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Antimicrobial Activity of Ferulic Acid Purified from Maize (*Zea mays* L.) Bran Against *Streptococcus mutans* Causing Dental Caries

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ABSTRACT

Dental caries is a common chronic disease that impacts the demineralization of tooth tissues by the production of acids during the fermentation of dietary carbohydrates by bacteria, using *Streptococcus mutans* as a prime pathogen. This research explored the antimicrobial effect of ferulic acid isolated as a purified product of maize (*Zea mays* L.) bran against *S. mutans*. Ferulic acid was prepared by optimized alkaline hydrolysis and methanol treatment. It was purified by silica gel chromatography and characterized by several chromatographic techniques. Thirty dental plaque samples were obtained, and five *S. mutans* isolates were detected. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of the erythromycin and ferulic acid in microtitre plates were determined employing *S. mutans*. The MICs of ferulic acid ranged from 4 to 32 ug/mL, while its MBC ranged from 16 to 128 ug/mL. Ferulic acid also demonstrated a better bactericidal activity than erythromycin and inhibited the growth of bacteria, biofilm, and key virulence factors. The finding suggests that maize bran could be a source of bioactive ferulic acid that has the potential to be used as a natural treatment agent. Future research ought to determine clinical use and synergistic effect with established antimicrobials in combating oral antibiotic resistance.

1. INTRODUCTION

Major phenolic chemicals must be extracted from agricultural crop wastes to create value-added goods from renewable byproducts [1]. Ferulic acid is the most prevalent hydroxycinnamic acid present in plant cell walls that are covalently bound to lignin and polysaccharides. It is one of the useful chemicals that may be isolated from agricultural biomass [2]. The cell walls of several plants, especially Graminaceae among monocots, have comparatively large quantities of ferulic acid (4-hydroxy-3-methoxycinnamic acid) [3]. Maize bran is a low-cost agro-industrial byproduct that is a good source of bound phenolic compounds, especially ferulic acid. Maize bran has economic and environmental benefits compared to other sources like wheat bran or sugar beet pulp because it is readily available and cheap [4]. The most prevalent type of phenolic chemicals found in cereal grains and numerous other plants is phenolic acids [5]. The leaves and seeds of many plants, but particularly those of grains, contain the phytochemical ferulic acid. It is the most common phenolic acid, with as much as 90 percent of all phenolic compounds in common cereals [6]. Some of the many physiological roles of ferulic acid that have been reported include anti-inflammatory, antimicrobial, antioxidant, and anticancer properties. Free radicals are neutralized by this antioxidant [7].

On the outside of teeth, dental plaque forms as a soft, sticky film. Another name for dental plaque is biofilm, which is mostly made up of water, extracellular polymeric substances (EPS), bacteria, and their byproducts. Some of the sequential events that culminate in a biofilm include the development of a conditioning layer, the initial microbial adhesion, and the growth of the plaque [7]. A serious oral condition affecting the teeth, dental plaque is brought on by a variety of foods, bacteria, and their byproducts [8, 9]. Within dental plaque, many microorganisms cover themselves with extracellular matrix [10]. *Streptococcus mutans* is a part of most dental plaque bacterial isolates [9]. The bacterial species is the central player in dental caries formation because of its outstanding acidogenicity, aciduricity, and ability to form a biofilm [11]. It is an effective dietary fermentable carbohydrate metabolizer, especially sucrose, glucose, and fructose, via glycolytic mechanisms, generating organic acids, e.g., lactic acid, as metabolic by-products. These acids accumulate to cause localized pH reduction at the tooth surface, which facilitates the demineralization of enamel and dentin, which is a characteristic of caries formation [12]. Besides being acidogenic, *S. mutans* is highly aciduric, which implies its ability to survive and be metabolically active in highly acidic conditions that prevent the growth of most other oral microorganisms [13]. This adaptive benefit enables it to prevail on the cariogenic biofilm, particularly in situations of high frequency of sugar consumption and low standard of oral care. Meanwhile, however, it is known that dental plaque consists of a wide range of different species of bacteria [14].

