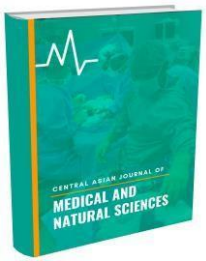

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Article

Antibacterial activity of ferulic acid extracted from corn bran (Zea mays L) against dental decay causing Lactobacillus spp.

Hala M.Sabre[1]

1. Al-Karkh University of Science 'Baghdad'Iraq
* Correspondence: larsa@kus.edu.iq

Abstract: One of the main causes of dental caries in humans has been identified as lactobacilli. Numerous secondary metabolites, also known as phytochemicals, are produced by plants and are known to play a part in defense mechanisms.Most of these metabolites are known to have antibacterial qualities and other positive health impacts. Using 1 M sodium hydroxide, ferulic acid was extracted from corn bran. Higher amounts of ferulic acid were purified for 12 hours using a methanol solvent. The FT-IR spectrum and HPLC analysis were used to describe the purification of ferulic acid. The contents of the products extracted under the experimental circumstances varied. Dental caries was primarily caused by L. fermentum. High bactericidal effectiveness against all Lactobacillus species was demonstrated by ferulic acid. The L. fermentum showed minimal MIC levels with higher activity. This promoted the usage of corn bran's ferulic acid as an additive in the most widely used mouth paste.

Keywords: Ferulic acid, corn bran, Lactobacillus spp.

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1. Introduction

Secondary metabolites called phenolic compounds are produced by plants as they grow (1). Of the phenolic acids found in many plants, ferulic acid (4-hydroxy-3-methoxycinnamic acid) is the most prevalent (2). FA ranks among the most significant phenolic chemicals (3,4). The hydroxyl of the α-L-arabinosyl side chains of xylans is often ester-bonded to the carboxylic acid group of cell wall polymers by ferulic acid in brans (5). Bran made from corn has the greatest ferulic acid concentration (4,5).

Among the most significant phenolic acids, ferulic acid is thought to have the most effective effect in preventing the oxidation of proteins and lipids (6). Ferulic acid's antibacterial and antioxidant qualities make it a candidate for use as a food industry preservative (7). Esters of ferulic acid are present and covalently bound to polysaccharides in cell walls, specifically to pectins, hemicelluloses, and cereal pentosans (8Because they are inexpensive and widely accessible, lignocellulosic materials such as barley bran , eucalyptus wood, , maize cobs, oat fibers, and corn leaves which are regarded as appropriate ferulic acid sources (9,10).

Ferulic acid performs a variety of biological tasks and is not harmful (11). Due to its anti-microbial, anti-inflammatory, anti-oxidant, and anti-cancer properties as well as its ability to prevent heart disease (10,11, 12).

The continuous demineralization of dental enamel on the proximal surfaces, under the gum line, and on the tooth surface itself as a result of acids produced by bacteria in the plaque microbiota digesting sugar is the clinical manifestation of dental caries (13).

Recent findings, such as data from the human oral microbiome project, have demonstrated that Dental caries is caused by dysbiotic plaque microbiota that reacts to host and environmental stressors such inadequate saliva, poor oral hygiene, and continuous fermentable sugar ingestion (14). Mutans streptococci, lactobacilli, and bifidobacteria are among the highly acidogenic and aciduric species that are disproportionately more prevalent in a cariogenic plaque microbiota (15).

It is commonly known that a bacteria must possess the following characteristics in order to induce carious lesions: the capacity to colonize and remain on the surface of the tooth, the capacity to withstand the acids and the resulting low pH environment, as well as the ability to catabolize carbohydrates and generate weak acidic metabolites (13,15). The aim of this study was to extract and purify of ferulic acid from corn bran using different amounts of NaOH and methanol solvent. Ferulic acid was identified in the extracted materials. Lactobacillus species were isolated from dental cavities, and the effectiveness of ferulic acid against these bacteria was determined.

