ORIGINAL ARTICLE

Ethyl acetate fractions of *Cordyline fruticosa* leaf: Chemical composition and cariogenic antimicrobial activity

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ABSTRACT

Introduction: Early childhood caries (ECC) is a prominent oral health problem, especially among low socioeconomic status (SES) groups. The development of herbal agents for caries prevention is essential due to the elevated costs and potential adverse effects linked to existing synthetic pharmaceuticals. *Cordyline fruticosa (L.) A. Chev.* (*CF*), prevalent in Indonesian rural regions, is an ornamental plant recognised for its medicinal properties, especially its antimicrobial efficacy. This study aimed to identify the chemical compounds in the ethyl acetate fraction of *CF* leaf extract and evaluate its antibacterial and antifungal activities against *Streptococcus mutans* (*S. mutans*) and *Candida albicans* (*C. albicans*), the main microorganisms associated with early childhood caries. **Methods**: This study used a laboratory experimental method. *CF* leaf extract was fractionated using ethyl acetate. Phytochemical and GC-MS screening were performed to identify secondary metabolites and chemical compounds. *S. mutans* and *C. albicans* were isolated from supragingival plaque samples of pediatric patients with severe ECC. The Minimum Inhibitory Concentration (MIC) was determined via serial dilution and ELISA spectrophotometry by comparing absorbance or optical density (OD) values before and after incubation.**Results**: Phytochemical screening confirmed the presence of phenolic compounds, flavonoids, tannins, and triterpenoids. GC-MS identified 4 chemical compounds, of which the largest peak area (73.88%) corresponding to 5-hydroxymethylfurfural. The MIC for *S. mutans* was 3.125% and 1.56% for *C. albicans*. **Conclusion**: The ethyl acetate fraction of the *CF* leaf extract exhibits antibacterial and antifungal properties and may function as a cariogenic antimicrobial agent.

KEYWORDS

Cordyline fruticosa (L.) A. Chev., *Streptococcus mutans*, *Candida albicans*, antimicrobial activity, early childhood caries

INTRODUCTION

Early childhood caries (ECC) is a significant oral health problem, particularly in low socioeconomic status (SES) populations. ECC is a condition affecting one or more primary teeth in children aged 71 months (5 years) or younger, characterised by the presence of carious lesions, missing teeth, or restorations due to caries. Severe ECC is defined as any smooth-surface caries in children younger than three years, or for children aged three to five, the presence of one or more cavities, missing teeth, or filled teeth on the front teeth, or a score of four or more for three-years-old, five or more for four-years-old, and six or more for five-years-old.[1] The prevalence of ECC differs among social strata, with 85% of affected children originating from low SES.[2] In Indonesia, the prevalence of ECC continues to rise, with research indicating that children aged 3–4 years largely

contribute to this high prevalence.[3] The primary risk factors of ECC can be classified into microbiological, dietary, and environmental categories.

Microbiologically, *S. mutans* is the primary bacterial species responsible for dental caries.[4] *C. albicans,* a type of fungus, also contributes to ECC pathogenesis. The commensal characteristics of *C. albicans* may transform into pathogenicity in an environment of dysbiosis due to immune system disruption or localised infection in the oral cavity.[5] *C. albicans* exists at higher quantities in the dental plaque of children with ECC than in caries-free children.[6,7] *S. mutans* and *C. albicans* have a significant association with the development of cariogenic biofilms, especially in children with ECC*. S. mutans* promote adhesion and produce lactic acid, which acts as a carbon source for the development of *C. albicans*. Several studies also show that *C. albicans* facilitates the proliferation of *S. mutans* through interactions with its yeast cells.[7,8] The interaction between *S. mutans* and *C. albicans* could lead to new methods for the prevention of ECC, such as exploring caries preventive agents that can effectively inhibit both microorganisms.[7]

Chlorhexidine is the gold standard antimicrobial agent for caries prevention, inhibiting the development of dental plaque. Chlorhexidine may exhibit either bacteriostatic or bactericidal properties depending upon the dosage administered. Chlorhexidine is effective against a range of bacteria, including both gram-positive and gram-negative strains.[9] Chlorhexidine mouthwash usage may result in various side effects, such as tooth discoloration, xerostomia, and alterations of taste if used long-term, as well as potential cytotoxic consequences following oral surgery.[10,11] Consequently, it is essential to research alternative caries preventative materials that exhibit minimal side effects, particularly for caries prevention in children.

