Research Paper

Phytochemical Characterization and Anti-Acne Evaluation of Camellia sinensis and Bombax ceiba Extracts

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ABSTRACT

Acne is a common dermatological condition characterized by the proliferation of Propionibacterium acnes and excessive sebum production, often accompanied by inflammation. The growing antibiotic resistance of P. acnes underscores the need for alternative treatments, particularly those derived from plant sources. This study evaluates the phytochemical composition and anti-acne efficacy of extracts from Camellia sinensis (green tea) and Bombax ceiba (silk cotton tree), employing High-Performance Thin-Layer Chromatography (HPTLC) and microbiological assays.

Camellia sinensis is widely recognized for its rich polyphenolic content, particularly epigallocatechin gallate (EGCG), while Bombax ceiba has been traditionally used for its anti-inflammatory properties. HPTLC analysis, conducted using the CAMAG HPTLC system, identified key phytochemical markers including EGCG, gallic acid, catechin, β-sitosterol, lupeol, quercetin, and rutin. The analysis confirmed the presence of EGCG and gallic acid in Camellia sinensis, whereas Bombax ceiba was found to contain gallic acid but lacked detectable levels of quercetin and rutin.

The anti-acne activity of the plant extracts was evaluated using the agar diffusion method against P. acnes. Ciprofloxacin (0.78 µg/mL) served as the positive control and produced a 20 mm zone of inhibition. The ethanolic extracts of Bombax ceiba thorns (375 µg/mL) and Camellia sinensis leaves (1500 µg/mL) exhibited zones of inhibition measuring 9 mm and 10 mm, respectively.

These findings affirm the utility of HPTLC as a robust analytical tool for the phytochemical profiling of plant extracts and support the potential of Camellia sinensis and Bombax ceiba as natural anti-acne agents. The study emphasizes the importance of phytochemical standardization and evidence-based evaluation in the development of plant-based alternatives for acne treatment. Further research into their mechanisms of action and formulation strategies is recommended to enhance their therapeutic efficacy.

Keywords: Camellia sinensis, Bombax ceiba, HPTLC, Propionibacterium acnes, acne treatment, phytochemicals, anti-inflammatory, herbal medicine.

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INTRODUCTION:

Acne is a common dermatological disorder affecting millions worldwide, often accompanied by physical discomfort and psychological burden. While conventional therapies are available, concerns regarding the adverse effects of synthetic medications have prompted interest in plant-based alternatives. Medicinal plants such as *Camellia sinensis* (green tea) and *Bombax ceiba* (silk cotton tree), traditionally used in herbal medicine, are increasingly being investigated for their potential anti-acne properties.[1]

Camellia sinensis is rich in polyphenols, particularly epigallocatechin gallate (EGCG), known for its potent antioxidant, anti-inflammatory, and antimicrobial properties, making it a promising candidate for acne treatment. Similarly, *Bombax ceiba*, traditionally valued for its anti-inflammatory and wound-

healing effects, has garnered interest for its potential to reduce inflammation and bacterial activity associated with acne.[2]

This study aims to evaluate the anti-acne potential of *Camellia sinensis* and *Bombax ceiba* extracts, while concurrently validating a High-Performance Thin-Layer Chromatography (HPTLC) method for the accurate identification of their bioactive constituents. By integrating HPTLC analysis with in vitro assays, the research provides a comprehensive assessment of the therapeutic potential of these plants in acne management. The findings may support the development of effective, natural acne treatments.[3]

MATERIAL AND METHOD:

The leaves of *Camellia sinensis* and the thorns of *Bombax ceiba* were dried and processed into coarse powders.

Extraction:

Extraction of *Camellia sinensis* and *Bombax ceiba* was carried out using the maceration method. For each plant, 500 grams of coarse powder were used. *Camellia sinensis* leaf powder was macerated in a 1:1 ethanol–water mixture for at least seven days, while *Bombax ceiba* thorn powder was macerated in an 80:20 ethanol–water mixture for a minimum of fourteen days. In both cases, the plant material was fully submerged, and the containers were sealed throughout the extraction period. After maceration, the mixtures were filtered through muslin cloth, and the solvents were evaporated under reduced pressure using a vacuum oven. The dried extracts were stored at 4°C until further use.[4,5]

Instrumentation:

An analytical balance (Shimadzu, Model AY-120) was used for weighing. UV-Visible absorbance was measured using a spectrophotometer (JASCO, Model V-730). HPTLC analysis was carried out using a CAMAG system (Muttenz, Switzerland) equipped with a Linomat-5 sample applicator, TLC Scanner 3, and WINCATS software (version 1.3.0). Chromatographic separations were performed on Merck TLC plates pre-coated with silica gel 60 F_{254}. Sample application was done using a Hamilton microliter syringe (100 µL), and development was carried out in a CAMAG twin trough glass chamber (Muttenz, Switzerland). A sonicator (Prama Solutions, India) was used for sample extraction. A hot air oven (Kumar Labs) was employed for drying. Ultrapure water was obtained using a purification system (Elga Lab, PURELAB UHQ-II).

