment was carried out using the method of Ohakawa et al[17] and Upadhyay et al[26] and modified to suit laboratory conditions. 250 µl of 10% (v/v) egg yolk was added to each individual test tube and mixed with PBS buffer (pH 7.4). After that, 50 µl of samples dissolved in 10% DMSO PBS buffer at different concentrations were added to each test tube followed by an addition of 200 µl of buffer to reach a final volume of 500 µl in each tube. 50 µl of $FeSO_4$ 70 mM was ultimately added to the mixture and the reaction was allowed to proceed for 30 minutes at 37°C to initiate the lipid peroxidation process. After incubation, 750 µl of 20% acetic acid (pH 3.5), 1000 µl of 0.8% TBA (w/v) prepared in 1.1% (w/v) sodium dodecyl sulfate (SDS) and 25 µl of 20% TCA (w/v) were added to each tube. The mixture was then heated at 95°C for 60 minutes. After cooling, 3 ml of 1-butanol was added to each tube and the tubes were centrifuged at 3000 rpm for 10 minutes. The absorbance of the organic layer was measured at a wavelength of 532 nm.

Anti-inflammatory activity determination by protecting albumin from denaturing: The experiment was carried out by the method established by Verma et al[0]. 0.16% (w/v) solution of bovine serum albumin (BSA) was prepared in a phosphate buffer at a pH of 5.5. The extract was subsequently diluted to various concentrations using distilled water. In a test tube, 1 ml of phosphate buffer pH 5.5, 2 ml of 0.16% BSA solution and 1 ml of the diluted stock solution were added. The resulting mixture was incubated at 37°C for 15 minutes. Subsequently, the mixture was heated to 74°C for 5 minutes. After this step, the test tube was promptly transferred to an ice bath at 4°C for 2 minutes and the absorbance was measured at a wavelength of 660 nm to record the optical density value after denaturation.

Antibacterial susceptibility assay

Agar-well diffusion method: For the diffusion assay, the method was conducted in accordance with Tran et al[22]. *C. acnes* and *S. epidermidis* were activated in liquid TSA medium for 24 hours. The bacterial solution was adjusted to an OD_{625nm} of ~ 0.1 (equivalent to 10^8 cfu/mL). 100 µL of the bacterial solution was spread on a Petri dish containing 3 - 4 mm thick TSA agar medium. After about 10 minutes, an agar well with a diameter of 7 mm was made. 50 µL of the test solution (100 mg/ml for extracts, prepared with 5% DMSO) was added to the well. The Petri dish was kept in the dark at room temperature. The antibacterial disc diffusion (mm) results were observed after 24, 48 and 72 hours.

Determination of minimum growth inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) by broth dilution method

MIC: The experiment was conducted according to Balouiri et al's method[4]. *C. acnes* and *S. epidermidis* were activated in liquid TSA medium for 24 hours to reach an $OD_{625\ nm}$ of ~ 0.1 (equivalent to 10^8 cfu/mL). The experiment was performed on a sterile 96-well plate. The reaction mixture consisted of 100 µL of the test sample and 100 µL of bacterial solution (equivalent to 10^6 cfu/mL). The plate was kept in the dark at 33–37 °C in 24 hours. After that, MIC values were observed and recorded. The MIC was calculated in the well with the lowest extract concentration that could inhibit bacterial growth. Wells that showed turbidity compared to the control wells indicated bacterial growth, while sterilized wells indicated no. When the bacteria did not grow at the lowest concentration, the result was recorded as less than or equal to that concentration. If bacterial growth

was observed at the highest concentration, the result was regarded as greater than that concentration.

MBC: The experiment was carried out using the method of Balouiri et al[4] which involved spreading various concentrations of bacteria that were not visible on the Petri dish containing medium (100 µL/dish). The plates were incubated in the dark at room temperature (33–37 °C) for 24-48 hours to determine the minimum bactericidal concentration (MBC). The lowest concentration recorded was on a plate with a maximum of 3 colonies of growth observed.