Research on natural products has demonstrated the ability to substantially enhance contemporary medicine. Their sustainable nature and varied bioactivity correspond with the prevailing trend towards safer, natural, and eco-friendly medicinal product alternatives.[12] The efficacy of natural ingredients as caries-preventive agents is associated with the antibacterial and antioxidant properties of the chemicals found in medicinal plants.[13,14]

Cordyline fruticosa (L.) A. Chev. (*CF*) is a medicinal plant originating from the Polynesian region, including Hawaii in the north and New Zealand in the south. The purple, lanceolate leaf is pointed at the tip and base, features wavy edges and pinnate venation resembling fish bones. It exhibits monopodial stem growth and has fibrous roots.[15] In Indonesia, this plant, known as *Andong merah* or *Hanjuang*, is primarily recognised for its decorative value rather than its medical properties. This plant grows abundantly and is commonly located in rice fields and gardens in rural regions. Given its abundance, researchers should continue developing this potential through sustained studies to ensure its renewability for Indonesia's future pharmaceutical industry.[16]

Researchers have performed several *in vitro* studies on the antimicrobial activities of *CF*. The methanol extract of this plant's leaf exhibits antimicrobial properties and contains antioxidants.[17] An additional *in vitro* study has shown that the methanol extract of *CF* possesses antifungal activity against *C. albicans*.[18]

Previous studies have demonstrated that the *CF* leaf fraction shows antibacterial activity against *Escherichia coli* and *Salmonella typhimurium*. The ethyl acetate and methanol fractions exhibit broad-spectrum antibacterial activity, demonstrating moderate to strong efficacy against *Salmonella typhi, Escherichia coli, Staphylococcus aureus*, and *Bacillus subtilis*.[19]

The ethyl acetate fraction of *CF* stem has greater antibacterial activity than other fractions.[16] However, there is limited empirical data directly associating its phytochemical components with cariogenic antimicrobial activity. Moreover, the majority of current studies seem to concentrate on crude extracts, resulting in insufficient examination of fractionated components, such as the ethyl acetate

fraction, despite the fact that fractionation frequently isolates bioactive compounds with stronger antimicrobial activity.

Additionally, synthetic antimicrobial agents, such as chlorhexidine, are frequently associated with undesirable side effects. This highlights the pressing necessity for safer, natural alternatives that specifically inhibit cariogenic microbes. Consequently, a knowledge gap exists in the identification and characterisation of bioactive compounds in the ethyl acetate fraction of *CF* leaf, as well as in the assessment of their antimicrobial activity against cariogenic pathogens. Addressing this gap will facilitate the advancement of innovative, natural, and potentially safer agents for caries prevention. The aim of this study was to identify the composition of ethyl acetate fraction from *CF* leaf and evaluate its antimicrobial activity against *S. mutans* dan *C. albicans*.

METHODS

The research method used was descriptive experimental study aimed at identifying the chemical substances present in the fractions and determining the minimum inhibitory concentration. The research samples were *S. mutans* and *C. albicans* isolated from supragingival plaque specimens and ethyl acetate fraction from *CF* leaf extract with concentrations of 50, 25, 12.5, 6.25, 3.125, and 1.56%. The positive control used was 2% chlorhexidine and the negative control was Brain Heart Infusion (BHI) broth and Sabouraud Dextrose Broth (SDB). This research examined various concentrations, as plant fractions frequently exhibit MICs in the low percent to sub-percent range. Initiating at 50% ensures detectable inhibition when the fraction exhibits moderate activity; 1.56% represents 1/32 of the maximum concentration to identify low MICs.

CF leaves were collected from the Bogor Botanical Garden in West Java. The plant species was identified and validated at the National Research and Innovation Agency (Indonesian: Badan Riset dan Inovasi Nasional, BRIN) in Cibinong, Bogor (No. B-2158/II.6.2/IR.01.02/7/2024). The *CF* leaves were washed with running water, dried, and drained until no moisture remained on their surface. They were then air-dried at room temperature, approximately 25-30°C, for 7-10 days. After that, the dried leaves were cut into small pieces. Forty-five grams of dried *CF* leaves were extracted in 70% methanol at a 1:2 ratio using the maceration technique.

