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Antiulcer Properties of Guava and Chameli Leaves: A Phytochemical Exploration for Oral Ulcer Management

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Abstract

This study explores the therapeutic potential of *Psidium guajava* (Guava) and *Jasminum grandiflorum* (Chameli) leaves for oral ulcer treatment. Traditionally used in Ayurveda, these plants are rich in bioactive compounds. Ethanolic extracts were assessed for phytochemical content, antioxidant, and anti-inflammatory properties. Thin-layer chromatography (TLC) confirmed the presence of flavonoids, phenolics, tannins, saponins, terpenoids, steroids, glycosides, and alkaloids. Total phenolics and flavonoids were quantified using colorimetric assays. Antioxidant activity was evaluated through total antioxidant capacity (TAC) and ferric reducing power (FRAP) assays. Anti-inflammatory activity was assessed via albumin denaturation inhibition, anti-protease activity, and membrane stabilization assays. Antibacterial efficacy against *Bacillus subtilis* and *Escherichia coli* was tested using agar well diffusion. Guava extract exhibited higher total phenolic content (121.6 mg GAE/g) and comparable flavonoid content (280 mg QE/g) to Chameli. Both extracts demonstrated notable antioxidant activity, with Guava showing superior anti-inflammatory effects across all assays and antibacterial activity against both bacterial strains. These findings highlight the bioactive compounds contributing to the therapeutic potential of Guava and Chameli leaves. The developed herbal oral paste was effective in promoting healing, supporting its potential for oral ulcer treatment, although further clinical validation is needed.

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INTRODUCTION

Mouth ulcers, or oral ulcers, are lesions of the mucous membrane lining the oral cavity [1] [2]. Several factors can contribute to their formation, including nutritional deficiencies**,** physical trauma**,** medical conditions**,** irritants and genetics [3] [4]. Conventional treatments for mouth ulcers include steroids, painkillers, and antiseptic mouthwashes [5]. While effective, these can have side effects like tooth discoloration, bad breath, and hormonal imbalances [6]. The growing interest in natural remedies has led to a surge in the popularity of herbal medicines for various health conditions,

including mouth ulcers. Many people perceive herbal remedies to be a more natural and holistic approach to healthcare, with fewer side effects compared to conventional medications [7]. Herbal treatments can be more affordable and easier to access than conventional medications, particularly in regions with limited access to healthcare resources. The World Health Organization (WHO) and the Government of India recently collaborated to establish a Global Centre for Traditional Medicine, highlighting the growing recognition and integration of traditional healing practices into modern healthcare systems [8].

This study explores the potential of *Psidium guajava* (Guava) and *Jasminum grandiflorum* (Chameli) leaves for treating mouth ulcers. Traditionally used in Ayurveda, these plants possess various bioactive compounds with potential therapeutic benefits. Guava is a tropical fruit native to India, Indonesia, Bangladesh, and South America [9]. It has a long history of use in traditional medicine, with extracts from its leaves exhibiting various properties, including anti-cancer, anti-malarial, anti-diabetic, antioxidant, anti-diarrheal, and antimicrobial properties [10]. Guava leaves are also believed to help with high cholesterol, vision problems, blood sugar control, as well as heart, and liver health [11] [12]. The anti-inflammatory and immune-boosting properties of guava leaves are attributed to their high content of ascorbic acid (vitamin C) and citric acid [13]. Guava leaf extracts (aqueous, chloroform, ethanol, and methanol) have also demonstrated potent antimicrobial activity [14].

Jasminum grandiflorum, commonly known as Chameli, is prized for its beautiful, fragrant flowers. In Ayurveda, Chameli leaves are used clinically for wound healing [15]. They have been traditionally employed to treat a wide range of ailments, including body aches, stomach aches, toothaches, boils, ulcers, nausea, constipation, detoxification, etc [16]. Topical application of Chameli leaf extract is believed to promote wound healing and relieve itching. Its high phenolic content and antioxidant capacity contribute to its anti-inflammatory properties [17]. Chameli leaves are also chewed for oral hygiene, as they are thought to strengthen teeth and gums and alleviate stomatitis (inflammation of the mouth lining). Fresh leaf juice is used as a topical remedy for corns, and the leaves themselves are chewed for mouth ulcers [18].

Both Guava and Chameli leaves are rich in phytochemicals, naturally occurring compounds with potential health benefits. The presence of these phytochemicals is linked to the anti-ulcer activity of these plants. Drawing on prior research suggesting the anti-ulcer properties of Guava and Chameli leaves, this investigation employed in vitro phytochemical assays to assess the antioxidant and anti-inflammatory potential of their ethanolic extracts. Our ultimate objective is to evaluate the suitability of these extracts for formulating an efficacious oral paste to treat mouth ulcers. In particular, this study aims to determine if the herbal formulation can alleviate pain and expedite the healing process of mouth ulcers.