Biofilm quantification using the 96-microtiter plate: The experiment was conducted following the method of Di Lodovico et al[8] with some minor adjustments. The experiment was performed on a 96-well plate, each well consisting of 100 µL of the test sample and 100 µL of *C. acnes* (10^6 cfu/mL). The plate was incubated at 37°C for 96 and 120 hours. A micropipette removed the floating liquid and the non-adherent bacteria were washed off with 1X PBS buffer. The plate was air-dried in a sterile incubator for 30 minutes. The wells were fixed with 200 µL of methanol and then dried at 50°C for 10 minutes. The samples were stained with 0.1% (w/v) crystal violet solution for 5 minutes. After removing the excess stain, the wells were rinsed with sterile distilled water three times. The stain was solubilized in 200 µL of 33 % (v/v) acetic acid and the optical density of crystal violet was measured at 595 nm.

Statistical analysis: The experimental treatments were set up entirely randomly and repeated three times, except for preparing extracts. The data obtained from the experiments were statistically analyzed using SPSS 20.0 and Microsoft Office Excel 2020. The results are presented as mean values ± standard deviation and the values were ranked using Duncan's multiple range test with a significance level of p = 0.05.

Results and Discussion

Preparation of total ethanol extract from parts of 4 plant species collected: The results of preparing extracts of the parts of four plant species are shown in the table 1, showing that *H. tiliaceus*, *P. betle* and *H. cordata* have higher moisture content than *C. grandis*. The stem of *H. cordata* has the highest moisture content because it is an herbaceous, succulent plant.

Meanwhile, the roots of *C. grandis* have the lowest moisture level because the harvested part that grows closest to the stem has turned into wood, so it is relatively dry.

Different herbs result in high volumes of herbal extracts and different extraction efficiencies. Turker et al[25] showed that herbal extracts extracted with ethanol and methanol will have a higher antibacterial effect than water in Gram-negative and Gram-positive bacteria. Ethanol can dissolve both compounds. It is also water- and oil-soluble. It can also evaporate quickly, producing a clean, pure extract without any trace of solvent in the finished product.

Besides, it provides suitable collection efficiency. As an active ingredient, ethanol is a solvent that meets safety and environmental friendliness requirements when applied in cosmetics and health care products, in general.

Evaluation of the antioxidant activity of ethanol extracts of parts of 4 plant species

DPPH radical scavenging ability: The results of the antioxidant activity evaluation of the extracts of the four plant species shown in the figure 1 show that all extracts can neutralize DPPH free radicals. This study looked at different extracts. The leaves and roots of *C. grandis*, the stems and roots of *P. betle* had the highest antioxidant activity, with IC50s of less than 100 µg/mL.

Table 1
Preparation of total ethanol extract from parts of 4 harvested plant species.

Plant name	Parts of plant	Wet weight (in gram)	Dry weight (in gram)	Moisture content (%)	Weight of extract (in gram)	Extract yield (%)
C. grandis	Leaves	70,99	30,04	57,68	6,91	23,00
	Stem	3,89	2,01	43,32	0,12	5,97
	Root	3,19	2,03	36,36	0,12	5,91
H. tiliaceus	Leaves	3,49	1,04	70,20	0,14	13,46
	Stem	4,02	2,00	50,24	0,30	15,00
	Root	86,67	30,34	64,99	2,18	7,18
P. betle	Leaves	158,64	30,04	81,06	4,58	15,24
	Stem	163,28	30,20	81,61	9,48	31,39
	Vines	195,07	30,08	85,59	8,25	27,42
	Root	138,53	30,02	80,06	4,23	14,09
H. cordata	Leaves	194,61	30,00	84,58	5,98	19,93
	Stem	200,00	24,36	87,82	1,57	6,44
	Root	188,87	30,05	84,09	8,49	28,25

Extracting leaves of *C. grandis* and stems of P. *betle* had the best activity with IC50s of 52.3 and 54.1 µg/mL respectively, not significantly different from the ascorbic acid as a positive control (29.2 µg/mL). Next were the extracts of *P. betle* and *C. grandis* roots with IC50s of 66.3 and 72.8 µg/mL respectively. Extracts of *C. grandis* stem, leaves and vines of *P. betle* and *H. cordata* leaves had a lower activity with IC50 less than 500 µg/mL. The remaining extracts showed no DPPH radical scavenging potential, with IC50 values all exceeding 1000 µg/mL.

Ferric reducing power capacity: The reducing power assay technique relies on the principle that substances with reduction potential react with potassium ferricyanide (Fe^{3+}) to produce potassium ferrocyanide (Fe^{2+}) which subsequently interacts with ferric chloride to form a ferric-ferrous complex that exhibits a maximum absorption at 700 nm^0. The antioxidant activity values quantified for each species correspond to an extract concentration of 2 mg/mL. The study looked at the antioxidant activity of extracts from four different types of plants, as shown in the figure 2. Among the extracts, the leaves and stems of *P. betle*, the extracts of *C. grandis* and the leaves of *H. cordata* were more active than the other extracts with OD > 0.8.