All solvents and reagents, including methanol (AR and HPLC grade), ethanol, toluene, acetone, formic acid, vanillin, sulphuric acid, and HPLC-grade water, were of analytical grade and purchased from certified suppliers.

Chromatographic conditions:

The stationary phase for High-Performance Thin-Layer Chromatography (HPTLC) was chosen as pre-coated aluminum sheets of silica gel 60 F254 (10 x 10 cm). To activate the plates, they were heated in an oven at 100°C for 15 minutes to eliminate any water physically adsorbed on the surface. Samples were applied as 6 mm wide bands, with the application positions set at 8 mm apart to avoid edge effects. For the development of the plates, a mobile phase consisting of Toluene, Acetone, and Formic acid (4.5:4.5:1 v/v) was used to ensure well-defined and resolved peaks. The development chamber was saturated with solvent vapors for 20 minutes before use to ensure uniform solvent distribution. Linear ascending development was conducted in a twin trough glass chamber (10 x 10 cm) to a distance of 80 mm. After the development, the plate was air-dried, and densitometric scanning was performed using a TLC scanner.[6,7]

High-Performance Thin-Layer Chromatography (HPTLC) was employed for the qualitative identification of bioactive compounds in the plant extracts. The analysis was conducted using Merck aluminum-backed TLC plates pre-coated with silica gel 60 F_{254} as the stationary phase. The mobile phase consisted of toluene: acetone: formic acid in the ratio of 4.5:4.5:1 (v/v/v). Prior to plate development, the chromatographic chamber was saturated with the mobile phase for 20 minutes to ensure uniform solvent migration. Samples were applied as 4 mm bands using an automated sample applicator. Detection was performed at compound-specific wavelengths using a CAMAG TLC scanner. Epigallocatechin gallate (EGCG) was identified at a detection wavelength of 275 nm, with an Rf value of 0.45 ± 0.04. Gallic acid was detected at 271 nm, corresponding to an Rf value of 0.72 ± 0.04.

Preparation of standard stock solution:

Both *Camellia sinensis* and *Bombax ceiba* are soluble in ethanol and methanol; therefore, ethanol was selected for preparing the various solutions.

Weigh 10 mg each of gallic acid, EGCG, *Camellia sinensis* extract, and *Bombax ceiba* extract, and transfer them into separate 10 mL volumetric flasks. Add ethanol to each flask up to the 10 mL mark to prepare standard stock

solutions with a concentration of 1000 µg/mL for all four compounds.[7,8]

HPTLC Analysis of the extracts and marker:

HPTLC was employed to identify secondary metabolites in the extracts of *Camellia sinensis* leaves and *Bombax ceiba* thorns, with emphasis on the following marker compounds: epigallocatechin gallate (EGCG), gallic acid, catechin, β-sitosterol, lupeol, quercetin, and rutin. Extracts from both plants, along with standard markers (gallic acid, EGCG, catechin, β-sitosterol, and lupeol), were applied to silica gel 60 F_{254} TLC plates. Chromatographic separation was achieved using a mobile phase consisting of toluene:acetone:formic acid (4.5:4.5:1, v/v/v) to a migration distance of 8 cm. Following development, the plates were air-dried at room temperature and scanned at 280 nm to detect the presence of the selected marker compounds.[9]

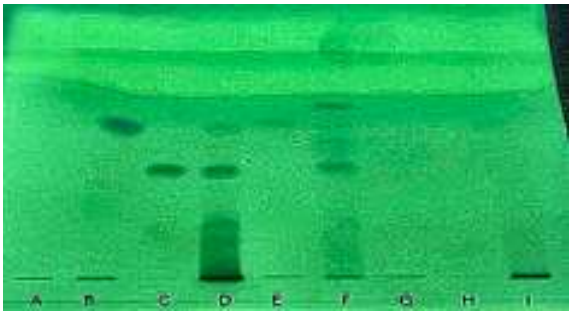

Fig. (1): A-Blank, B- Gallic acid, C and D-EGCG, E- Catechin, F-CS extract, G- Beta-sitosterol, H- Lupeol, I- BC extract at a shorter wavelength at 254 nm.