MATERIALS AND METHODS

Collection of Plant Material and Plant Extract Preparation:

Guava and Chameli leaves were obtained from locally grown plants and air-dried for 48 hours (Figure 1). The dried leaves were then mechanically ground into a coarse powder (Figure 2). Five grams of each powdered material (Guava and chameli) were separately weighed and immersed in 25 mL of ethanol for 24 hours at room temperature. The mixtures were then filtered using Whatman filter paper No. 42. The resulting filtrates were collected and used as the Guava and Chameli leaf extracts for further experiments.

Figure 1: A) Guava and B) Chameli Leaves

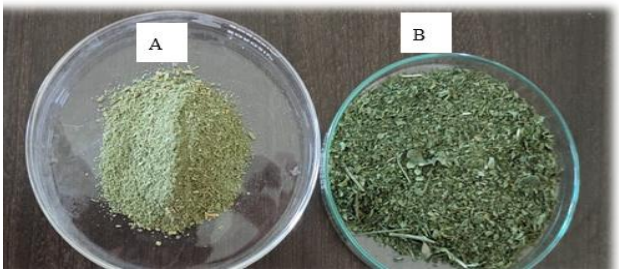

Figure 2: A) Guava and B) Chameli Leaves Dried Powder

Thin Layer Chromatography:

Silica gel G (400 mesh) was used as the stationary phase for TLC plates prepared in the laboratory. The extracts were applied 2 cm from the bottom edge of the plates using a micropipette. The developed plates were obtained using a chloroform-methanol solvent system (15:1 v/v) in a chromatography chamber. After air-drying, the plates were visualized under visible light. Retention factors (Rf values) were calculated for each visible band [19].

Phytochemical Evaluation:

Extracts from Guava and Chameli leaves were subjected to various qualitative tests to determine the presence of specific phytochemical classes by standard biochemical test [20].

Flavonoids: The alkaline reagent test was performed by mixing 2 mL of extract with 2 mL of 40% NaOH solution. A deep yellow coloration indicated the presence of flavonoids.

Phenolic compounds: The ferric chloride test was employed. Briefly, 1-2 mL of extract was treated with 1 mL of 5% $FeCl_3$ solution. A blue-black appearance confirmed the presence of phenolic compounds.

Tannins: Braymer's test was used. 1 mL of extract was treated with 2 mL of 10% $FeCl_3$ solution. A blue-black coloration indicated the presence of tannins.

Saponins: The foam test was conducted. 2 mL of extract were diluted with 5 mL of distilled water in a test tube and vigorously shaken. Stable foam formation suggested the presence of saponins.

Terpenoids: The Salkowski test was performed. 2 mL of extract were treated with 1 mL of chloroform. Concentrated H_2SO_4 was carefully added along the test tube wall to form a layer. A reddish-brown coloration at the interface indicated the presence of terpenoids. The same test was performed for identification of steroids.

Glycosides: The Keller-Killiani test was used. 5 mL of extract were treated with 2 mL of glacial acetic acid and 1 mL of 5% $FeCl_3$ solution. After gentle heating and cooling, 2 mL of concentrated H2SO4 were added. A reddish-brown color at the junction of the two liquids and a bluish-green color in the acetic acid layer indicated the presence of glycosides.

Quantitative Estimations:

Total Phenolic Content (TPC):

TPC was determined using a modified Folin-Ciocalteu method [21]. A gallic acid standard solution (1 mg/mL) was prepared and used to generate a calibration curve. Serial dilutions of the extracts were prepared. The reaction mixture for each sample dilution and standard concentration included 2.5 mL of Folin-Ciocalteu reagent (FC reagent) and 2 mL of sodium carbonate solution. After incubation, the optical density (OD) was measured at 630 nm against a blank. The TPC of the extracts was calculated based on the gallic acid calibration curve and expressed as milligram gallic acid equivalents (GAE) per gram of sample (mg/g).

Total Flavonoid Content (TFC):

The aluminum chloride colorimetric method was employed to quantify TFC. A calibration curve was constructed using quercetin standard solutions (1 mg/mL) prepared [21]. The extracts were also serially diluted. The reaction mixture for each standard and sample dilution contained 0.1 mL of 10% $AlCl_3$ solution, 0.1 mL of 1M potassium acetate solution, and 2.2 mL of water. After incubation at room temperature for 30 minutes, the optical density (OD) was measured at 430 nm against a blank. The TFC of the extracts was calculated based on the quercetin calibration curve and expressed as quercetin equivalents (QE) per gram of sample (mg/g).

Antioxidant Activities:

Production of free radicals can lead to oxidative stress (OS), a condition implicated in various diseases due to its detrimental effects at the molecular, cellular, and tissue levels. Antioxidant compounds play a crucial role in mitigating these effects by scavenging free radicals, thereby minimizing their harmful influence. By neutralizing these reactive molecules, antioxidants can potentially prevent or delay various forms of cellular damage. Additionally, they contribute to processes that maintain cell viability and promote DNA repair mechanisms.