The stem and leaf extracts of *P. betle* with root extract of *C. grandis* had the best ability to show the highest OD absorbance, with absorbed ODs of 1.124, 1.160 and 1,113 respectively followed by leaves, stem extract *C. grandis and* leaves extract *H. cordata* with OD absorption of 0.949, 0.875 and 0.692 respectively. The remaining extracts had weak antioxidant activity in the reducing ability with OD < 0.5.

ABTS radical scavenging activity: The results of the investigation of the antioxidant activity of extracts of plant parts of four plant species shown in the figure 3 showed that all extracts can neutralize free radicals by the ABTS method. Among the extracts, all parts of *C. grandis*, the stem and roots of *P. betle* and the leaves of *H. cordata* were more active than the extracts of the other parts. The parts of *C. grandis*, the stem *P. betle* IC50 have the ability to neutralize free radicals better than the positive control (Trolox) in this evaluation with IC50 smaller than Trolox and they have the same statistical rank. Next is the root extract of *P. betle* which has the ability to neutralize ABTS free radicals with IC50 of 0.18 mg/mL. The remaining extracts in the study showed the ability to neutralize free ABTS with IC50 greater than 0.2 mg/mL.

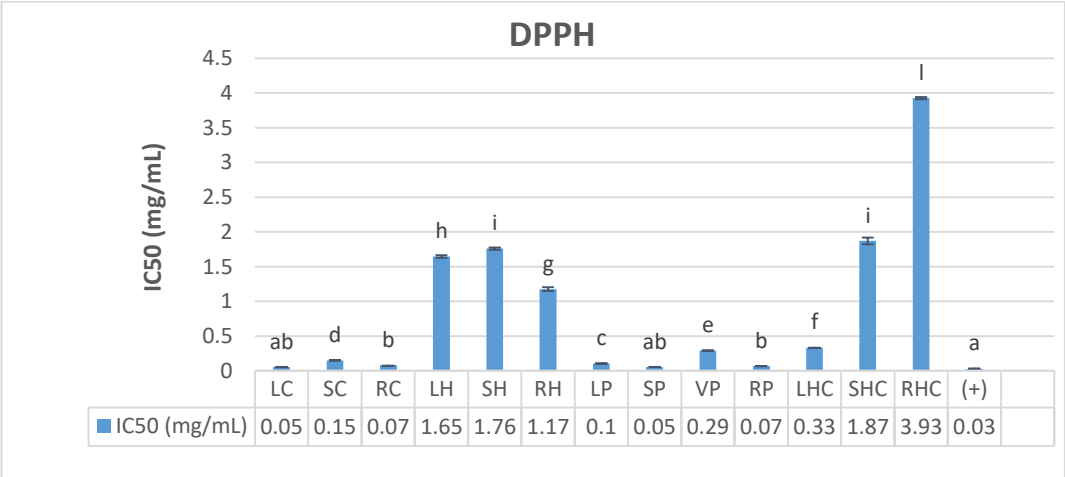

Figure 1: IC50 values for DPPH free radical neutralization of plant extracts.
Different letters on the graph represent statistical differences at the p=0.05 level.