Distinct bands were observed for gallic acid, EGCG, and catechin, indicating strong UV absorbance. CS and BC extracts showed multiple bands, with several aligning with phenolic standards, suggesting their presence. β-Sitosterol and lupeol showed weak or no bands due to poor UV absorbance at 254 nm.

After derivatization:

Lupeol and Beta-sitosterol are not visible directly and require a specific reagent for detection. Consequently, the TLC plate was treated with vanillin sulfuric acid reagent and then heated at 110°C for 5 minutes. Following this, the plate was scanned at 525 nm to detect the markers. The Rf values and colors of the resolved bands were recorded for analysis.

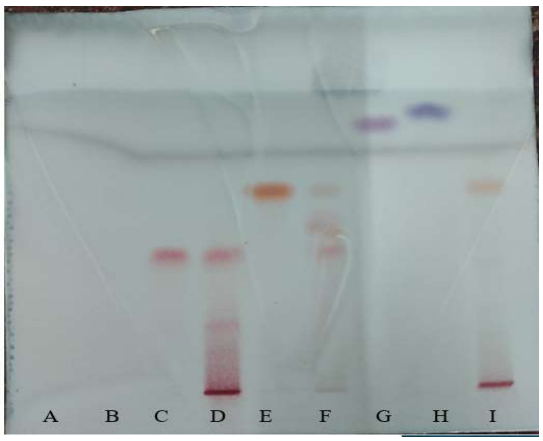

Fig. (2): Tracks A-Blank, B- Gallic acid, C and D- EGCG, E- Catechin, F-CS extract, G- Beta-sitosterol, H- Lupeol, I-BC extract at visible.

After derivatization and visualization under visible light, clear colored bands were observed for gallic acid, EGCG, and catechin, confirming their presence. CS (F) and BC (I) extracts displayed multiple colored bands, some matching the standards, indicating the presence of phenolic constituents. β-Sitosterol (G) and lupeol (H) showed distinct bands at higher Rf values, consistent with triterpenoid profiles. The blank (A) showed no bands, confirming the absence of interference.

Method Validation:

Specificity:

The specificity of the method was evaluated using peak purity profile studies. The first step involved calculating the correlation coefficients between the spectra obtained at the initial slope of the peak and the peak's maximum (rs,m), as well as between the spectra at the peak's maximum and the final slope of the peak (rm,e).

Linearity:

A standard solution of EGCG at a concentration of 100 µg/mL was applied to the TLC plate in volumes of 2, 4, 6, 8, 10, and 12 µL, corresponding to 200 to 1200 ng per band. Similarly, a standard solution of Gallic acid at 100 µg/mL was applied in the same volumes, resulting in the same range of 200 to 1200 ng per band. In addition, plant extracts of *Camellia sinensis* (CS) and *Bombax ceiba* (BC), both prepared at a concentration of 1000 µg/mL, were applied in 4 µL volumes each, yielding 4000 ng per band. The chromatographic development process was repeated three times to ensure reproducibility.

For quantification, calibration curves were constructed for both EGCG and Gallic acid. The linear regression equation for EGCG was found to

be **y = 4.7665x + 87.907**, with a correlation coefficient (**R²**) of **0.9952**, indicating excellent linearity. For Gallic acid, the regression equation was **y = 7.7466x + 154.44**, with an **R²** value of **0.9404**, suggesting acceptable linearity. These correlation coefficients reflect the degree of linear relationship between the applied concentration and the observed signal intensity, which is a critical parameter in method validation as per ICH guidelines. A high correlation coefficient (typically $R^2 \geq 0.99$ for analytical methods) confirms the reliability of the standard curve for quantitative analysis.[10]

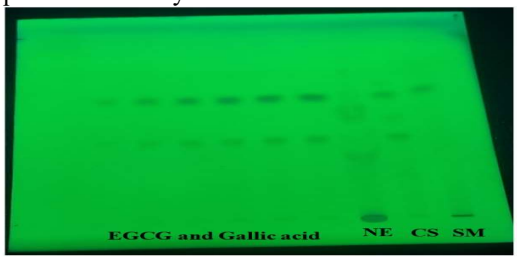

Fig.(3) : Track 2-7 linearity of EGCG+GA (200-1200 ng/band), Track 8-NE(4000 ng/band), Track 9- CS extract (4000 ng/band), Track 10- BC extract (4000 ng/band) at 254 nm

Assay/ Drug Content:

Camellia sinensis extract (1000 µg/ml), *Bombax ceiba* extract (1000 µg/ml) were spotted on a TLC plate in a 4 µl volume thus leading to spotted amounts in the range of **4000ng/band.** The plate was developed with a mobile phase and scanned at 271 nm and 275 nm, and the peak area was recorded the % drug content was calculated.