The antimicrobial agents present in toothpaste and

mouthwash have been reported to be inefficient in regard to their ability to kill bacteria that are present in dental plaques [15, 16]. A number of plant-based products have been employed in the recent past in the treatment of dental plaque biofilms [17]. Comparing these molecules to man-made chemicals reveals numerous advantages [18]. Utilizing plant extracts and compounds in dentistry offers a cost-effective solution for a large number of patients worldwide, tackling the accessibility problems with fluoride for dental caries prevention and the global disparity in preventive dental treatment [19]. Antimicrobial plant extracts function as broad-spectrum antibiotics by lowering virulence factors, preventing the development of resistance, preventing microbial growth, and demonstrating antibiofilm activity [20].

The research gap of this study lies in several critical areas. First, the current techniques used to extract bioactive compounds are usually characterized by low productivity and the need to use severe conditions during the processing, which can damage the stability and bioactivity of the target compounds. Second, there is a shortage in research that directly compares the efficacy and nature of purified ferulic acid with purified extracts, and as such, there are doubts as to how they perform comparatively in relation to each other and what their possible uses are. Lastly, the efficacy of these compounds against multidrug-resistant (MDR) strains also lacks a substantial amount of data, which is also a matter of concern in the context of increasing antimicrobial resistance.

The current study attempted to examine the antimicrobial properties of ferulic acid extracted from maize (*Zea mays* L.) bran against *Streptococcus mutans*, one of the major causative agents of dental caries, by assessing its inhibitory properties in the growth of bacteria and its use as a natural substitute in caries prevention and control. The research offers (i) efficient extraction and purification of ferulic acid in maize bran under alkaline conditions (ii) and a thorough assessment of the antibacterial effects of ferulic acid against *Streptococcus mutans*, which is clinically isolated, and MDR strains. Moreover, the research examines the relative efficacy of purified ferulic acid, which provides information on its possible use as a natural therapeutic agent in the treatment of dental caries and the treatment of oral pathogens that are resistant to antibiotics.

2. MATERIALS AND METHODS

2.1 Ethical approval

Ethical approval for the collection of dental plaque samples from participants was obtained from the Ethics Committee of the Department of Biology, University of Mustansiriyah. Also, informed consent was obtained from all participants.

2.2 Plant material

Plant material was sourced in the local store, and it consisted of maize bran (*Zea mays* L.). The dried maize bran was blended using a blender to a fine powder and kept in airtight containers to be subsequently utilized.

2.3 Preparation of ferulic acid extract

Ferulic acid was extracted from 15 g of maize bran for 48 hours at a hydrolysis temperature of 50 ℃ using concentrations of 1, 2, 3, 4, and 5 M sodium hydroxide. The flask was shaken at 200 rpm for five hours prior to use to guarantee full hydrolysis. After cooling, the solid residue was separated by centrifugation. After being neutralized with 6 M HCl, the ferulic acid-containing supernatant was concentrated using a rotary evaporator [21].

2.4 Purification of ferulic acid using methanol extraction

An aliquot of 100 mL of methanol solution was mixed with the resultant extract and left at 60 ℃ for 2, 4, 6, 8, 10, and 12 hours [22]. Following lignin precipitation with HCl (6 M), the methanolic extracts' pH was raised to 2.0. The filtrate was centrifuged for two minutes at 10,000 rpm after the mixture had been filtered. Vacuum-evaporating the supernatant removed any excess methanol. The concentrated extract was then put on the silica gel column.

2.5 Purification of ferulic acid employing silica gel chromatography

A silica gel column was used to extract ferulic acid in the methanolic extract. The mobile phase contained methanol and water (80:20, v/v). The flow rate was 0.5 mL/min. Absorption was found at 320 nm. The quantity of ferulic acid was measured using the single ferulic acid standard, and a calibration curve was generated [23].