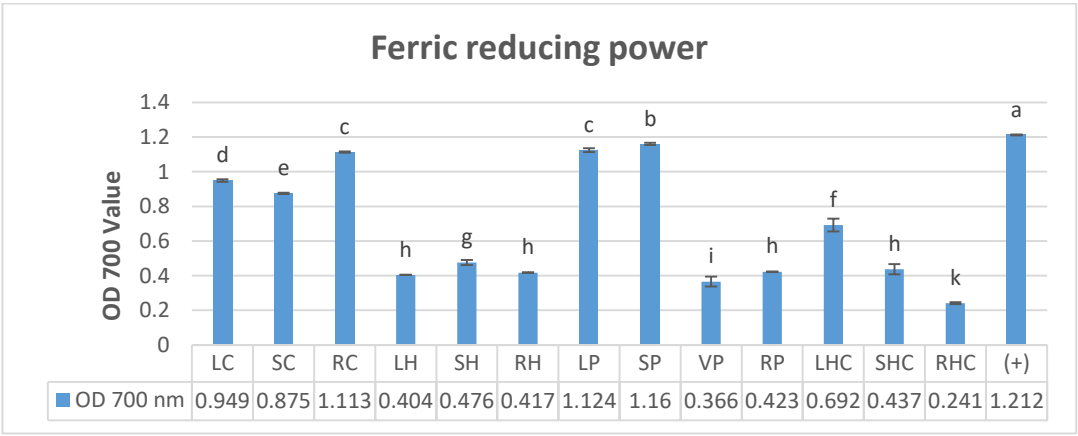

Figure 2: The OD700 absorbance represents the iron reduction capacity of plant extracts.
Different letters on the graph represent statistical differences at the p=0.05 level.

Antioxidant capacity in anti-lipid peroxidation by TBA assay: The antioxidant activity values quantified for each species correspond to extract concentrations of 2 mg/mL in a concentration-dependent manner. Among the four plant species surveyed, parts of *C. grandis*, *P. betel* and *H. cordata* exhibit better resistance to lipid peroxidation than *H. tiliaceus*. These extracts, specifically all parts of *P. betel* and the stem and root of *C. grandis*, could prevent lipid peroxidation by 40% or more. Notably, the stem of *P. betel* and *C. grandis* showed the highest percentages of anti-lipid peroxidation in the survey as 58.28% and 48.60% respectively.

Until now, research on the antioxidant activity of *C. grandis* has mainly focused on the leaves. Studies worldwide have recorded that the antioxidant capacity of *C. grandis* leaves is consistent with this study. Arul et al[3] showed that at the same concentration tested, ethanol extract of *C. grandis* leaves neutralized DPPH free radicals by 87.90%, compared to 81.94% in this study. Prada et al[19] studied the ability to neutralize ABTS free radicals of ethanol extract of *C. grandis* leaves with an IC50 of 0.72 µg/mL while this study found it to be 0.85 µg/mL. This study notes that all parts of *C. grandis* exhibit effective antioxidant activities in the four surveyed models.

Similarly, research on the antioxidant activity of *P. betel* has also mainly focused on the leaves. Research by Nguyen et al[16] revealed that hydroxyl chavicol, eugenol and gallic acid are the main compounds in determining the ability to neutralize free radicals, obtained most effectively in betel plants with ethanol solvent. This study notes that all parts of *P. betel* have effective resistance to oxidation, with the leaves, stems and roots having more effective ability to neutralize free radicals than vines.

Evaluation of the anti-inflammatory activity of ethanol extracts of parts of 4 plant species using the protein denaturation method: The anti-inflammatory activity values quantified for each of the species corresponding to an extract concentration of 2 mg/mL in a concentration-dependent manner.

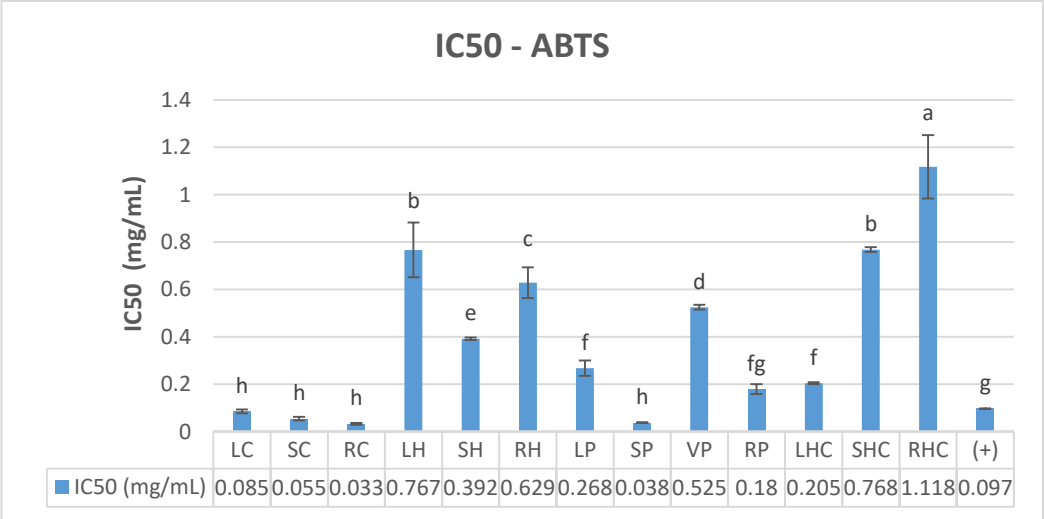

Figure 3: IC50 values for ABTS free radical neutralization of plant extracts.
Different letters on the graph represent statistical differences at the p=0.05 level.