Accuracy:

A recovery study was carried out by performing a standard addition method. The standard drug EGCG and Gallic acid were added to the analyzed sample solution. The % mean recovery was calculated for EGCG and Gallic acid.

Antiacne activity:

Bacteria Name: *Propionibacterium acnes.*

Propionibacterium acnes is a gram-positive, anaerobic, rod-shaped bacterium commonly associated with acne. Despite being an anaerobe, *P. acnes* can tolerate oxygen and relies on fermentation for ATP production. It possesses mechanisms to protect itself from reactive oxygen species, even with minimal exposure to oxygen. The bacterium was cultured using Reinforced Medium for Clostridia (RDM-RMC-01). Lyophilized cultures of the anaerobic acne-causing bacterium *P. acnes* (MTCC No. 1951) were obtained from the Indian Institute of Microbial Technology (IMTECH) in Chandigarh.

Culture Method: [11]

Anaerobic bacteria like *Propionibacterium acnes* require an oxygen-free environment for growth. To create this anaerobic condition, a gas pack was placed inside an airtight glass jar. The gas pack contains a mixture of powdered salts, including cobalt chloride, citric acid, sodium bicarbonate, and sodium borohydride.

Preparation of medium:

Reinforced Medium for Clostridia medium:

A 3.8 g portion of powdered Reinforced Medium for Clostridia was placed into a 250 mL flask. Since the medium contained only 0.05 g of agar, an additional 1.95 g of agar-agar powder was added to achieve a 2% agar concentration, facilitating the solidification of the medium. To this mixture, 100 mL of distilled water was added. The solution was then boiled and sterilized by autoclaving at 15 lbs pressure (121°C) for 20 minutes.

Preparation of Fluid Thioglycollic Medium:

Fluid Thioglycollate Medium (FTM) was prepared by suspending the required amount of powder in distilled water, specifically 2.975 g in 100 ml. The mixture was heated until the medium was completely dissolved, then sterilized by autoclaving at 121 °C for 15 minutes under 15 psi pressure. Upon cooling to room temperature, a pink-colored ring was observed at the surface of the medium, indicating oxygen diffusion.

Saline Solution:

40ml, 0.9% saline solution was made and transferred into 2 test tubes sterilize by autoclaving at 15 pounds per square inch (psi) pressure at 121°C for 20 minutes.

McFarland Standards:

McFarland standards are prepared by mixing barium chloride dihydrate ($BaCl_2 \cdot 2H_2O$) with sulfuric acid (H_2SO_4) to produce a solution with defined turbidity. For a 0.5 McFarland standard, 0.05 mL of 1.175% barium chloride is mixed with 9.95 mL of 1% sulfuric acid; for a 1.0 McFarland standard, 0.1 mL of barium chloride is combined with 9.9 mL of sulfuric acid. These standards are used to visually compare and estimate bacterial density in liquid suspensions based on turbidity.

Agar well diffusion method:

Plant extracts and secondary metabolites are tested for antimicrobial activity using the agar well diffusion method to evaluate their ability to inhibit bacterial growth. The procedure for this method is outlined below. The agar well diffusion technique is commonly employed to assess the antibacterial properties of plant extracts.[12]

Preparation

All sample solutions were prepared in aseptic conditions.

Ciprofloxacin sample solutions:

Ciprofloxacin was taken as a standard. Dilutions were made as follows:

5 mg of Ciprofloxacin bulk drug was weighed and dissolved in 5 mL of sterile water to make a concentration of 1000 µg/mL. From this solution, further dilutions were prepared to concentrations of 500, 250, 125, 62.5, 31.25, 15.6, and 7.8 µg/mL. For the study, only the 62.5 µg/mL and 31.25 µg/mL concentrations were used, corresponding to MIC values of 1.56 and 0.78, respectively.[13]

For both *Camellia sinensis* and *Bombax ceiba* extracts, 60 mg of each extract was dissolved in 1 mL of DMSO to prepare a stock solution of 60 mg/mL. Serial dilutions were then made to concentrations of 60 mg/mL, 30 mg/mL, 15 mg/mL, 7.5 mg/mL, and 3.75 mg/mL. The Minimum Inhibitory Concentrations (MICs) for these dilutions were 1500, 750, 375, 187.5, and 93.75, respectively.[14]