Methanol was used as a solvent due to its efficacy in extracting diverse chemicals, including flavonoids, terpenoids, and phenolic compounds. This study used 70% methanol to optimize extraction while minimizing evaporation and reducing toxicity risks.[20] The extract was filtered and evaporated to achieve a thick viscosity. The procedure subsequently progressed to fractionation. Twenty grams of methanol extract were placed into a container, followed by the addition of 400 ml of hot water.

The extract was combined and placed in a separating funnel, to which 400 ml of ethyl acetate solvent was added in a 1:1 ratio. The mixture was agitated for one minute and subsequently permitted to rest until the solution stratified into two distinct layers. Additionally, the researchers collected the ethyl acetate fraction filtrate, evaporated it until they produced a viscous extract, and determined the yield. Extraction, fractionation, and phytochemical screening were performed at the Chemical Application Laboratory and Service, Faculty of Mathematics and Natural Sciences, Padjajaran University, Bandung, West Java.

Phytochemical screening was carried out to identify secondary metabolites using specific reagents: 5% $FeCl_3$ for phenolic compounds and 1% $FeCl_3$ for tannins, 10% NaOH for flavonoids, 2N HCl for saponins, H_2SO_4 for triterpenoids and steroids, and Wagner's reagent, which consists of iodine and potassium iodide, to detect alkaloids.[20] The GC-MS analysis was conducted at the Regional Health Laboratory (Indonesian: Laboratorium Kesehatan Daerah, Labkesda) in

Jakarta, Indonesia. One microliter of the ethyl acetate leaf fraction was injected into the GC-MS instrument via direct injection at 270°C.

The helium gas pressure used as the carrier gas was sustained at 1 kg/cm², whilst the pressures of hydrogen and nitrogen gases were set at 0.5 kg/cm². The flow rates were established at 30 mL/min for hydrogen, 400 mL/min for oxygen, 30.1 mL/min for nitrogen, and 46.4 mL/min for helium. The column temperature was initially set at 130°C and subsequently raised to 230°C at a rate of 4°C per minute. The detector temperature was set at 280°C, the ion source temperature set at 250°C, and the mass spectrometer operated at 70 eV. The mass spectrum analysis using GC–MS was performed using the National Institute of Standards and Technology (NIST) 2020 database. Concentrations of ethyl acetate fractions of 50%, 25%, 12.5%, 6.25%, 3.125%, and 1.56% were prepared through serial dilution (1:2 v/v).

S. mutans and *C. albicans* were isolated from supragingival plaque specimens of pediatric patients with severe early childhood caries (severe ECC/ S-ECC) at the Special Dental and Oral Hospital, Faculty of Dentistry, University of Indonesia. Identification of *S. mutans* involved spreading supragingival plaque samples onto Trypticase Yeast Sucrose (TYS) Agar and incubating for 24 hours under anaerobic conditions at 37°C. Following incubation, *S. mutans* colonies were extracted and transferred to Eppendorf tubes containing TYS Broth. Identification was performed via PCR. PCR products were analysed by agarose gel electrophoresis at 60 V and 400 mA for 75 minutes. DNA fragments generated were visualised using ultraviolet light.

The identification of *C. albicans* was conducted using CHROMAgar. *C. albicans* colonies exhibit a green coloration following 48 hours of incubation under aerobic conditions at 37° C. *albicans* colonies were extracted from CHROMAgar, transferred to Eppendorf tubes with Sabouraud Dextrose Broth (SDB), and incubated for 48 hours at 37°C. The DNA samples were identified using PCR. The PCR products were subjected to electrophoresis on a 1.0% (wt/vol) agarose gel at 94 V for 20 minutes following amplification.

We conducted the minimum inhibitory concentration (MIC) assay via the microdilution method and evaluated its antimicrobial activity using an enzyme-linked immunosorbent spectrophotometer (ELISA).[23] Suspensions of *S. mutans* and *C. albicans*, both adjusted to a standard concentration of McFarland 0.5 in 1 mL, were added to Eppendorf tubes with different concentrations of extracts (50%, 25%, 12.5%, 6.25%, 3.125%, and 1.56%); Chlorhexidine (CHX) 2% was used as a positive control, Brain Heart Infusion (BHI) broth was the negative control for *S. mutans*, and Sabouraud Dextrose Broth (SDB) was the negative control for *C. albicans*. Each test tube containing CF leaf extracts and suspensions of *S. mutans* and *C. albicans* was stirred with a vortex mixer.