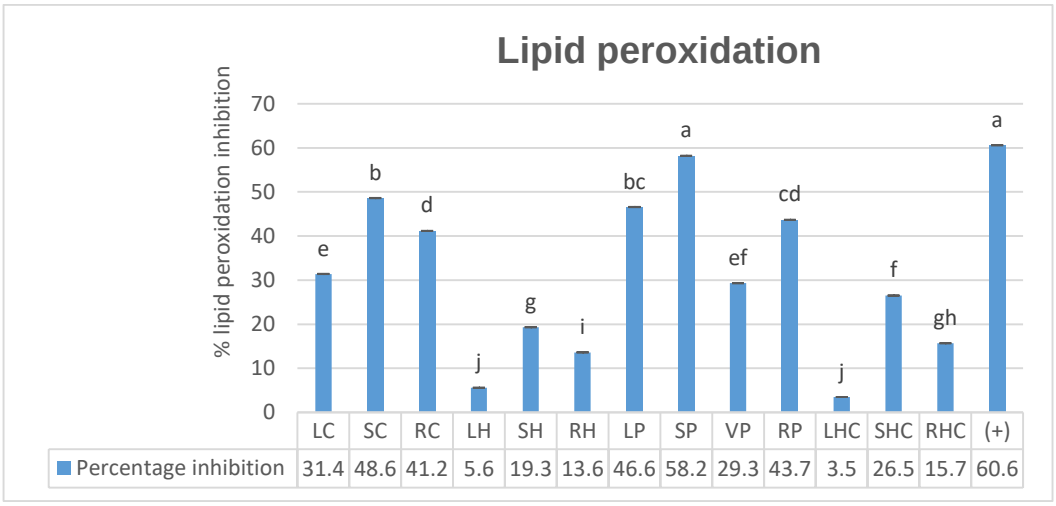

Figure 4: Percent inhibition of lipid peroxidation of plant extracts.
Different letters on the graph represent statistical differences at the p=0.05 level.

The results of investigating the antioxidant activity of extracts of parts of four plant species presented in the figure 5 show that all extracts have the ability to prevent protein denaturation. Among the extracts, extracts of the leaves, stems and roots of *P. betle* and *C. grandis*, leaves of *H. cordata* and *H. tiliaceus* have an anti-denature percentage of >30%. Notably, the stem and leaves of *P. betle* exhibit the strongest anti-inflammatory activity, achieving inhibition percentages of 47.94% and 45.73% respectively.

Currently, only the study by Jain et al[12] recorded the anti-inflammatory ability of carrageenan *C. grandis* leaves in mice. This study highlights that all parts of *C. grandis* possess anti-inflammatory potential, with the leaves and stem demonstrating more effective properties than the roots, ranking just behind the leaves and stems of the betel plant.

Studies mainly investigate the anti-inflammatory ability of the leaves of *P. betel*. Dohi et al[9] noted that ethanol extract from *P. betel* leaves has anti-inflammatory properties. The authors also pointed out that eugenol, one of the main components of *P. betel* leaves, has been shown to have anti-inflammatory effects in many animal models with different inflammatory agents. As mentioned in antioxidant activity, ethanol is the solvent that captures most of these compounds. In particular, the leaves and stems of *P. betel* have the most effective anti-inflammatory properties compared to parts of the other three plant species in this survey.