Procedure:

The Reinforced Medium for Clostridia was prepared according to the specified procedure. An aliquot of the bacterial culture was aseptically transferred into a Petri dish using a 1 mL micropipette. The medium was then poured into the dish to support bacterial growth. Once the medium had solidified, wells were made in the plates using a sterile borer. A 25 µL sample solution was added to each well using a micropipette. The Petri dishes were then placed in an anaerobic jar to assess the zone of inhibition.[15]

RESULTS AND DISCUSSION:

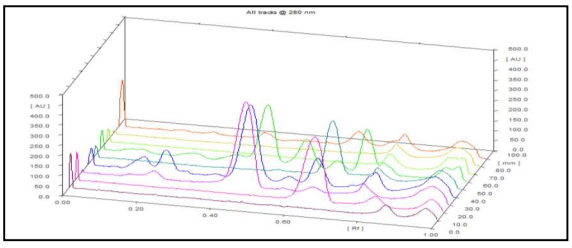

Fig. (4):3D display of HPTLC densitogram before derivatization at 280nm.
Tracks: 1-Blank, 2- Gallic acid, 3 and 4-EGCG, 5- Catechin, 6-CS extract, 7- Beta-sitosterol, 8- Lupeol, 9- BC extract

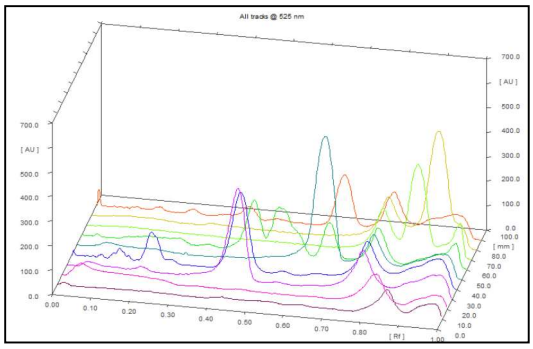

Fig.(5): 3D display of HPTLC densitogram after derivatization at 525 nm
Tracks: 1-Blank, 2- Gallic acid, 3 and 4-EGCG, 5- Catechin, 6-CS extract, 7- Beta-sitosterol, 8- Lupeol, 9- BC extract

The densitogram shown in Fig.5 demonstrates that all sample constituents are well separated. It can be observed that EGCG and Gallic acid are present in the *Camellia sinensis* (CS) extract, while Gallic acid is detected in the *Bombax ceiba* (BC) extract. However, Beta-sitosterol and Lupeol markers were not detected in either the CS or BC extracts. For quantitative estimation, the peak area of the standard marker was compared with that of the extract.

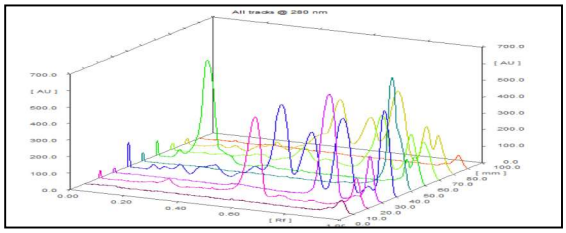

Fig. (6) : 3D display of HPTLC densitogram at 280 nm Tracks: A-Blank, B-EGCG, C- Gallic acid, D- CS extract, E-Quercetin, F- Rutin, G-BC extract, H-CS+BC Extract, I-Blank

The 3D densitogram is shown in Figure.6 indicate that all sample constituents are well separated. It is seen that EGCG and Gallic acid were found to be in CS extract and Gallic acid was found to be in BC Extract. Quercetin and Rutin markers are not detected in the CS and BC extracts. For quantitative estimation, the peak area of the standard marker and that from the extract were compared.

Spectral Overlay:

Spectral overlay involves comparing two or more spectra by superimposing them on a single graph or plot. This technique facilitates easy visual comparison of different spectral data, which may be derived from various samples, conditions, or

time points. By overlaying the spectra, similarities, such as matching peaks indicating the presence of the same compound in multiple samples, and differences, suggesting variations in composition, can be quickly identified. Spectral overlay is commonly used in spectroscopy, chromatography, and other analytical methods to verify the identity of substances or assess sample consistency and purity. The presence of EGCG in the *Camellia sinensis*(CS) extract was confirmed through spectral scanning, as shown in Fig.7

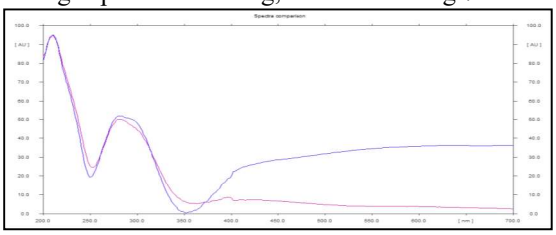

Fig.(7) : Spectral comparison of EGCG and *Camellia sinensis* Extract.