Upon achieving homogeneity, 200 mL of each combination was extracted and placed into 96-well plates to ascertain the initial absorbance value or turbidity level using a spectrophotometer before incubation. Well plates containing the fraction and *S. mutans* were distinguished from those with the fraction and *C. albicans* based on their incubation at different temperatures.

The 96-well plates were incubated anaerobically at 37°C for 24 hours for *S. mutans* and aerobically for 48 hours for *C. albicans* following initial absorbance measurements. Following incubation, the absorbance of each mixture was reassessed using a spectrophotometer. The Minimum Inhibitory Concentration (MIC) was determined by comparing the absorbance or optical density (OD) values before and after incubation. A positive difference in optical density measurements, where pre-incubation values exceed post-incubation values, signifies a reduction or inhibition of microbial growth. A higher disparity in optical density values corresponds to a reduced quantity of microorganisms.

If the OD values prior to incubation were lower than those following incubation, this indicated that the bacteria persisted in growth or were not suppressed, leading to a negative difference in OD values. We established the MIC

as the minimal concentration that inhibits microbial proliferation, defined as the lowest concentration showing a positive OD difference.[21] Every experiment was performed in duplicate. All laboratory experiments were conducted at the Oral Biology and Oral Science Research Centre, Faculty of Dentistry, Universitas Indonesia.

RESULTS

Polyphenolic and flavonoid compounds are widely recognized as key secondary metabolites contributing to the pharmacological properties of medicinal plants, particularly in relation to their antioxidant, antimicrobial, and anti-inflammatory activities. Quantitative analysis of these compounds provides essential information for understanding the therapeutic potential of plant extracts. These measurements reflect the relative concentration of bioactive constituents present in the ethyl acetate fraction of *CF* leaf. The detailed results are presented in the following table.

Table 1. Identification total polyphenol and flavonoid

Test parameters	Result	Unit
Total polyphenol	15.13	%
Total flavonoid	5.11	%

[1]The result based on test method IK-36/LAKP/SOP/.10

In this study, the ethyl acetate fraction of *CF* leaf contained approximately three times the total polyphenols (15%) compared to its flavonoid content (5%), as seen in Table 1. Phytochemical screening was performed to ascertain the presence of secondary metabolites in the extract. The findings revealed the presence of several bioactive compounds typically linked to antibacterial, antioxidant, and therapeutic effects. The following table presents a comprehensive summary of the identified phytochemical constituents.

Table 2. Phytochemical screening result.

Secondary metabolites	Reagent	Result
Phenolic	5% $FeCl_3$	+
Tannin	1% $FeCl_3$	+
Flavonoid	10% NaOH	+
Saponin	2N HCl	-
Triterpenoid	$H2SO_4$	+
Steroid	$H2SO_4$	-
Alkaloid	Wagner's	-

[2]Description: (+) detected, (-) not detected

The phytochemical analysis revealed the presence of phenolics, tannins, flavonoids, and triterpenoids as secondary metabolites, as indicated in Table 2. No saponins, steroids, or alkaloids were found. GC–MS analysis was performed to identify the chemical composition of the ethyl acetate fraction of *CF* leaf. The comprehensive list of detected compounds, along with their retention times, peak areas, and potential biological significance, is provided in the following table.

Table 3. GC-MS screening result.

No.	Compound	Quality	RT	Area (%)
1.	Xylopyranoside, methyl 4-azido-4-deoxyl-, beta.-L-	32	3.308	3.01
2.	1,3,5-Triazine-2,4,6-triamine	64	6.817	4.54
3.	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-	96	8.120	11.94
4.	5-Hydroxymethylfurfural	95	12.109	73.88

[3]RT= Retention Time

Four chemical compounds were detected in the ethyl acetate fraction from the CF leaf using GC-MS (Table 3). The largest peak area (73.88%) corresponded to 5-Hydroxymethylfurfural, which had a retention time of 12.109 minutes.