Total Antioxidant Capacity (TAC):

TAC is a measure of a sample's ability to scavenge free radicals, reflecting its overall antioxidant capacity or reductive potential. The assay principle relies on the inhibition of a substrate's oxidation by

antioxidants. In this method, when a reducing agent (antioxidant) in the test solution reacts with Mo(VI) ions in the acidic phosphomolybdate reagent, it reduces them to Mo(V) ions. This reduction is accompanied by a color change from colorless to bluish green [22]. Ascorbic acid (1 mg/mL) is used as a standard, and a calibration curve is generated using various concentrations (25-125 µg/mL). Both the standard and sample solutions are allowed to react with the phosphomolybdate reagent at 95°C for 15 minutes. The optical density (OD) is then measured at 670 nm. The TAC of the samples is determined using the calibrated ascorbic acid standard curve and expressed as ascorbic acid equivalents (AAE) per gram of sample (mg/g).

Ferric Reducing Power Assay (FRPA):

The FRPA relies on the basic and key property of transition metals, i.e., they generate/change color when their oxidation state is changed. An antioxidant will reduce Fe^{3+} (ferric ion) to Fe^{2+} (ferrous ion) under acidic conditions [23]. Different concentrations of ascorbic acid (standard) and extracts in sodium phosphate buffer (0.2M, pH 6.6) were mixed with 1% Potassium ferricyanide and incubated at 50°C for 20 minutes. Then 10% TCA was added along with 0.1% $FeCl_3$. The mixture was left aside at RT for 10 minutes and then OD was recorded at 670 nm. The FRPA for aliquots was determined by a calibrated ascorbic acid standard curve and expressed as ascorbic acid equivalents (AAE) in mg/g of the sample.

Anti-Inflammatory Activity:

Inhibition of Albumin Denaturation:

The anti-inflammatory potential of the extracts was assessed using a modified protein denaturation assay [24]. Briefly, 100 µL of each extract, distilled water (negative control), and aspirin solution (150 mg/10 mL in water, positive control) were separately incubated with 0.4 mL of 1% bovine serum albumin (BSA) in 3 mL of phosphate-buffered saline (PBS, pH 6.4) at 37°C for 5 minutes. The mixtures were then heated in a water bath at 70°C for 15 minutes. After cooling, the absorbance of each sample was measured at 670 nm. The following formula will be used to calculate the percentage inhibition of protein denaturation:

$$\text{Percentage inhibition} = \frac{\text{abs control} - \text{abs sample}}{\text{abs control}} \times 100$$

Anti-Protease Action:

The anti-protease activity of the extracts was evaluated using a modified method [25]. Briefly, various concentrations of the extracts, along with a reference drug (aspirin) and a control (water), were incubated with trypsin (50 µL, 10 µg/mL), Tris-HCl buffer (0.5 mL, 20 mM, pH 7.4) at 37°C for 5 minutes. Subsequently, 0.5 mL of 1% BSA was added to each test tube, followed by an additional incubation at room temperature for 20 minutes. The reaction was terminated by adding 1 mL of 10% TCA. The Biuret test was employed to assess anti-protease activity. Biuret reagent (0.5% $CuSO_4$ and 10% NaOH) was added to each tube, and a separate reaction with 1% BSA and the reagent served as a positive control for color intensity comparison. The color produced by the extracts was compared to the control and BSA reactions to assess their anti-protease potential.

RBC Membrane Stabilization:

The anti-inflammatory activity of the extracts was assessed using the HRBC (human red blood cell) membrane stabilization assay with slight modifications [21].

Preparation of 10% RBC suspension:

Fresh whole human blood was obtained from the pathology department. The blood was then centrifuged at 3000 rpm for 5 minutes to separate plasma from red blood cells (RBCs). The supernatant plasma was discarded. The RBC pellet was washed twice with saline to remove residual plasma components. The packed RBC volume was measured, and a 10% (v/v) suspension was prepared by resuspending the RBCs in fresh PBS at pH 7.4.

Heat induced hemolysis:

Various concentrations of the extract, the reference drug (aspirin), and the control (PBS) were each mixed with 3.5 mL of PBS buffer (pH 7.4) and 100 µL of a 10% RBC suspension. The tubes were incubated at room temperature for 10 minutes, followed by a 20-minute incubation in a water bath at 54°C. After cooling, the reaction mixtures were centrifuged at 2500 rpm for 3 to 5 minutes, and the supernatant absorbance was measured at 540 nm.

Assuming the hemolysis in the control to be 100%, the percentage of hemolysis was calculated. The percentage inhibition of hemolysis was determined using the following formula:

$$\text{Percentage inhibition} = \frac{\text{abs control} - \text{abs sample}}{\text{abs control}} \times 100$$

Antibacterial Activity

This study investigated the antibacterial activity of Guava and Chameli leaf extracts using a modified agar well diffusion method [26]. A Gram-positive and a Gram-negative bacterial culture grown in laboratory were used for the assay. Four wells were created in solidified nutrient agar plates. Each plate was then inoculated with the respective bacterial culture using a streaking technique. Subsequently, 50 µL and 100 µL aliquots of both Guava and Chameli leaf extracts were pipetted separately into the designated wells on each plate. The plates were allowed for a 30-minute diffusion period followed by overnight incubation at 37°C. Following incubation, the presence of clear zones surrounding the wells was indicative of antibacterial activity by the extracts.