2.6 Characterization of ferulic acid by Fourier transform infrared spectroscopy

A Fourier transform infrared spectroscopy (FT-IR) spectrometer (Alpha II, Bruker, Germany) was used to characterize isolated ferulic acid and standard spectra in the 400–4000 cm^{-1} region using a KBr disc that contained 1% finely ground samples.

2.7 Characterization of ferulic acid by high-performance liquid chromatography analysis

One millilitre of methanol was combined with 0.05 milligrams of the purified sample and standard ferulic acid using a vortex mixer. Using a gradient of mobile phase made up of solvents A (1% acetic acid in high-performance liquid chromatography (HPLC)-grade water) and B (acetonitrile), the mixtures were filtered using a 2.5 µm disposable filter and run in an HPLC system utilizing C18 reverse-phase chromatography. The gradient program included 0–5 min (10% B), 5–15 min (10–60% B), 15–20 min (60–90% B), and a flow rate of 1 mL/min. The results were determined at 310 nm wavelengths [24].

2.8 Characterization of ferulic acid by thin-layer chromatography

Chloroform: acetone: formic acid (75:16.5:8.5) was used as a mobile phase to apply a few drops of standard ferulic acid and the purified sample (1 mg in 1 mL absolute methanol) to activated silica gel. The Retention factor (Rf) values of the isolated spots were compared to those of typical ferulic acid after the produced plate was detected at 254 nm UV [24] as in the following equation: R*f* = Distance traveled by the solute (spot) / Distance traveled by the solvent front.

2.9 Isolation of *Streptococcus mutans* from plaque samples

Streptococcus mutans was isolated and grown on trypone-yeast-cysteine-sucrose-bacitracin agar (HiMedia, India) with selective antibiotics and nutrients (as per the instructions of the manufacturer) to facilitate its growth and inhibit the growth of unwanted oral microorganisms. The medium's composition, including sucrose and cysteine, supports biofilm formation and enhances the recovery of cariogenic strains from clinical samples, ensuring reliable and selective detection for downstream antibacterial testing and microbiological analyses. The tryptone-yeast-cysteine-sucrose-bacitracin agar was streaked with the growth from the brain-heart infusion broth, and thirty plaque samples were added. The samples were then placed in the brain-heart infusion broth and incubated for the entire day at 37 ℃ [25].

2.10 Diagnosis of *Streptococcus mutans*

The species and genus levels of each isolated bacterium were determined employing biochemical reaction-based methods, such as the catalase test, dextran synthesis, and sugar fermentation test. It was established if bacterial isolates could ferment sorbitol, sucrose, and inulin. Bacterial isolates were also identified under a microscope using traits of the microorganisms, including appearance, organization, and Gram reactions [25]. Identification was limited to phenotypic and biochemical characterization, which may not fully confirm species-level identity.

2.11 Antibacterial activity of ferulic acid against *Streptococcus mutans*

Using a broth dilution technique of analysis, the minimum inhibitory concentration (MIC; the lowest concentration with no visible growth) of the pure ferulic acid and erythromycin was determined in 96-well microtiter plates. The quantities of erythromycin and ferulic acid were diluted twice, from 1,024 µg/mL to 1 µg/mL. The microtiter plate well was dispensed with 100 µL of the brain heart infusion broth, 80 µL of erythromycin or ferulic acid, and then 20 µL of bacterial suspension standardized to 0.5 McFarland (approximately 1.5 × 10^8 CFU/mL) was added to bring the final volume to 200 µL. Positive control (erythromycin), negative control (broth only), and sterility control were also included. The plates were incubated at 37 ℃ for 24 hours, followed by the addition of 20 µL of a 1 mg/mL tetrazolium salt solution and incubation for 30 minutes. Following the addition of the indicator, the assessed isolates were observed to be growing more in the wells that went purple and to be growing less in the wells that stayed yellow [26]. Subsequently, a loopful of broth from each well that had not shown growth was inoculated into a nutrient agar plate. Then, the agar plates were examined for growth after 24 hours at 37 ℃. The wells with the least ferulic acid concentration that showed no growth after the first 24 hours, and still no growth on the agar plate, were regarded as the minimum bactericidal concentration (MBC). All experiments were performed in triplicate.