2. Materials and Methods

Preparation of plant material

Corn bran was produced by an agro-industry dry milling process, ground into a fine powder, and then kept until it was needed.

Extraction ferulic acid

The extraction procedure was conducted using the methodology outlined in (16). In order to improve the extraction process, 5 g, 10, 15, and 20 g of maize bran were combined with 0.5, 1, 2, and 4 M sodium hydroxide concentrations, totaling 100 ml, at a hydrolysis temperature of 50°C. To ensure complete hydrolysis, the flask was shaken at 200 rpm for five hours. Centrifugation was used to separate the solid residue once it had cooled. The supernatant containing ferulic acid was concentrated using a rotary evaporator after it had been neutralized with 6M HCl.

Purification of ferulic acid

The resulting extract was combined with 100 milliliters of 99% v/v methanol solution, and ferulic acid was extracted by reflux for two, six, and twelve hours at 60 degrees Celsius (17). Then, using HCl 6 M for lignin precipitation, the pH of the methanolic extracts was brought to 2.0. Following the filtering of the mixture, the filtrate was centrifuged at 10,000 rpm for two minutes. Excess methanol was eliminated by vacuum-evaporating the supernatant. After that, the concentrated extract was freeze-dried and kept at 20 degrees Celsius until it could be examined.

Determination of ferulic acid concentration

Standard curve was used for all quantitative analyses, with absorbency at 320 nm and ferulic acid acting as the analytical standard (18).

Analysis of ferulic acid

1- FT-IR spectroscopy

FT-IR analysis An FT-IR spectrometer was used to record isolated and standard ferulic acid spectra in the 400–4000 cm-1 range.

2- HPLC characterization

A UV/Vis detector was connected online to the HPLC system, and the separated filtrate was pumped into it. The oven temperature was set at 35 degrees, and the flow rate was set at 1.0 milliliters per minute. The detector was tuned to 320 nm, and the mobile

phase was made up of methanol and water (35:65, v/v) with 1% acetic acid. The analytical standard used for all quantitative studies was ferulic acid (17).

Isolation of *Lactobacillus* spp. from dental caries

Using aseptic precautions, twelve samples were taken from dental caries patients, transferred to 1% saline solution, vortexed for one minute, and then plated on sterile MRS agar plates. The samples were then incubated for 24 hours at 37C. Following growth, the expanding colonies were described in accordance with (19).

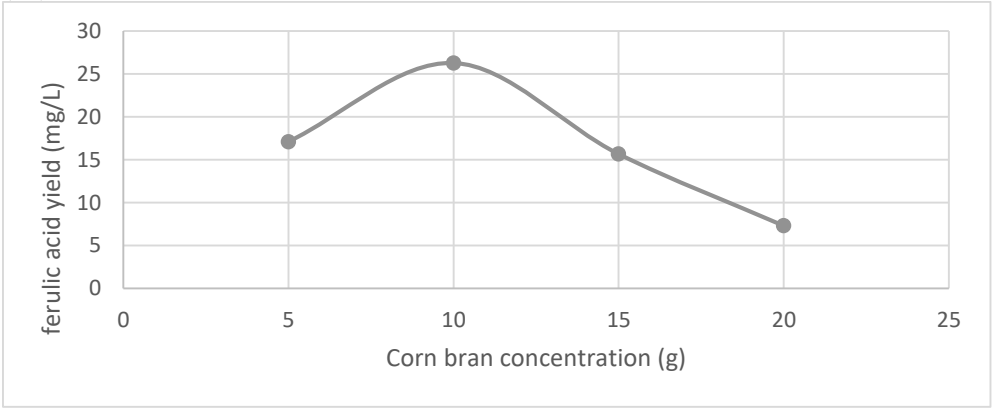
Evaluation of antibacterial activity of ferulic acid against dental caries causes dental caries