Figure 3: Formulated Herbal Paste for Oral Ulcer

Formulation of Herbal Paste and its Application

An oil-based herbal paste was formulated for the treatment of oral ulcers. The formulation included approximately 2 grams of Guava leaves and 0.5 grams of Chameli leaves. To prepare the base, coconut oil was infused with 20-25 pudina leaves by heating coconut cream with the leaves on low flame. The specified amounts of powdered Guava and Chameli leaves were then mixed with 6 mL of the infused coconut oil. Additionally, 1 gram of stevia powder (as a sweetener) and 0.01% sodium benzoate (as a preservative) were added to the mixture. The formulated product (Figure 3) was left to infuse overnight to ensure proper blending of the ingredients. The paste was subsequently tested on a 17-year-old male patient with frequent oral ulceration, applied consecutively for 5 days.

Statistical Analysis

All experiments were performed in triplicate to assess reproducibility. Data are presented as the mean ± standard deviation (SD) to reflect both the central tendency and variability of the measurements. Statistical calculations and generation of standard graphs were performed using Microsoft Excel software.

RESULTS

Percentage Yield and Color of Plant Extract:

Ethanol extraction was employed to isolate phytochemicals from the leaf powders. This process involved submerging the powders in ethanol, facilitating the solvation and subsequent extraction of the target compounds. The resulting crude extracts were then subjected to further analysis. The percentage yield and color of each extract are presented in Table 1, with a visual representation of the color provided in Figure 4.

Table 1: Extraction Yield and Color of Extracts

Parameters	Chameli extract	Guava extract
Obtained volume	7 ml	12.1 ml
Color	Bottle green color	Dark olive color
Odor	Grassy smell	Pleasant guava smell
Percentage yield	28%	48.4%

$$\text{*percentage yield} = \frac{\text{volume of extract obtained}}{\text{volume of ethanol used}} \times 100$$

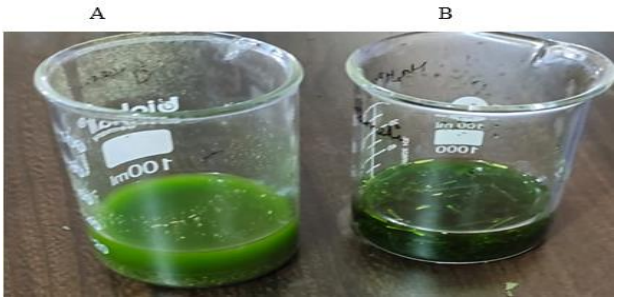

Figure 4: Crude Extracts of Guava and Chameli Leaves

Thin Layer Chromatographic Profiling:

Thin-layer chromatography (TLC) profiling was performed on the ethanolic extracts of Guava and Chameli leaves to assess the presence and diversity of colored phytochemicals. The observed colored spots under visible light are depicted in Figure 5. A comparative analysis of the TLC profiles is presented in Table 2. The varying Rf values of the extracts from both leaves suggested the presence of structurally similar phytochemicals with distinct banding patterns. These findings may be relevant to the antioxidant activity of this plant.

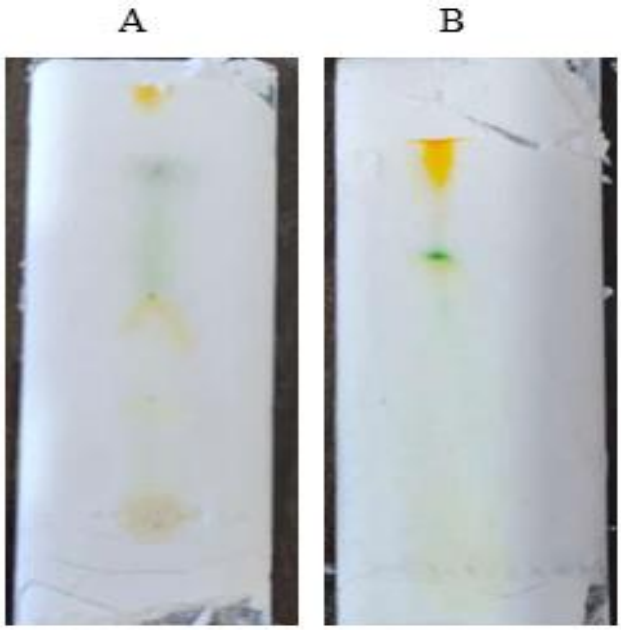

Figure 5: TLC Profile of Extracts of A) Guava and B) Chameli Leaves

Table 2: TLC Separation of Phytochemicals

Extracts	No. of spots	Color	R_f
Guava	4	Yellow	0.275
		Light green	0.5
		Dark green	0.793
		Orange	1
Chameli	4	Yellow	0.596
		Green	0.692
		Dark yellow	0.75
		Orange	1

Phytochemical Screening:

Qualitative phytochemical analysis of the ethanolic extracts from Guava and Chameli leaves revealed the presence of a broad spectrum of secondary metabolites, including flavonoids, polyphenols, terpenoids, glycosides, and saponins (Table 3). Notably, all identified phytochemicals, except saponins, were found to be more abundant in the Guava leaf extract compared to Chameli leaves. Notably, flavonoids and polyphenols are well-documented for their diverse biological activities, including antioxidant and anti-inflammatory properties. The observed presence of these compounds in both leaf extracts potentially supports the traditional medicinal uses of Guava and Chameli leaves. Figure 6 depicts the qualitative screening results based on colorimetric reactions.