2.12 Statistical analysis

Data were analyzed using the analysis of variance (ANOVA), and $p < 0.05$ was considered significant. The Statistical Package for the Social Sciences (SPSS; version 21) was utilized for the statistical analysis.

3. RESULTS

3.1 Ferulic acid extraction

The results unequivocally demonstrated that using a 2 M NaOH solution, ferulic acid was extracted with a greater yield of 48.2 mg/L. As observed in Figure 1, additional increases in the concentration of NaOH resulted in noticeably poorer extraction yields.

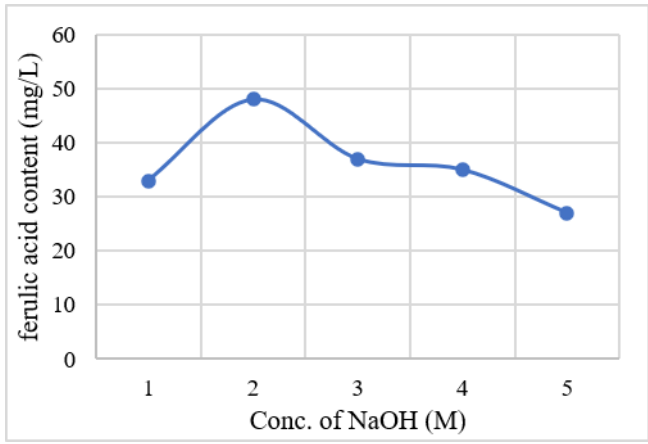


Figure 1. Extraction of ferulic acid with different concentrations of NaOH solution
Note: Data are presented as mean ± standard deviation (n = 3).

3.2 Purified ferulic acid

When methanol was used as an extraction system, ferulic acid was extracted with maximum yield after 10 hours, with the greatest concentration reaching 58.7 mg/L, as shown in Figure 2. FT-IR, HPLC, and thin-layer chromatography (TLC) were used to identify the structural identity of the purified ferulic acid, and the results were found to be equal to the standard spectra, which confirms the extraction and purification schemes used in the present research.

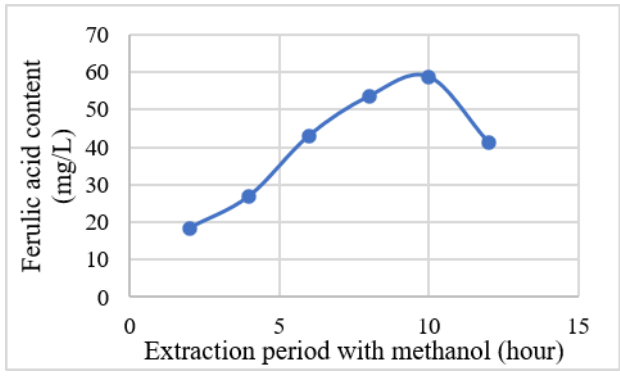


Figure 2. Extraction of ferulic acid with methanol solvent at different extraction periods
Note: Data are presented as mean ± standard deviation (n = 3).

Following the elution stage, one major peak and one minor peak emerged after loading the extracted ferulic acid with methanol on the silica gel column. The second large peak showed a final recovery of 62.08 mg/L, as shown in Figure 3.