A microbroth dilution test was used in 96-well flat-bottom sterile microplates to assess the impact of ferulic acid on Lactobacillus species. The MIC and MBC were calculated as follows: Using an overnight growth culture that was standardized using the 0.5 McFarland turbidity standard, an inoculum was created. Each well contained 80 µl of ferulic acid in varying doses (1–1024 µg/ml), 20 µl of microbial inoculum, and 100 µl of Mueller-Hinton broth. Sterility and growth controls were also considered for observation. The microtiter plates were kept at 37 °C for a whole day. The plates were read by observing the medium's color change upon the addition of 20 µL of resazurin. The existence of bacterial growth in this experiment is indicated by the medium's distinctive color change from blue to red, whilst the absence of growth is indicated by the medium's constant blue hue. The MIC was defined as the lowest concentration that prevented the organism's visible growth. In triplicate, each experiment was conducted (20). After 24 hours of incubation at 37°C on a Mueller-Hinton agar plate, the MBC was determined to be the lowest concentration at which no live cells were detected.

3. Results and discussion

Extraction of ferulic acid

The findings clearly show that the 10 g samples had the highest average ferulic acid production, measuring 26.29 mg/L. Significantly lower extraction yields have resulted from further increases in the initial weight of maize (figure 1a). One M NaOH solution yielded the highest average yields, at 54.67 mg/L. The yields of ferulic acid did not significantly alter with further increases in the concentration of NaOH, as shown in figure (1b).



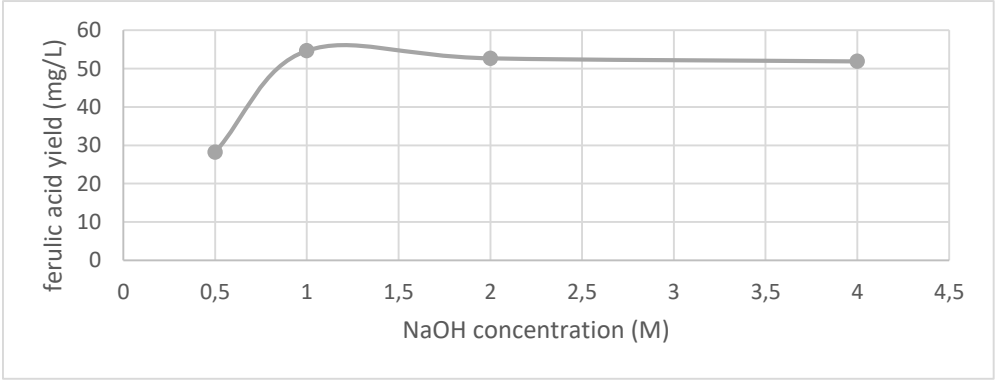


Figure (1): a) Detection the best concentration of corn bran for ferulic acid yield, b) detection best concentration of NaOH for ferulic acid yield

It is economically favorable to employ hydroxide with low concentrations, which is crucial for the probable industrial synthesis of ferulic acid (17). Compared to methanol, alkaline hydrolysis releases phenolic chemicals more effectively. An alkaline hydrolysis considered as efficient method of releasing phenolic compounds from polysaccharides with breaking the ester link that phenolic acids have with the cell wall (16,17).

Actually, lignin is dissolved by alkaline treatments that cleave the ester bonds in lignin–polysaccharide complexes. A considerable amount of these compounds degrade at high alkali concentrations and temperatures, generating phenolic acids (21). For the release of ferulic acid, the alkali content and hydrolysis time were identified as critical variables. Due to the fact that modest treatments typically result in minimal solubilization, while extreme conditions may induce product degradation. Our findings are consistent with the results that have been reported (22, 23).

Purification of ferulic acid

By using 12 hours as the reaction time, the maximum ferulic acid yield was extracted from the extraction with methanol solvent with the greatest concentration of 72.33 mg/L, as shown in figure (2).