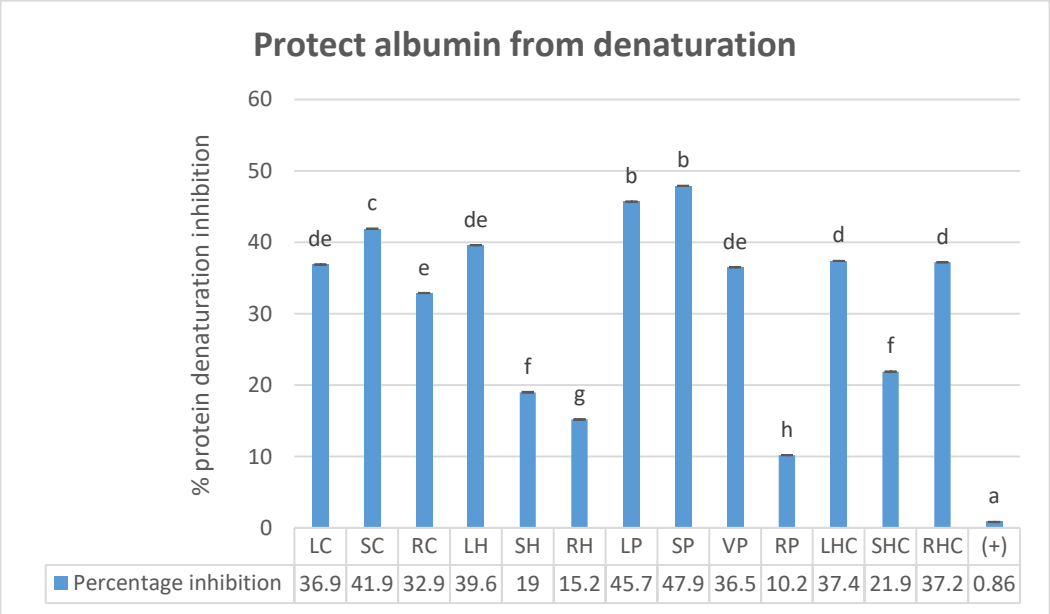

Figure 5: Percentage inhibition of albumin denaturation of plant extracts.
Different letters on the graph represent statistical differences at the p=0.05 level.

Table 2
Antibacterial ring diameter of *C. acnes* of the extracts.

Plant name	Parts of plant	Zone of Inhibition *C. acnes* diameter (mm) according to time frames		
		24h	48h	72h
C. grandis	Leaves	$19,33 \pm 1,15^{b}$	$19,00 \pm 1,00^{b}$	$19,00 \pm 1,00^{b}$
	Stem	$17,67 \pm 1,53^{b}$	$17,33 \pm 1,15^{b}$	$17,00 \pm 1,00^{c}$
	Root	$18,00 \pm 1,00^{b}$	$17,67 \pm 0,58^{b}$	$17,00 \pm 0,00^{c}$
H. tiliaceus	Leaves	-	-	-
	Stem	-	-	-
	Root	-	-	-
P. betle	Leaves	$8,67 \pm 0,58^{d}$	$8,67 \pm 0,58^{f}$	$8,00 \pm 1,00^{e}$
	Stem	$11,67 \pm 0,58^{c}$	$11,00 \pm 0,00^{e}$	$9,33 \pm 1,53^{e}$
	Vines	-	-	-
	Root	$8,67 \pm 0,58^{d}$	$8,33 \pm 0,58^{f}$	$7,67 \pm 0,58^{e}$
H. cordata	Leaves	-	-	-
	Stem	-	-	-
	Root	-	-	-
(+)		$25,33 \pm 0,58^{a}$	$25,33 \pm 0,58^{a}$	$25,33 \pm 0,58^{a}$

In a row, average values with different letters are statistically different at p=0.05.
Note: "-": inactive; "+": positve control as doxycycline 0.14 mg/ml

Evaluation of the the antibacterial activity against C. acnes and S. epidermidis of ethanol extracts of parts of 4 plant species

By agar disk diffusion method: The results of investigating the antibacterial activity of the extracts against *C. acnes* are presented in table 2. Extracts of parts of *H. tiliaceus* and *H. cordata* do not have the ability against *C. acnes*. Extracts of parts of *C. grandis* and *P. betle* (except the vines) have inhibitory activity against *C. acnes*. Among the 4 plant species surveyed, all parts of *C. grandis* maintained antibacterial activity against *C. acnes* after 72 hours of survey. The most prominent of these are the leaves and stems of *C. grandis* because the diameter of the antibacterial ring is large and stable during 24 to 48 hours of survey.

The results of investigating the antibacterial activity of the extracts against *S. epidermidis* are presented in table 3. Extracts of parts of *C. grandis*, *P. betle* (except vines), *H. cordata* leaves and *H. tiliaceus* leaves have the ability to inhibit *S. epidermidis*. The most prominent are the leaves and large stems of *C. grandis* because they have the largest antibacterial ring diameter, in which the leaves have a stable antibacterial ring within 24 to 48 hours of survey and the antibacterial ring diameter of the *C. grandis* stem is stable for 24 hours, then decreased by 0.33 – 1.00 mm after the next 48 and 72 hours.