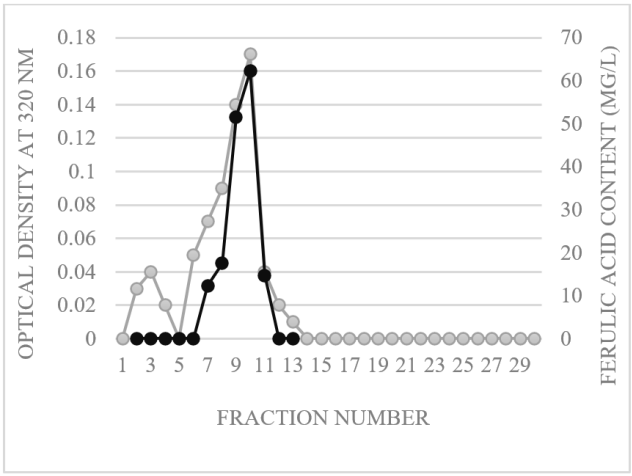
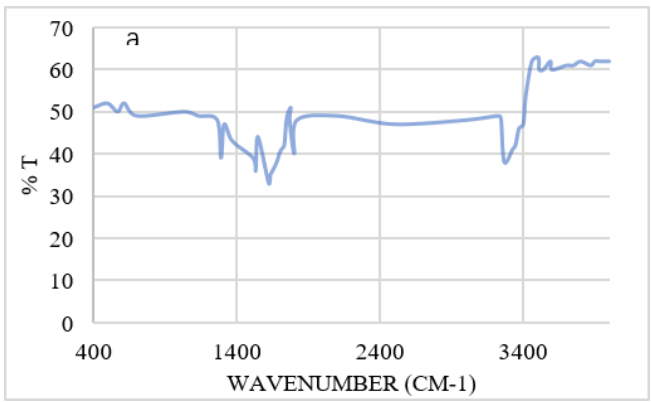


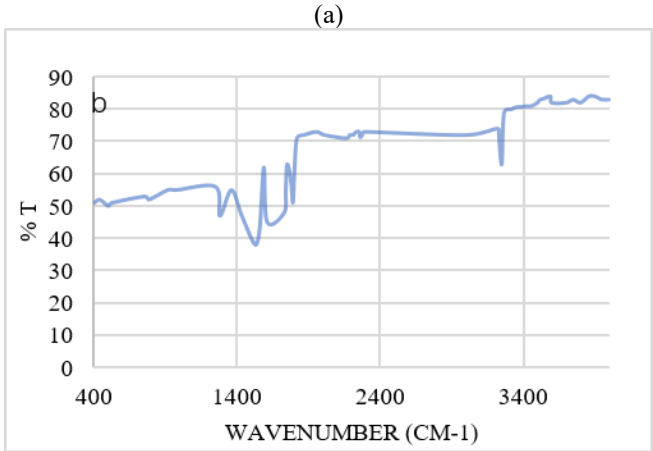
Figure 3. Silica gel column for the purification of ferulic acid

3.3 Characteristics of ferulic acid as determined by Fourier transform infrared spectroscopy

The infrared spectra confirmed the structure of ferulic acid as illustrated in Figure 4, which depicts the presence of peaks at 3250 cm^{-1} (carboxylic acid O-H stretching), 1790 cm^{-1} (carboxylic acid C = O stretching), 1280 cm^{-1} (carboxylic acid C-O stretching), and 1527 cm^{-1} (aromatic C = C stretching). The IR spectra of the purified molecule and the standard ferulic acid spectrum were presented in Figures (a) and (b), respectively. Consequently, the infrared spectrum of the recovered compound entirely corresponds with the proposed structure of ferulic acid.



(a)

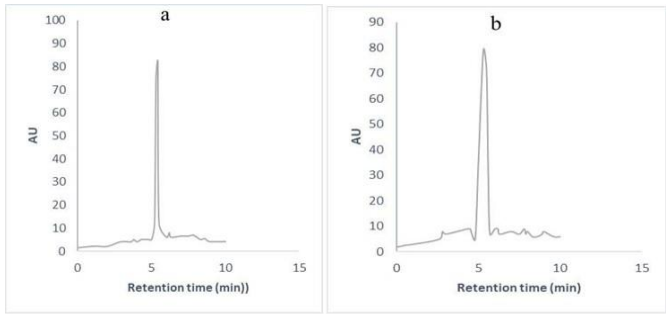


(b)

Figure 4. Fourier transform infrared spectroscopy (FT-IR) spectrum of (a) standard ferulic acid and (b) ferulic acid purified from maize bran