Figure(2):Detection the effect of time on purification of ferulic acid by methanolic solvent

Analysis of ferulic acid

1- FT-IR spectroscopy

The presence of the primary functional groups in the ferulic acid structure was evident in the precipitate sample's FT-IR spectra, which was compared to ferulic acid standard the spectrum (Figure-2). The OH group in phenolic compounds is identified by the broad and strong band at 3133.08 cm^{-1}. The aromatic ring's C-H stretching occurred at the 2895 cm^{-1} band. The carbonyl group (C = O) is represented by the band at 1643 cm^{-1}. The methyl group's C-H vibration is characterized by a stretching band at 1228 cm^{-1}, whereas the aromatic ring's C= C vibration is located at a 782 cm^{-1} band (figure-3).

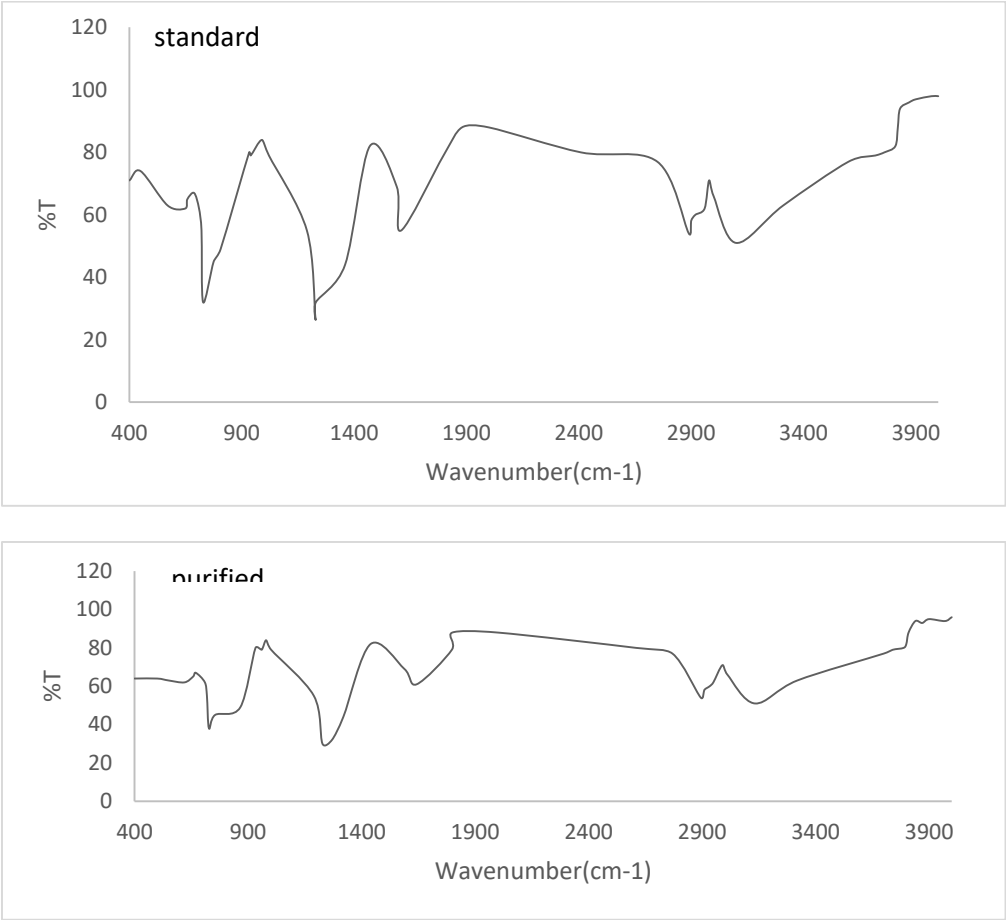


Figure (3): FT-IR spectroscopy for detection the functional groups in the standard and purified ferulic acid

2- HPLC analysis

Sharp peaks that emerged at a retention period of 3.36 minutes compared to the standard at 3.33 minutes, as shown in figure (4), showed that the alkaline hydrolysate of corn bran contained ferulic acid by HPLC analysis.