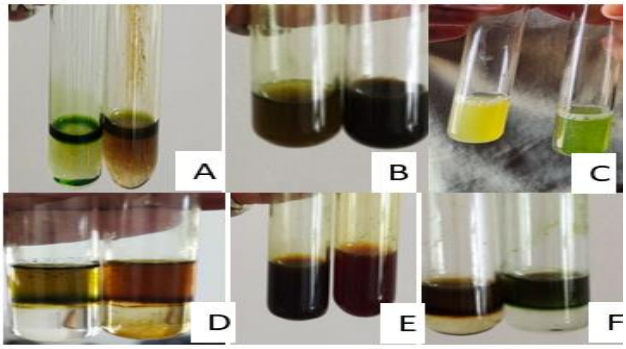

Figure 6: Colored Reactions of Phytochemicals A) Flavonoid, B) Polyphenol, C) Saponin, D) Glycosides, E) Alkaloids, F) Terpenoids for Chameli (left) and Guava (right) Leaves

Table 3: Results of Qualitative Phytochemical Screening

Test	Guava	Chameli
Flavonoids	++	+
Polyphenols	++	+
Tannins	+++	++
Saponins	+	++
Terpenoids	++	+
Steroids	++	+
Glycosides	+++	+
Alkaloids	++	+

Strongly present +++ Moderately present ++ Fair +

Total Phenolic Content (TPC):

The total phenolic content of the ethanolic extracts from Guava and Chameli leaves was determined using gallic acid as a standard based on a calibration curve (Figure 7 A). The Guava leaf extract exhibited a significantly higher phenolic content (121.6 ± 0.124 mg GAE/g of sample) compared to the Chameli leaf extract (29.5 ± 0.341 mg GAE/g of sample), with Guava leaves containing

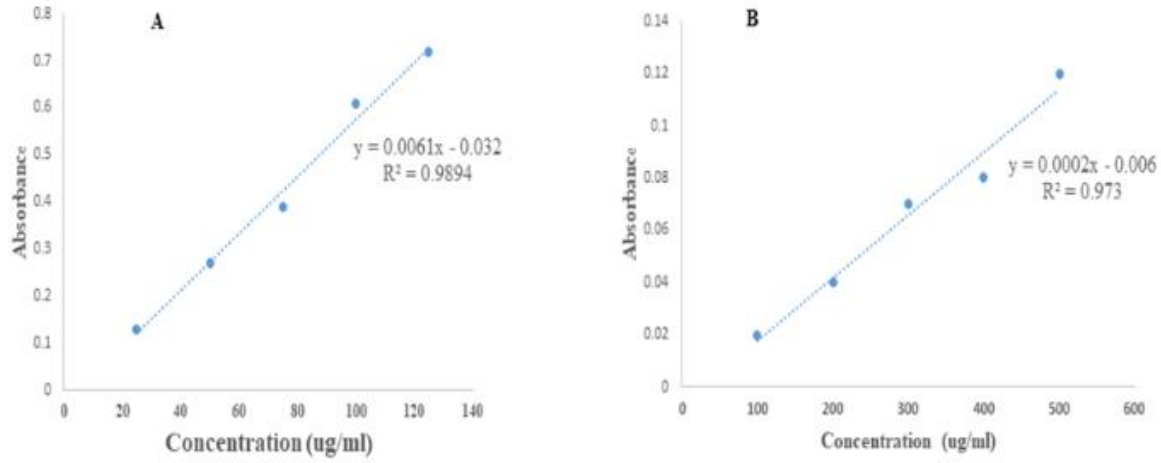

Figure 7: Standard graph of (A) Gallic Acid, B) Quercetin

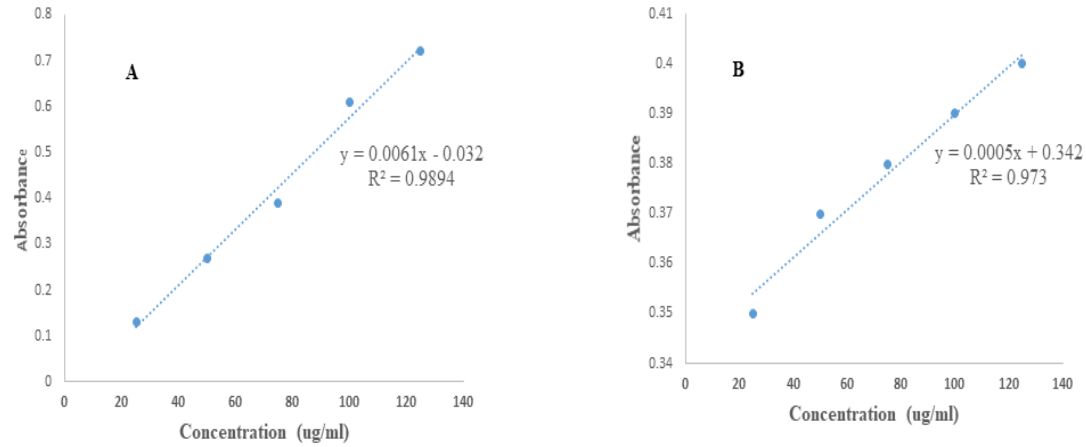

Figure 8: Calibration curve of Ascorbic Acid for A) TAC and B) FRPA

Table 4. Total Phenolic, Flavonoid Content, and Antioxidant Activity Quantification by TAC and FRAP in Guava and Chameli Extracts