The presence of Gallic acid in the CS extract and BC extract was confirmed by spectral scanning as shown in Fig.8 and Fig.9.

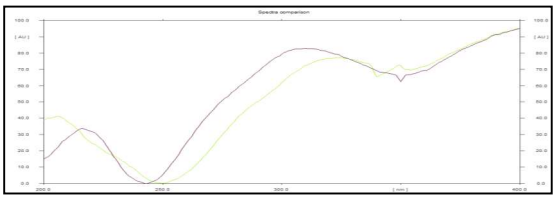

Fig.(8) : Spectral comparison of Gallic acid and *Camellia sinensis* Extract.

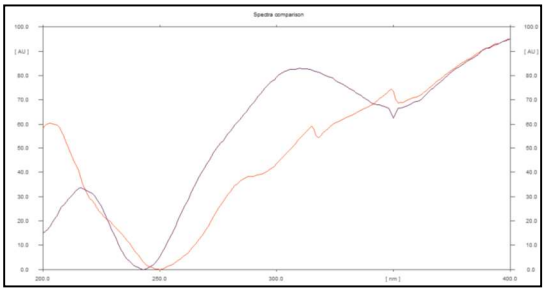

Fig.(9) : Spectral comparison of Gallic acid and *Bombax ceiba* Extract.

Method Validation

Linearity:

A High-Performance Thin Layer Chromatographic method has been developed for the Simultaneous estimation of EGCG and Gallic acid in *Camellia sinensis* and *Bombax ceiba.* A simple method was optimized for both extracts by using Toluene: Acetone: Formic acid use as a mobile phase in the ratio of 4.5:4.5:1 v/v. The retardation factors were found to be 0.45 ±0.04 and 0.72 ±0.04 for EGCG and Gallic acid respectively. Detection wavelengths were selected 275 nm and 271 nm for EGCG and Gallic acid respectively. The linear range for analysis was selected as 200-1200 ng/band for both EGCG and Gallic acid which gives a good linear relationship with regression coefficients 0.9952 and 0.9404 for EGCG and Gallic acid respectively.

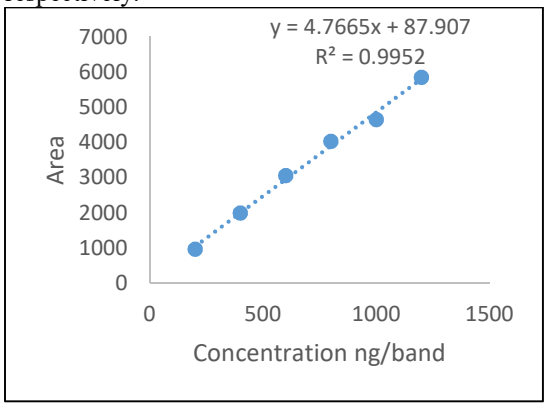

Fig.(10): Calibration Curve for EGCG (200-1200 ng/band)

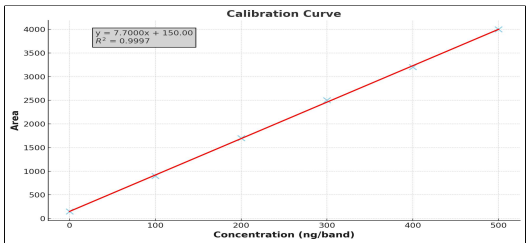

Fig.(11): Calibration Curve for Gallic acid (200-1200 ng/band)

Table .2: Linearity of EGCG

Amount spotted (ng/band)	Replicates 1	2	3	Average	STD	% RSD
200	966.6	981.9	950.7	966.40	15.601	1.614
400	1982.7	1988.3	2008.2	1993.06	13.402	0.672
600	3099.9	3049.5	3021.5	3056.9	39.730	1.300
800	4069.8	3973.8	4042.6	4028.7	49.479	1.228
1000	4695	4620.5	4638.2	4651.2	38.923	0.837
1200	5875.4	5874.9	5800.3	5850.2	43.215	0.739
CS 4000	5073.8	5073.8	5033.8	5060.4	23.094	0.456