Figure 1 below shows the chromatographic profile of those detected compounds. The chromatographic profile offers a detailed depiction of the volatile and semi-volatile compounds present in the ethyl acetate fraction of CF leaf. The differing peak intensities reflect variations in relative abundance, providing preliminary insights into the predominant and minor components of the extract. This chromatographic pattern underscores the chemical diversity of the sample and provides a basis for subsequent compound identification and interpretation of bioactive potential.

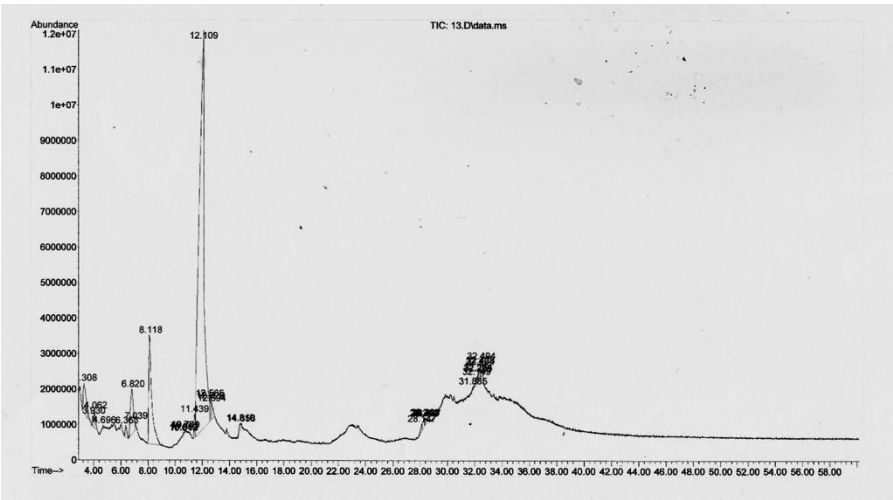

Figure 1. Chromatographic profile of ethyl acetate fraction conducted using gas chromatography (GC)

Figure 1 illustrates that the highest peak occurred at retention time 12.109 (belong to 5-Hydroxymethylfurfural), whereas the lowest appeared at 3.308 (belong to Xylopyranoside, methyl 4-azido-4-deoxyl-, beta.-L-). The mass spectrum confirms the presence of 5-Hydroxymethylfurfural in the ethyl acetate fraction of *CF* leaf. The characteristic fragmentation peaks serve as diagnostic markers confirming the identity of 5-Hydroxymethylfurfural, thereby supporting its detection and correlation with previously reported phytochemical data, as seen in Figure 2. The molecular structure of 5-Hydroxymethylfurfural, which showed the largest peak area, was validated using mass spectroscopy as seen in figure 2.

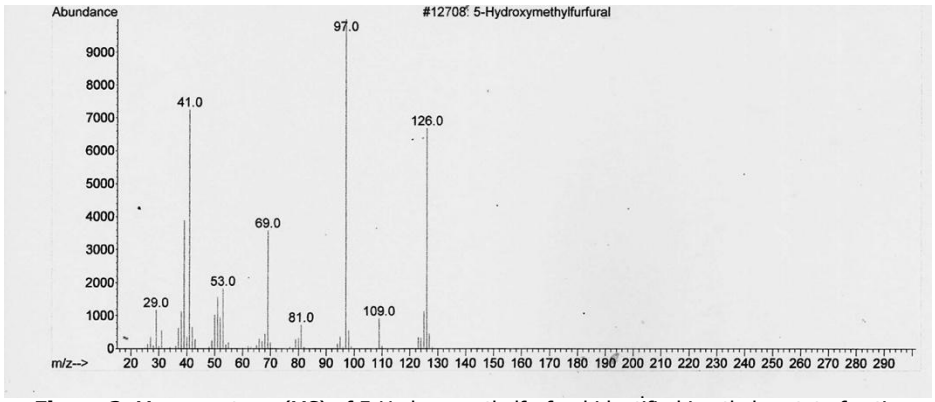

Figure 2. Mass spectrum (MS) of 5-Hydroxymethylfurfural identified in ethyl acetate fraction

The antimicrobial activity of ethyl acetate fraction from *CF* leaf against *S. mutans* and *C. albicans* is presented in Table 4. The minimum inhibitory concentration (MIC) assay was performed using the dilution method and analysed

with an enzyme-linked immunosorbent spectrophotometer (ELISA) to evaluate antimicrobial activity. MIC values represent the averages of duplicate experiments.