Sample	Total phenolics (mgGAE/g	Total flavonoids (mgQE/g)	TAC (mgAAE/g)	FRAP (mgAAE/g)
Guava	121.6±0.124	275.65±0.325	129.35±0.112	160.15±0.436
Chameli	29.5±0.341	286.768±0.212	68.84±0.217	145.85±0.398

nearly fivefold the amount of phenolics. The detailed experimental results for the extracts are presented in Table 4.

Total Flavonoid Content (TFC):

Quantification of total flavonoid content (TFC) was performed using the aluminum chloride colorimetric assay with quercetin as a standard. A standard curve of absorbance against quercetin concentration (Figure 7B) was used to calculate the unknown flavonoid concentration (quercetin equivalent/QE) in Guava and Chameli leaf extracts. Upon addition of $AlCl_3$, yellow-colored Al (III)-flavonoid complexes formed (complexes between

Table 5: Effect of Extracts of Guava and Chameli on Albumin Denaturation

Sample	Conc. (mg/ml)	Volume (µl)	Albumin denaturation (% inhibition)
Guava	0.2	10	78.93±0.451
Chameli	0.2	10	57.87±0.145
Aspirin	15	100	70.32±0.523

Values are expressed as mean ±SD for three determinations

Table 6: Effect of Guava and Chameli on Membrane Stabilization

Sample	Concentration (mg/ml)	Volume (µl)	% hemolysis inhibitio
Guava	0.2	10	70.00±0.15
Chameli	0.2	10	56.00±0.32
Aspirin	15	100	83.3±0.45

Values are expressed as mean ±SD for three determinations

Al+3 and carbonyl and hydroxyl groups of flavonoids). Their absorbance was measured at a specific wavelength (430 nm) within the range of 410–440 nm. Guava and Chameli extracts exhibited total flavonoid concentrations of 275.65 ± 0.325 mg and 286.768 ± 0.212 mg QE/gm extract, respectively, indicating similar flavonoid content in both extracts (Table 4).

Total Antioxidant Capacity (TAC):

The phosphomolybdate assay was employed to assess the overall antioxidant capacity of the extracts. Higher absorbance values correspond to a greater reduction potential of molybdenum ions, thereby indicating stronger antioxidant potential. The total flavonoid content of Guava and Chameli extracts was determined by extrapolation from the standard curve generated using quercetin (Figure 8A). As shown in Table 4, the Guava extract exhibited a nearly twofold higher antioxidant capacity (129.35 ± 0.112 mg AAE/gm extract) compared to the Chameli extract (68.84± 0.217 mg AAE/gm extract) in reducing molybdate ions.

Ferric Reducing Power Assay (FRPA):

The ferric reducing antioxidant power assay (FRPA) is based on the reduction of ferric ions by the antioxidant. A higher absorbance value indicates a greater reducing potential of the sample. A calibrated ascorbic acid standard curve (Figure 8B) was used to determine the FRPA of Guava and Chameli extracts, expressed in terms of mg AAE/gm of extract. As shown in Table 4, the Guava extract exhibited a slightly higher FRPA potential (160.15 ± 0.436 mg AAE/gm extract) compared to the Chameli extract (145.85 ± 0.398 mg AAE/gm extract).

Anti-Inflammatory Studies:

Inhibition of albumin denaturation:

Protein denaturation is an indication of inflammation. Heat exposure can induce protein denaturation, resulting in increased turbidity measurable by spectrophotometry. Lower absorbance values indicate greater inhibition of protein denaturation by the extract, suggesting its potential anti-inflammatory activity. In this study, we investigated the ability of Guava and Chameli leaf extracts to inhibit heat-induced albumin denaturation. As shown in Table 5, Guava extract exhibited significantly stronger inhibition compared to Chameli extract. The maximum inhibition observed for guava extract was 78.93 ± 0.451% at 10 µL, while Chameli extract displayed a lower inhibition of 57.87 ± 0.145% at the same concentration. Aspirin, used as a positive control, showed an inhibition of 70.32 ± 0.523% at 100 µL.