3.4 Characteristics of ferulic acid as determined by high-performance liquid chromatography analysis

Ferulic acid was eluted in the HPLC analysis at 5.30 minutes, and the peak for the same was discovered at 5.42 in the standard ferulic acid, as depicted in Figure 5.



a b

Figure 5. High-performance liquid chromatography (HPLC) chromatogram of ferulic acid in C18 reverse-phase chromatography at 310 nm (a) standard; (b) purified ferulic acid (peak of ferulic acid = 5.30 min)

3.5 Characteristics of ferulic acid as determined by thin-layer chromatography

Thin-layer chromatography was used to compare pure ferulic acid to standard ferulic acid, as shown in Figure 6. The spots' determined Rf value of 0.61 suggests that ferulic acid may be present in the extract.

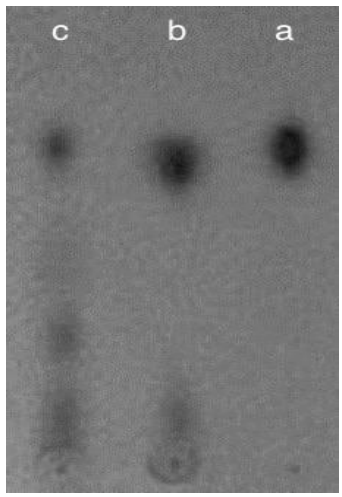


Figure 6. Thin-layer chromatography for ferulic acid extracted from maize bran

Note: a: standard ferulic acid; b: purified ferulic acid; c: partially purified ferulic acid.

3.6 Isolates of *Streptococcus mutans* from plaque samples

In 30 plaque samples taken from patients with dental caries, five *Streptococcus mutans* were found. Among the five isolates, three were obtained from female patients and two from male patients.

3.7 Antimicrobial activity of ferulic acid on *Streptococcus mutans*

The effects of erythromycin and ferulic acid on the

Streptococcus mutans isolates obtained from dental cavities are shown in Figure 7. When it came to inhibiting MDR *Streptococcus mutans*, ferulic acid was the most effective drug of choice because its MICs ranged from 4 to 32 µg/mL, whereas erythromycin's MICs varied from 32 to 256 µg/mL (break point ≤ 0.5 µg/mL). So that all isolates were considered resistant to erythromycin. Ferulic acid also showed a significant level of bactericidal effectiveness against *Streptococcus mutans* isolates. On the other hand, the values for erythromycin and ferulic acid varied from 128 to 1024 µg/mL and 16 to 128 µg/mL, respectively.

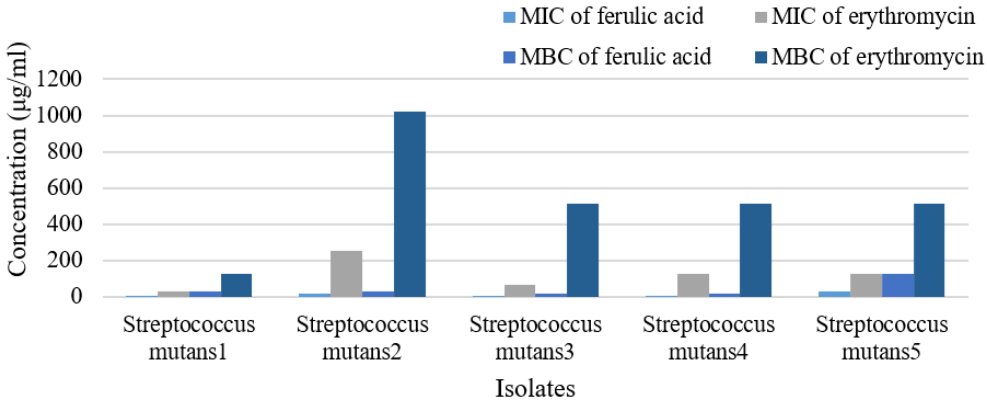


Figure 7. Detection of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of ferulic acid against *Streptococcus mutans*