Table 4. MIC based on optical density (OD) values.

No.	Concentration (%)	*S. mutans*			*C. albicans*		
		before	after	difference	before	after	difference
1.	50	2.688	2,663	0,025*	1.230	0.333	0.897*
2.	25	2.719	2.553	0.166*	1.085	0.156	0.929*
3.	12.5	2.588	2.013	0.575*	0.840	0.145	0.695*
4.	6.25	2.222	1.04	1.182*	0.769	0.127	0.642*
5.	3.125	1.557	0.171	1.386*	0.422	0.153	0.270*
6.	1.56	0.051	0.789	-0.738**	0.708	0.066	0.642*
7.	CHX 2%	1.547	0.407	1.14*	0.450	0.450	0.000*
8.	Medium	0.050	0.256	-0.206**	0.055	0.937	-0.882**

[4]Description: before= OD before incubation; after= OD after incubation; difference= OD before-OD after; *= inhibited (OD before > OD after, so the difference value= (+) or > 0); **= not inhibited (OD before < OD after, so the difference value= (-) or < 0)

According to Table 4, the ethyl acetate fraction of *CF* leaf exhibits MICs of 3.125% for *S. mutans* and 1.56% for *C. albicans*. Chlorhexidine, as the positive control, inhibited both *S. mutans* and *C. albicans*. In contrast, the medium (BHIB for *S. mutans* and SDB for *C. albicans*) demonstrated no inhibitory activity as the negative control.

DISCUSSION

The quantitative analysis of the ethyl acetate fraction from CF leaf revealed that total polyphenols (15.13%) were present at levels higher than flavonoids (5.11%), as seen in Table 1. This result aligns with previous studies which found that polyphenols are the major class of compounds commonly found in most plants with therapeutic benefits.[24–26] Polyphenols are low-molecular-weight organic compounds characterised by an aromatic ring (benzene or phenol) bearing one or more hydroxyl groups. Polyphenols are a class of phytochemicals known to confer health benefits.[26] Polyphenols are categorised as flavonoids (flavonols, flavanols, flavones, flavanones, isoflavones, and anthocyanins) and non-flavonoid compounds (phenolic acids, hydroxycinnamic acids, lignans, stilbenes, and tannins).

The bioavailability of polyphenols differs across various classes. Physiological-biochemical, molecular-genetic, and environmental factors influence the synthesis and accumulation of polyphenols in plants. Polyphenols demonstrate strong antioxidant activity, supporting their effective application in pharmacology for the treatment of disorders with diverse causes.[26] The effects of polyphenols include capillary strengthening, antibacterial, antiviral, antitoxic, and neuroprotective properties.[25]

The phytochemical analysis identified four secondary metabolites: phenolics, flavonoids, tannins, and triterpenoids (Table 2). Previous studies revealed similar findings regarding secondary metabolites that identified phenolics, flavonoids, tannins, and triterpenoids in methanol extract of Cordyline fruticosa (L.) A. Chev. leaves.[18,25] Phenolic compounds have long been used as medicines and nutritional supplements due to their high biological activity.26 Flavonoid-type polyphenols have intricate structures, characterised by two aromatic rings linked by a three-carbon segment. Flavonoids break down proteins in cell walls, which weakens their structure and changes how easily substances can pass through microsomes, lysosomes, and microbial cell walls. Flavonoids induce the denaturation of proteins within cell walls, compromising structural integrity and altering membrane permeability in microsomes, lysosomes, and microbial cell walls.[27]

Tannins have demonstrated antifungal properties.[19] Tannins compromise cell membrane integrity and hinder cell wall production.[28] This fraction also contains triterpenoids. Triterpenoids are terpenoid compounds with various biological actions, including anticancer, anti-inflammatory, antioxidant, antiviral, antibacterial, and antifungal activities.[29] Additionally, earlier studies indicated that triterpenoids damage cell membranes by interfering with ergosterol production, reducing acid levels in the medium, and ultimately disrupting normal functioning of the plasma membrane.[30] These metabolites act against microorganisms by reducing the surface tension of sterol membranes, degrading cell walls, and inhibiting the synthesis of new cell walls, ultimately leading to microbial eradication. Their activities enhance antibacterial efficacy against S. mutans.[31] Prior research has shown that flavonoids and tannins in CF leaf extracts have antibacterial effects against S. mutans and antifungal effects against C. albicans, which are significant factors in the development and virulence of cariogenic biofilms.[32,33] This fraction did not contain saponins, alkaloids, or steroids.