Inhibition of Protease:

Inhibition of protease activity is a recognized strategy for anti-inflammatory agents. We investigated the potential of Guava and Chameli leaf extracts to inhibit trypsin, a protease enzyme. The assay assessed whether the extracts could bind to trypsin, preventing its activity on bovine serum albumin (BSA). Trypsin activity cleaves BSA, resulting in smaller peptides detectable by the biuret reagent, which produces a light violet color. Conversely, inhibition of trypsin by the extracts would preserve intact BSA, leading to a dark purple color with the biuret reagent. As anticipated, the negative (-) control (trypsin + BSA) exhibited a light violet color due to trypsin activity. The positive (+) control (BSA only) displayed the

darkest shade of violet, indicating intact protein. Both Guava and Chameli extracts demonstrated anti-proteinase activity, as evidenced by a shift towards a darker purple color compared to the negative control (Figure 9). Notably, the Guava extract exhibited superior trypsin inhibition, reflected by a darker purple/blue color, suggesting a stronger ability to preserve BSA from trypsin-mediated proteolysis compared to the Chameli extract.

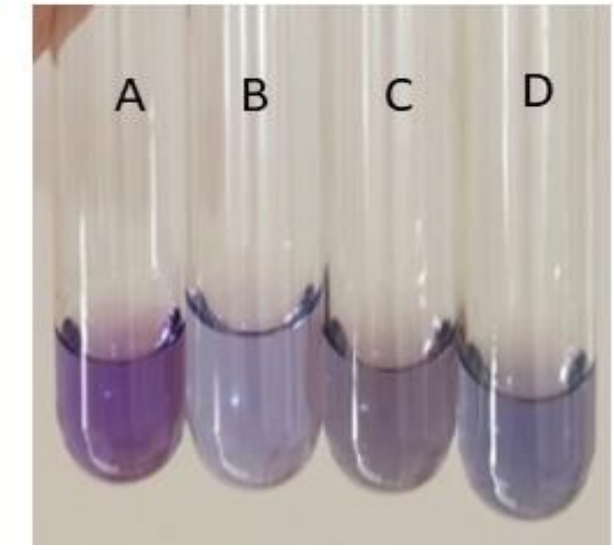

Figure 9: Antiproteinase Activity A) Control (+), B) Control (-), C) Guava, D) Chameli

Heat Induced Hemolysis Inhibition:

The anti-inflammatory potential of Guava and Chameli leaf ethanolic extracts was further investigated by assessing their ability to inhibit heat-induced hemolysis of red blood cells (RBCs). This assay is based on the rationale that the structure of RBCs is analogous to lysosomes, organelles involved in inflammation. By inhibiting RBC lysis, the extracts might mimic the action of some anti-inflammatory drugs that prevent the release of lysosomal enzymes from neutrophils, thereby reducing tissue damage at the inflammation site. As shown in Table 6, the guava leaf extract exhibited significant inhibition of heat-induced hemolysis, with a maximum of 70.0 ± 0.15% inhibition at 200 µg/ml. The Chameli extract displayed a lower inhibition percentage (56.0 ± 0.32%), while Aspirin, the standard drug used at 15 mg/ml, showed an inhibition of 83.3 ± 0.45%. These findings suggest that the Guava extract possesses a stronger ability to inhibit hemolysis compared to the Chameli extract.

Antibacterial Activity Evaluation:

This study explored the potential application of plant extracts as topical agents for oral ulcers. The rationale lies in the ability of such extracts, containing antimicrobial compounds, to combat bacterial growth often associated with these lesions. Our findings revealed promising antibacterial activity of Guava leaf extract against both Gram-positive and Gram-negative bacteria. This was evidenced by the presence of clear zones of inhibition surrounding the wells containing the extract on agar diffusion plates (Figure 10). In contrast, Chameli leaf extract, at similar concentrations, lacked such inhibitory action against the tested bacteria. The observed antibacterial properties of Guava extract might be attributed to the presence of various bioactive antioxidant components within the leaves. Further investigation is necessary to identify and characterize the specific compounds responsible for this effect.

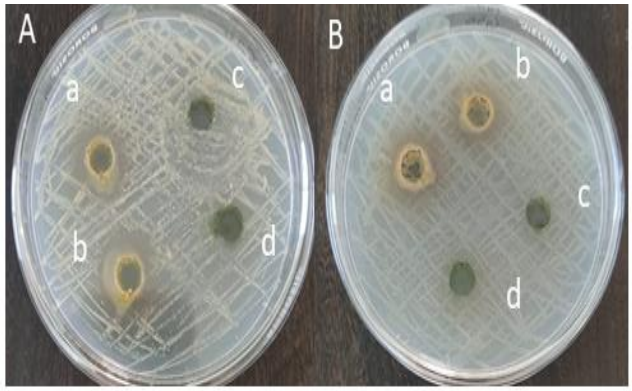

Figure 10: Antibacterial Activity Against A) Gram Negative and B) Gram Positive a) Guava (50µl), b) Guava (100µl), c) Chameli (50 µl) and d) Chameli (100µl)

Application of Herbal Paste:

It was discovered that the herbal paste formulation was safe and did not cause irritation to the skin. The patient noticed a cooling effect as well as pain and inflammation reduction following use of the specially designed solution. When herbal paste was applied to the damaged area for five consecutive days without any negative side effects, the mouth ulcer was totally healed. Figure 11 shows the day-by-day variations in the healing process.