4. DISCUSSION

The alkali content and hydrolysis duration were found to be crucial factors for the release of ferulic acid. Because mild treatments usually cause little solubilization, whereas harsh conditions can cause product deterioration [2]. In order to reduce the number of experimental steps and energy expenses while also increasing the extraction yield (up to 470 mg of FA per 100 g of BSG), a reduction of the standard post-hydrolysis processes is also suggested [23]. The most important variables affecting ferulic acid extraction were time, temperature, and NaOH concentration. For all temperature/time circumstances, a high alkali concentration had a detrimental dissociation effect on the ferulic acid content [23]. The alkaline hydrolysis (NaOH) method is used to extract and purify ferulic acid from sugar beet pulp. The effects of parameters on extraction are evaluated, and the ideal conditions for extraction time, temperature, and NaOH concentration are 12 hours, 41 °C, and 2M, respectively [21]. The samples containing 5 g of bran had the highest average yields (2.91 mg/g), while the samples containing 15 g of bran were nearly impossible to stir during hydrolysis, which reduced the hydrolysis's effectiveness and produced much lower ferulic acid yields (1.90 mg/g) [22].

Alkaline hydrolysis is more efficient at releasing phenolic compounds than methanol. By severing the ester bond that phenolic acids have with the cell wall, alkaline hydrolysis is thought to be an effective technique for releasing phenolic chemicals from polysaccharides [21, 27]. Higher phenolics were maintained after an alkaline treatment, which may be because alkaline hydrolysis effectively releases phenolic compounds from polysaccharides by breaking the ester linkage that binds phenolic acids to the cell wall. It is evident that hydrolyzing the covalent esteric linkages in chemical processes is a more effective way to remove phenolic chemicals [23]. The highest concentration of ferulic acid was obtained during extraction with 2 M NaOH, which demonstrates the relevance of alkaline hydrolysis in the successful release of bound phenolic compounds by the plant cell walls. These findings are consistent with the existing literature that mild alkaline conditions are efficient in breaking up ester bonds between phenolic acids and polysaccharides, maintaining bioactivity and avoiding degradation [28]. The methanol extraction step further enhanced the yield, corroborating observations that organic solvents can stabilize and concentrate ferulic acid after initial hydrolysis [24].

A range of polar and non-polar macroporous resins were employed to enhance the ferulic acid from *Cynomorium songaricum* extracts. Both polar and non-polar resins could be utilized for ferulic acid adsorption since it comprises both polar multi-hydroxyl groups and non-polar phenyl groups [27]. Ferulic acid was extracted and purified from apple peels using C-18 column chromatography [29]. Silica gel chromatography purification yielded high ferulic acid (62.08 mg/L), which is consistent with plant-derived phenolics, including apple peels and other cereal residues, where column chromatography enabled high-purity compounds to be isolated and subjected to antimicrobial testing [29].

Ferulic acid can be quantitatively analyzed via TLC. Numerous studies have looked at the commercial manufacture of ferulic acid in good yield from the bound form present in plant material, such as maize hulls, using enzymatic techniques; nonetheless, this is still a challenging task. For routine high-throughput detection and measurement of any ferulic acid in such research, TLC separation of crude extracts and visualization by UV light or other spray reagents provides a quick procedure [24]. Furthermore, selective screening for the generation of higher-value compounds, like vanillin or acetovanillone, would be possible with the application of specific spray reagents [30].

According to the findings of the study on the factors that contribute to dental caries and how they differ in men and women, women are at risk for dental caries due to differences in salivary composition and flow rate, hormonal fluctuations, dietary habits, genetic variations, and specific social roles within their family [31]. Saliva and plaque samples are both good sources for isolating *S. mutans*. Even though there was no substantial relation between the caries history and the amount of *S. mutans* in either source, the occlusal plaque was

more sensitive in determining high levels of *S. mutans* [32].