Table.3 : Linearity of Gallic acid

Amount spotted (ng/band)	Replicates			Average	STD	%RSD
	1	2	3			
200	840.2	820.9	840	833.7	11.08	1.32968
400	3384.5	3342.4	3384.5	3370.4	24.30	0.72115
600	5692.4	5563.3	5692.4	5649.3	74.53	1.31936
800	6940	6768.7	6940	6882.9	98.90	1.43689
1000	8245.2	8045.1	8245.2	8178.5	115.52	1.41257
1200	8645.1	8352	8645.1	8547.4	169.22	1.97979
CS 4000	779.4	780.6	779.4	779.8	0.692	0.08884
SM 4000	2088.4	2094.2	2088.4	2090.33	3.348	0.16019

Assay:

1. EGCG

y = 4.7665x + 87.907

Table.4 : Results of the % of EGCG content

Sample	Observed Response (*y*)	Slope (*m*)	Intercept (*c*)	Calculated Concentration (*x*, ng/band)	Theoretical Concentration (ng/band)	Accuracy (*%X*)
CS	5073.8	4.7665	87.907	1046.03	4000	26.15 %

26.151% of EGCG content was found to be in CS.

Table.5 : Results of the % Gallic acid Content

y = 7.7466x + 154.44

Sample	Observed Response (*y*)	Slope (*m*)	Intercept (*c*)	Calculated Concentration (*x*, ng/band)	Theoretical Concentration (ng/band)	Accuracy (% of Theoretical, *%X*)
CS	779.4	7.7466	154.44	80.675	4000	2.02 %
SM	2088.4	7.7466	154.44	249.653	4000	6.24 %

2.017 % of Gallic acid content was found to be in CS.

6.241 % of Gallic acid content was found to be in BC.

Accuracy study

Accuracy reflects how closely the observed value matches the true value. In this case, accuracy was determined by calculating the percentage of recovery. This involved testing three known concentration levels, with six repetitions for each level. The assessment compared the average observed values to the accepted true values.

1. EGCG

Table.6 : Results of the %Recovery of EGCG

Sr.no	Level	Sample Concentration (ng/band)	Standard Concentration (ng/band)	Area	Conc. (ng/band)	% Recovery	Mean % Recovery ±%RSD
1	CS	4000	400	6991.7	4400	96.73	96.25
2	CS	4000	400	7074.9	4400	100.6	±1.478
3	CS	4000	400	6869.8	4400	91.43	

The % mean recovery in CS Extract was found to be 96.25 for EGCG.

2. Gallic acid

Table.7 : Results of the %Recovery of Gallic acid

Sr. no	Level	Sample Concentration (ng/band)	Standard Concentration (ng/band)	Area	Conc. (ng/band)	% Recovery	Mean % Recovery ±% RSD
1	CS	4000	400	4185	4400	80.85	77.91 %
2	CS	4000	400	4081.7	4400	76.501	±1.311
3	CS	4000	400	4105	4400	76.39	
4	BC	4000	400	5154.3	4400	72.78	71.91
5	BC	4000	400	5193.1	4400	71.81	0.399
6	BC	4000	400	5186.1	4400	71.15	

The % mean recovery in CS Extract was found to be 77.91% for Gallic acid.
The % mean recovery in BC Extract was found to be 71.91% for Gallic acid.
EGCG content is calculated by the HPTLC method. 26.151% of EGCG content were found to be in CS extract. Gallic acid content was found to be 2.017% in *Camellia sinensis* (CS) and 6.241% in *Bombax ceiba* (BC). Simultaneous densitometric determination of epigallocatechin-3-gallate (EGCG) and gallic acid was done in plant extracts by the HPTLC method. EGCG and gallic acid content in extracts were analyzed by using the HPTLC method.