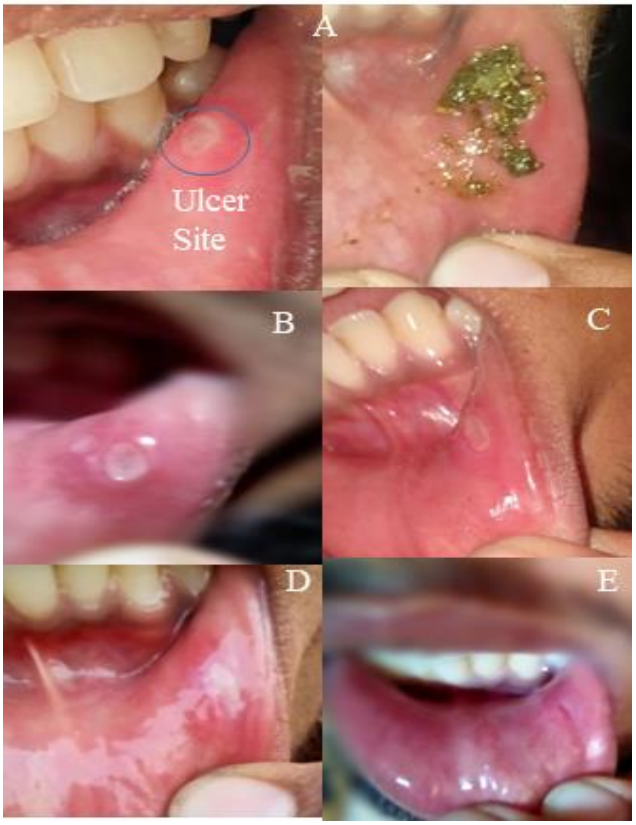

Figure 11: Efficacy of Herbal Oral Paste A) Day 1, B) Day 2, C) Day 3, D) Day 4 and E) Day 5. Day1: Application at Ulcerated Site, Day 2: Reduction of Ulcerated Site upon application of Herbal Paste, Day 3: Pain Relief, Day 4: No Swelling and Day 5: Healed Ulcerated Site

DISCUSSION

This study investigated the potential of Guava and Chameli leaf extracts as a source of natural anti-inflammatory and antioxidant agents. The ethanolic extracts were found to possess a broad spectrum of phytochemicals, including phenolics, flavonoids, tannins, terpenoids, glycosides, saponins, steroids, and alkaloids. The presence of these phytochemicals, particularly phenolics and flavonoids, aligns with previous research demonstrating their contribution to the anti-inflammatory and antioxidant properties of medicinal plants [27] [28].

Quantitative analysis revealed a higher content of phenolics and flavonoids in Guava compared to Chameli extracts. This correlated with a slightly greater total antioxidant capacity (TAC) measured by FRAP assay in the Guava extract. Both extracts exhibited similar ferric reducing potential (FRPA). The anti-inflammatory potential of the extracts was investigated through their ability to inhibit protein denaturation, suppress protease activity, and stabilize membranes. In all these assays, the Guava extract displayed a slightly stronger inhibitory effect compared to Chameli. Notably, both extracts demonstrated greater anti-inflammatory activity than Aspirin, a common over-the-counter medication.

These findings suggest that the ethanolic extracts of Guava and Chameli leaves possess significant antioxidant, anti-inflammatory, and anti-hemolytic properties. Their diverse phytochemical profile, particularly the abundance of phenolics and flavonoids, provides a potential explanation for these observed activities. While these results are promising, further studies are warranted to explore the efficacy and safety of these extracts in a clinical setting.

CONCLUSIONS

This study supports the significant role of medicinal plants in managing various health conditions due to their diverse secondary metabolite content. The observed anti-inflammatory properties of Guava and Chameli leaf extracts potentially contribute to their potential for treating ulcers. The presence of flavonoids and other phytochemicals, along with their presumed biocompatibility and minimal side effects compared to conventional medications, warrants further investigation. While both leaf extracts contained a wide range of the tested phytochemicals, the Guava extract exhibited a higher abundance of these compounds, particularly phenolics and flavonoids. This finding aligns with the observed stronger antioxidant and anti-inflammatory activity of the Guava extract compared to Chameli. The formulated oil-based product derived from Guava and Chameli leaves demonstrated promising results in a preliminary user trial. Patient reported subjective experiences of pain relief, reduced redness, and a cooling sensation upon application to oral ulcers. These observations suggest the potential of this topical herbal paste for treating oral ulcers with minimal side effects.

Controlled clinical studies with a larger sample size are necessary to validate the efficacy and safety of this formulation for oral ulcer treatment. Additionally, investigating the effects on various

age groups and different ulcer types would provide valuable insights for broader application.

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Author Contributions

All the authors contributed in the conception, designing and execution of the study for manuscript preparation. They were involved in the collection of data and its analysis for drafting of the manuscript and its approval. The revision and editing of the manuscript by the authors ensured its accuracy and integrity.

Ethical Approval

Approvals to conduct hemolysis study on human blood cells were approved by the Institutional Ethical Committee of IILR (IEC No-19/07/21) Academy, Indore where work was done. The volunteer signed the informed consent for this study.

Conflict of Interest

The authors declare no conflict of interest, financial or otherwise.

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