By broth dilution method: The MIC and MBC of *C. acnes* and *S. epidermidis* of all extracts (Table 4a and 4b) have antibacterial activity. The MIC value was determined by the lowest concentration that inhibited bacterial growth in the microplate, indicated by the first turbid concentration. In contrast, the MBC value was identified as the lowest concentration that could kill nearly all bacterial colonies, as demonstrated by the last clear microplate.

The results of investigating the antibacterial activity of different types of extracts against *C. acnes* by the broth dilution method show that *P. betle* has better antibacterial ability against *C. acnes* than *C. grandis*. According to Canillac and Mourey[7], if the MBC/MIC ratio is less than or equal to 4, the test sample is considered to have bactericidal ability; if this ratio is greater than 4, the test sample has a bacteriostatic effect. Therefore, the extracts in this survey have the ability to kill *C. acnes*.

Until now, Luan et al's[14] research on *C. grandis* leaves has shown inhibition of different strains of *C. acnes* isolated from acne skin, with an antibacterial ring diameter of 15 - 25 mm at a concentration of 200 mg/mL and MIC= 30 mg/mL. Additionally, they identified three compounds quercetin, rutin and aloe-emodin that play a major role in combating *C. acnes*. This study further investigated the stem and roots of *C. grandis*, revealing that these parts produced an antibacterial ring diameter only 1 - 2 mm less than the leaves and maintained this effect for 72 hours. Moreover, when examining the MIC and MBC values, results indicated that the stem and roots exhibit stronger antibacterial activity against *C. acnes* than the leaves.

Notably, this study also demonstrates that all parts of the *C. grandis* plant inhibit another bacterium associated with acne formation: *S. epidermidis*. The stem and roots, in particular, perform on par with or even better than the leaves. Therefore, the entire plant contains multiple compounds with significant antibacterial properties against both *C. acnes* and *S. epidermidis*, with the stem and roots showing particularly strong potential. Therefore, the entire plant contains multiple compounds with significant antibacterial properties against both *C. acnes* and *S. epidermidis* with the stem and roots showing particularly strong potential.

Table 3
Antibacterial ring diameter of *S. epidermidis* of the extracts.

Plant name	Parts of plant	Zone of Inhibition *S. epidermidis* diameter (mm) according to time frames:		
		24h	48h	72h
C. grandis	Leaves	$17,67 \pm 0,58^{a}$	$17,67 \pm 0,58^{a}$	$16,33 \pm 0,58^{b}$
	Stem	$16,00 \pm 1,00^{b}$	$15,67 \pm 0,58^{b}$	$15,00 \pm 1,00^{c}$
	Root	$12,33 \pm 0,58^{c}$	$12,33 \pm 0,58^{c}$	$12,00 \pm 0,00^{d}$
H. tiliaceus	Leaves	$4,33 \pm 0,58^{f}$	$4,00 \pm 0,00^{g}$	$3,67 \pm 0,58^{h}$
	Stem	-	-	-
	Root	-	-	-
P. betle	Leaves	$5,67 \pm 0,58^{e}$	$5,67 \pm 0,58^{f}$	$5,33 \pm 0,58^{g}$
	Stem	$8,67 \pm 0,58^{d}$	$8,33 \pm 0,58^{e}$	$8,33 \pm 0,58^{f}$
	Vines	-	-	-
	Root	$8,67 \pm 0,58^{d}$	$8,33 \pm 0,58^{e}$	$8,00 \pm 1,00^{f}$
H. cordata	Leaves	$5,67 \pm 0,58^{e}$	$4,67 \pm 0,58^{g}$	$4,67 \pm 0,58^{gh}$
	Stem	-	-	-
	Root	-	-	-
(+)		$22,67 \pm 0,58^{a}$	$22,67 \pm 0,58^{a}$	$22,67 \pm 0,58^{a}$

In a row, average values with different letters are statistically different at p=0.05.
Note: "-": inactive; "+": positve control as chloramphenicol 0,5 mg/ml.

Table 4a
MIC and MBC concentrations of extracts against bacteria *C. acnes*.

Plant name	Parts of plant	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC
C. grandis	Leaves	10	12,5	1,25
	Stem	6,25	7,5	1,2
	Root	7,5	10	1,33
P. betle	Leaves	3,75	5	1,33
	Stem	2,5	3,75	1,5
	Root	3,75	7,5	2

Table 4b
MIC and MBC concentrations of extracts against bacteria *S. epidermidis*.