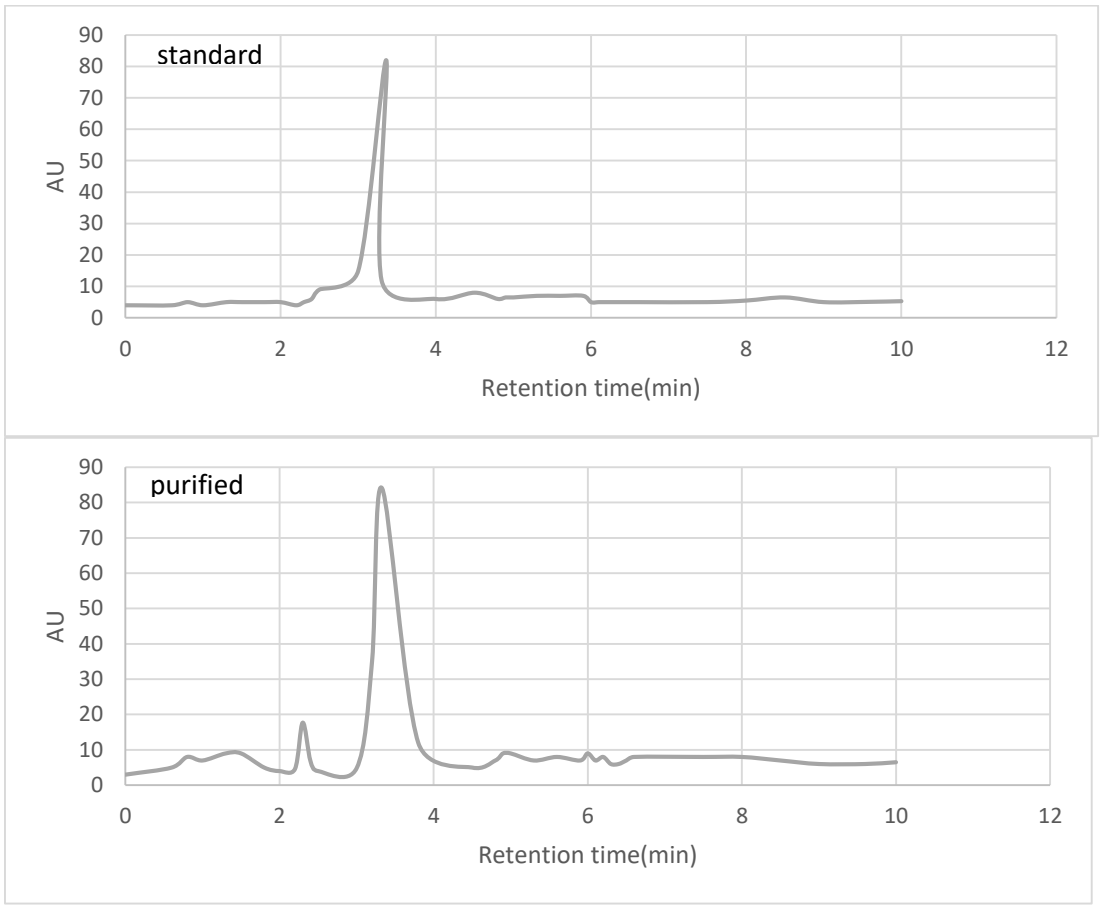


Figure (4): HPLC analysis for the standard and purified ferulic acid

Isolation of *Lactobacillus* spp. from dental caries

Five *Lactobacillus* species were identified in 12 samples obtained from individuals with dental caries, including 3 (60%) isolates of *Lactobacillus fermentum*, 1 (20%) isolate of *Lactobacillus acidophilus*, and 1 (20%) isolate of *Lactobacillus plantarum.* Depending on the extent of the lesion, different *Lactobacillus* species are associated with dental caries. The most common *Lactobacillus* in children was *L. fermentum* with moderate to high caries, whereas *L. salivarius* was the most common in children with mild caries (24).