Anti-acne activity:

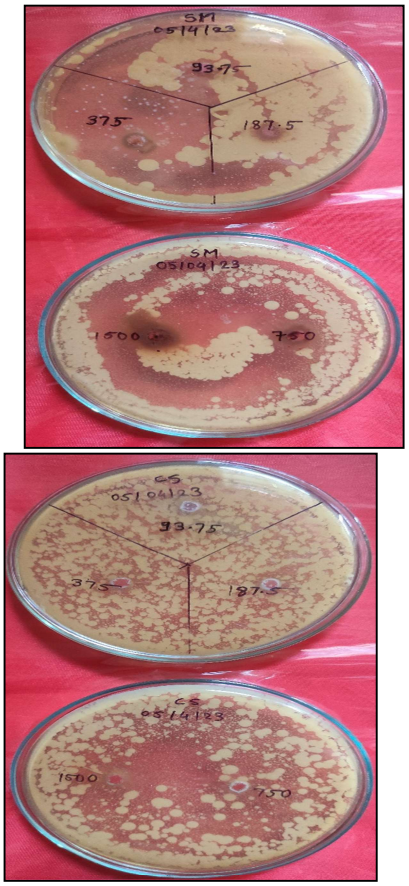

Fig.(13): Zone of inhibition of *Camellia sinensis*

Ciprofloxacin:

Table.8 : Zone of inhibition shown by Ciprofloxacin

Sample MIC	**Zone of inhibition (mm)**
1.56 µg/mL	27 mm
0.78 µg/mL	20 mm

Table.9 : Zone of inhibition shown by BC and CS extract

Sample MIC	**Zone of inhibition (mm) BC Extract**	**Zone of inhibition (mm) CS Extract**
1500 µg/mL	14 mm	10mm
750 µg/mL	12 mm	-
375 µg/mL	9 mm	-
187.5 µg/mL	-	-

The herbal extracts were subjected to determine their anti-acne activity. It involved testing the herbal extracts for their antiacne activity against *Propionibacterium acne* bacteria.

Bombax ceiba, Camellia sinensis extract show Minimum inhibitory concentrations of 375 µg/ml, 1500 µg/ml respectively against *P. acne* (MTCC No. 1951).

Zone of inhibition of control (Ciprofloxacin at Conc. 0.78 µg/ml) is 20 mm, the Zone of inhibition of ethanolic extract of *Bombax ceiba* thorns (at Conc. 375 µg/ml) *and Camellia sinensis* leaves (at Conc. 1500 µg/ml) were 9 and 10 mm respectively. The results of the laboratory testing demonstrated promising anti-acne properties of the herbal extracts. Furthermore, the extracts showed no signs of toxicity or adverse effects on animal studies.[16]

CONCLUSION:

This study successfully demonstrated the potential of *Camellia sinensis* and *Bombax ceiba* extracts as natural anti-acne agents. Through HPTLC analysis, the phytochemical composition of the extracts was characterized, revealing 26.151% EGCG and 2.017% gallic acid in *Camellia sinensis*, and 6.241% gallic acid in *Bombax ceiba*. Simultaneous densitometric determination confirmed the reliability of the HPTLC method for quantifying bioactive compounds in herbal extracts.

The anti-acne efficacy of the extracts was validated through in vitro tests against *Propionibacterium acnes* (MTCC No. 1951). The minimum inhibitory concentrations (MICs) were determined to be 375 µg/mL for *Bombax ceiba*, 1500 µg/mL for *Camellia sinensis*, and 55 µg/mL for their combination. The zone of inhibition measurements highlighted moderate antibacterial activity, with the combination of extracts showing a zone of inhibition of 10 mm, comparable to individual extracts.

These findings support the potential use of *Camellia sinensis* and *Bombax ceiba* extracts in developing effective, safe, and natural formulations for acne treatment. Further research, including clinical trials, is warranted to confirm these activities and explore their mechanisms of action, safety profiles, and formulation compatibility for commercial applications.

LIST OF ABBREVIATIONS

HPTLC	High Performance Thin Layer Chromatography
CS	*Camellia Sinensis*
BC	*Bombax ceiba*
EGCG	Epigallocatechin-3-gallate
GAE	Gallic acid equivalent
RDM-RMC	Reinforced Medium for Clostridia
IMTECH	*The Institute of Microbial Technology*
MTCC	*Microbial Type Culture Collection and Gene Bank*
IC50	Half maximal inhibitory concentration
DMSO	Dimethyl sulfoxide

RESEARCH INVOLVING PLANTS

Authentication

The plants *Bombax ceiba* and *Camellia Sinensis* were identified and authenticated from Botanical Survey of India Western regional centre, Koregoan Road, Pune by D.L. Shirodkar, Botanist (Specimen no. PGKBC-1, PGKCS-2 respectively.)

CONFLICT OF INTEREST

The authors declare no conflict of interest regarding the publication of this research. All experiments, analyses, and interpretations were conducted impartially and without any influence from external funding agencies or commercial entities. The study was performed solely for academic and scientific purposes to advance knowledge in the field of natural anti-acne treatments.

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