This is consistent with newly studied plant phenolics being effective at preventing biofilm formation, disrupting EPS, and reducing bacterial virulence [11]. The bactericidal activity is thought to be related to the ability of ferulic acid to inhibit the activity of glucosyltransferase (GTF) and weaken biofilm structure, as well as the synergistic effect with conventional antibiotics to be used as additional treatment or alternative therapeutic strategy for combating resistant oral bacteria [31].

Erythromycin (24.1%) and lincomycin (28.7%) had the highest resistance rates, followed by amoxicillin (14.9% in mother *S. mutans* isolates) and penicillin (14.9% in child *S. mutans* isolates). In conclusion, *S. mutans* clinical isolates from dental patients exhibit notable levels of penicillin, erythromycin, amoxicillin, clindamycin, and lincomycin resistance [33]. Selective antibiotic pressure, where erythromycin is used more frequently in children than in adults, can account for the high rate of erythromycin resistance in child isolates. This may be a significant factor in the development and dissemination of erythromycin resistance in child isolates [34].

In comparison with other studies, ferulic acid displayed significant activity against *Enterococcus faecalis* (MICs = 16 µg/mL) and also demonstrated prominent activity against planktonic *Staphylococcus aureus* with a MIC value of < 8 µg/mL [35]. It was found that ferulic acid had antimicrobial activity against the bacteria tested, with MICs of 100 µg/mL for *E. coli* and *P. aeruginosa*, 1100 µg/mL and 1250 µg/mL for *S. aureus* and *L. monocytogenes*, respectively, with FA. The MBC for *E. coli* was 2500 µg/mL. for S. aureus was 5000 µg/mL, for *L. monocytogenes* was 5300 µg/mL [36].

While the resistance rate can vary with locale, erythromycin is widely effective in suppressing the growth of *Streptococcus mutans*, a common bacterium of tooth cavities. Its activity may produce a post-antibiotic effect and slow bacterial growth [32]. By diminishing its biofilm-forming capability and inhibiting the activity of a key bacterial adherence enzyme, GTF, which plays essential roles in biofilm development and plaque formation, ferulic acid suppresses the *Streptococcus mutans* activity and reduces bacterial motility and pathogenicity as well as decreases the production of EPS of the biofilm matrix. Moreover, it has been found that ferulic acid exhibits synergistic activity with certain antibiotics, which means that new treatment plans might be developed [37]. The isolates were determined to be resistant to erythromycin, and no resistance to other antibiotics (e.g., tetracycline, penicillin) was evaluated and should be assessed in the further studies.

The heterogeneity of bacterial prevalence and dependence on host factors, such as the composition of the salivary and dietary components and oral hygiene, is also demonstrated by the selectivity of *S. mutans* in 5 out of 30 samples of dental plaque and confirms the previous findings that biofilms of the plaque are highly heterogeneous and depend on the host factors. The anti-browning effect of ferulic acid on these recalcitrant strains highlights the potential of ferulic acid as a viable and sustainable antimicrobial that is plant-based and has the potential to curb the growing menace of antibiotic resistance among the dental pathogens [35].

5. CONCLUSION

In the study, it was shown that the ferulic acid extracted as a pure substance from maize (*Zea mays* L.) bran is highly active as an antimicrobial against *Streptococcus mutans*, including those that are MDR, and it is better than erythromycin. Ferulic acid was extracted with maximum yield with 2M NaOH after 10 hours in methanol, with the greatest concentration reaching 58.7 mg/L, and the yield was increased after loading the extracted solution on the silica gel column, with final recovery of 62.08 mg/L, and it was verified by FT-IR, HPLC, and TLC. The compound was found to prevent bacterial growth, decrease the formation of biofilms, and disrupt virulence factors, including GTF activity, which demonstrates its possible application as a natural therapeutic agent to manage dental caries. Considering such findings, maize bran can be a cost-effective source of bioactive ferulic acid with sustainability. Future research ought to examine its clinical use, synergistic interactions with traditional antibiotics, and a wider spectrum of antimicrobial action against a wide range of oral pathogens to aid in its evolution as a natural oral health intervention.

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