Evaluation of antibacterial activity of ferulic acid against dental caries causes dental caries

Figure (5) illustrates how ferulic acid affected the *Lactobacillus* species that were isolated from dental cavities. Ferulic acid demonstrated a significant level of bactericidal effectiveness against every tested *Lactobacillus* species. For all *Lactobacillus* species with greater inhibitory activity against *L. fermentum*, the ferulic acid MIC and MBC ranged from 16 to 64 μg/ml and 32 to 256 μg/ml, respectively.

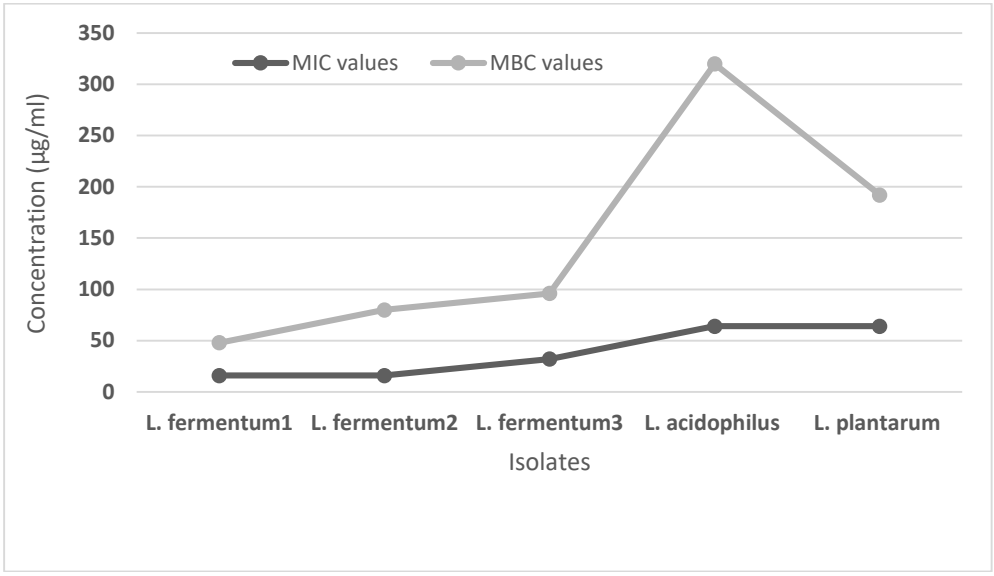


Figure (5): Detection of MIC and MBC levels of ferulic acid against *Lactobacillus* spp.

The charge, physicochemical properties and permeabilitythat related with cell membrane properties were irreversibly changed by gallic acid and ferulic acid, which also decreased the negative charge of surface, changed hydrophobicity, and caused desruption in cell membranes, allowing essential intracellular constituents to leak out (25). When it came to Gram-positive strains, ferulic acid had a quick bactericidal effect and a bacteriostatic effect on Gram-negative bacteria since when tested against *E. coli, P. aeruginosa, S. aureus*, and *B. subtilis*, hexyl ferulate had the strongest antibacterial properties. The impact on bacterial growth, biofilm, cell shape, cell contents leakage, and membrane potential is also included (26).

4. Conclusion

Ferulic acid was liberated in greater quantities after being isolated from corn bran using alkaline hydrolysis. Using its solubility to purify ferulic acid from alkaline extracts using a methanolic solvent simplifies the process significantly. L. fermentum was predominantly responsible for dental caries. All Lactobacillus species were shown to be very susceptible to the bactericidal effects of ferulic acid, with L. fermentum being the most susceptible.

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