GC-MS analysis revealed four bioactive compounds in this fraction (Table 3, figure 1). The predominant chemical was 5-hydroxymethylfurfural, comprising 73.88% of the total, as seen from the gas chromatography (GC) screening result in figure 1 and validated by mass spectroscopy (MS) in figure 2. This study is a novel attempt to quantify the ethyl acetate fraction components in the leaves of Cordyline fruticosa (L) A. Chev. To date, no research has analysed the ethyl acetate fraction content of Cordyline fruticosa leaves for comparison with our findings. Prior studies have identified two pure chemical compounds, namely stigmasterol and oleanolic acid, in the ethyl acetate fraction of the Cordyline fruticosa stem.[16] Previous study demonstrated that 5-hydroxymethylfurfural possesses antioxidant, antibacterial, anti-proliferative, and antiallergic properties.[34] This finding is consistent with prior study that found the concentration of furfural is almost ten times greater than other chemicals.[35] Other studies indicated that these substances have high antifungal efficiency by inhibiting fungal spore germination, hyphal extension, and mycelial proliferation, rendering them essential compounds for bioactive applications.[36]

The 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, accounted for 11.94% in this fraction. This compound is known to have antioxidant, anti-inflammatory, and antifungal properties.[37] Both 1,3,5-triazine and methyl 4-azido-4-deoxyl-beta-L-xylopyranoside were found in very small amounts (less than 5% each). Prior research demonstrated that 1,3,5-triazine and its derivatives possess considerable biological activity, including antibacterial, antifungal, antimalarial, anticancer, antiviral, antimicrobial, anti-inflammatory, anti-amoebic, and antitubercular properties;[38] meanwhile xylopyranoside exhibits vasorelaxant and hypotensive effects.[39]

Scientific information addressing the efficacy of ethyl acetate fraction of CF leaf extracts against S. mutans and C. albicans in the context of ECC has been inadequate. The antimicrobial activity was determined based on MIC evaluation results (Table 4). The MIC for S. mutans was 3.125%, but at 1.56%, bacterial proliferation occurred, evidenced by elevated absorbance values post-incubation. In contrast, the MIC for C. albicans was 1.56%, indicating the minimal inhibitory concentration observed in this investigation.

Based on earlier study, a higher concentration of the fraction correlated with increased microbial inhibition, likely because it contained more antimicrobial compounds present.[40] CHX (2%) served as a positive control, demonstrating significant efficacy against both microorganisms, whereas the Brain Heart Infusion (BHI) and Sabouraud Dextrose Broth (SDB) media served as negative controls, exhibiting no inhibitory activity. This study showed that the ethyl acetate fraction from CF leaf at low concentration was more effective at inhibiting C. albicans than S. mutans, based on MIC tests.

The observed phenomenon may be attributed to the presence of chemical compounds within the ethyl acetate fraction identified in this study, all of which

exhibit antifungal activity. Notably, one compound, 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl-, exhibits limited antibacterial activity yet demonstrates antifungal effects. Previous study revealed that 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP) may serve as a promising alternative in antifungal applications, such as in protecting rubberwood from fungal attacks.[39] Most recent studies identified 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- by GC-MS in plant extracts with documented antifungal and antibacterial activity.[40]

The limitation of this study lies in its emphasis on in vitro methods, with no toxicity studies conducted. Additional in vivo studies and toxicity assessments are required to determine the efficacy and safety of direct application.

CONCLUSION

The ethyl acetate fraction of *CF* leaf contained high levels of polyphenols and four major classes of secondary metabolites (phenolics, tannins, flavonoids, and triterpenoids) along with four bioactive compounds, with 5-hydroxymethylfurfural identified as the predominant constituent. These components demonstrated antibacterial activity against *S. mutans* and antifungal activity against *C. albicans*. The implication of these findings is that the ethyl acetate fraction from *CF* leaf extract has potential as an alternative natural antimicrobial agent for the prevention of ECC.

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