Plant name	Parts of plant	MIC (mg/mL)	MBC (mg/mL)	MBC/MIC
C. grandis	Leaves	10	12,5	1,25
	Stem	7,5	10	1,33
	Root	10	12,5	1,25
H. tiliaceus	Leaves	5	10	2
P. betle	Leaves	2,5	3,75	1,5
	Stem	3,75	7,5	2
	Root	7,5	10	1,33
H. cordata	Leaves	10	12,5	1,25

Table 5
Percent inhibition of *C. acnes* biofilm formation of the extracts.

Plant name	Parts of plant	% inhibition biofilm *C. acnes* formation	
		72h	96h
C. grandis	Leaves	$44,50 \pm 0,08^{a}$	$29,21 \pm 0,02^{a}$
	Stem	$34,97 \pm 0,02^{b}$	$22,97 \pm 0,01^{b}$
	Root	$17,21 \pm 0,03^{c}$	$3,93 \pm 0,01^{e}$
H. tiliaceus	Leaves	-	-
	Stem	$4,19 \pm 0,01^{d}$	-
	Root	-	-
P. betle	Leaves	-	-
	Stem	$11,67 \pm 0,02^{c}$	$8,48 \pm 0,01^{d}$
	Vines	-	-
	Root	$17,50 \pm 0,05^{c}$	$11,17 \pm 0,01^{c}$
H. cordata	Leaves	-	-
	Stem	-	-
	Root	-	-

In a row, average values with different letters are statistically different at p=0.05.
Note: "-": inactive

According to research published by Wirasuta et al[28], chavicol and eugenol are two compounds that determine the antibacterial ability of *P. betel* leaf extract against *C. acnes*. According to Purba et al[20], in addition to eugenol, compounds with antibacterial properties against *C. acnes* include kaempferol, apigenin, quercetin, myrecetin, ferrulic acid, p-coumaric acid, rutin, syringic acid, caffeic acid, gallic acid etc. This study found that the leaves, stems and roots of the betel tree can inhibit *S. epidermidis* and *C. acnes* and the betel tree's vine dose not have the ability to inhibit these two bacteria.

Evaluation of the antibiofilm C. acnes formation of ethanol extracts of parts of 4 plant species: The results of surveying the biofilm formation inhibition activity of extracts of parts of four plant species are given in table 5. At the surveyed concentrations at 10 mg/mL, the extracts of all parts of *C. grandis*, stem and root of *P. betle and* stem of *H. tiliaceus* had the ability to inhibit *C. acnes* biofilm formation after 72 hours.

In particular, leaves and stem of *C. grandis* extracts effectively inhibited the biofilm formation of *C. acnes* with inhibition percentages of 44.5% and 34.97% respectively. After 96 hours, the inhibition percentage of leaves and stem of *C. grandis* decreased by 1.9 - 1.5 times in which *H. tiliaceus* stem lost the ability to inhibit biofilm after 96 hours of investigation. The long-lasting acne condition and

difficulty in achieving effective treatment are largely due to *C. acnes* biofilm, which makes it difficult for active ingredients to reach acne-causing agents. Howlin et al[11] announced that tobramycin and vancomycin are the two antibiotics with the strongest ability to inhibit *C. acnes* but are less effective when *C. acnes* is surrounded by biofilm.

Besides, Howlin et al[11] commented that removing biofilms that have been formed, is very difficult. Therefore, it is necessary to inhibit biofilm formation rather than find ways to remove already formed biofilm. Among the four plants investigated, *C. grandis* exhibited the strongest ability to inhibit biofilm formation by *C. acnes*. Furthermore, the leaves and stems of *C. grandis* are promising sources and may contain various compounds with the potential to reduce biofilm formation, which could contribute to acne prevention.

Conclusion

In summary, all four surveyed plant species exhibit bioactivities that support the prevention of acne. Among them, *C. grandis* and *P. betle* are the most promising, demonstrating the best antioxidant, antibacterial, anti-albumin denaturation and biofilm inhibition activities against *C. acnes*. The study also reveals that not only the leaves but also the roots and stems of these two plant species possess significant bioactivities. These findings guide the search for natural materials capable of replacing current antibiotics used in acne treatment.

Additionally, it is essential to explore the potential compounds from *C. grandis* and *P. betle* to understand their acne prevention mechanisms and to apply these compounds in the production of natural formulations for future cosmetic use.

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