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Research article

Phytochemical profile and antimicrobial activity of *Jatropha curcas* extracts against oral microorganisms

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ABSTRACT

Introduction: The growing problem of antimicrobial resistance on a global scale has highlighted the need to investigate alternative antimicrobial agents with reduced side effects. Plant-derived secondary metabolites have emerged as potential contenders in tackling this challenge. *Jatropha curcas*, a perennial plant, has traditionally been utilized for the treatment of gum boils, toothaches, and infections. This plant exhibits a wide range of pharmacological properties. However, its potential as an antimicrobial agent against oral microorganisms has yet to be investigated. Hence, the objective of this study was to investigate the antimicrobial properties of *Jatropha curcas* extracts against selected bacteria and fungi commonly present in the oral cavity.
Methodology: Jatropha curcas samples were collected from Bagan Datuk, Perak, Malaysia, and subsequently identified at Universiti Malaya. The ethanolic extract of the leaves (ELJC) and the stem bark latex (LJC) of *Jatropha curcas* were tested against six species of oral microorganism: *Streptococcus sanguinis, Streptococcus mutans*, *Streptococcus mitis*, *Lactobacillus helveticus*, *Candida albicans, Candida tropicalis*, and a mixture of these microorganisms. The methods employed in this study were well diffusion assay, minimum inhibitory concentration, minimum bacterial concentration, live-dead assay, field emission scanning electron microscopy, and liquid chromatography with tandem mass spectrometry.
Results: ELJC and LJC demonstrated significant antimicrobial effects ($p < 0.05$). Treatment with ELJC and LJC resulted in morphological changes and increased death rates in the targeted microorganisms. ELJC was found to contain more than 300 bioactive compounds, with isovitexin, being the most abundant. In contrast, LJC exhibited over 1000 bioactive compounds with 2-hexyl-decanoic acid and 2,4,6-trihydroxybenzoic acid being the predominant constituents.
Conclusion: These findings suggest that the antimicrobial effects observed in ELJC and LJC against *S. sanguinis* and *S. mutans* can be primarily attributed to isovitexin, 2-hexyl-decanoic acid, and trihydroxybenzoic acid. However, further research and investigation are necessary to elucidate the mechanisms by which these constituents exert their antimicrobial effects on the microorganisms.

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1. Introduction

The oral cavity contains a diverse array of more than 700 microorganisms species [1–4] and the composition of these microbial communities varies among different components within the oral cavity [1,5] and throughout different stages of human life [1]. Among these microorganisms, *Streptococcus mutans*, *Streptococcus sanguinis*, and *Streptococcus mitis* are Gram-positive facultative cocci that are commonly found in the gingival crevice [5]. Additionally, *Lactobacillus* is a Gram-positive facultative rod-shaped bacterium prevalent in the same area [5]. *Candida tropicalis* and *Candida albicans* are the common species of oral fungi found in the oral cavity [6].

The microorganisms that resides in the oral cavity can have both advantageous and disadvantageous effects, which depend on several factors. These factors include the specific microbial species that are introduced into the oral cavity and their interactions with the existing oral microbiota, and the host's immune response [3]. It has been observed that certain probiotic microorganisms, such as lactobacilli when introduced into the oral environment, can improve oral health by fostering a harmonious microbial ecosystem [7]. For example, certain probiotic strains are effective in reducing the populations of cariogenic bacteria such as *Streptococcus mutans*, thereby mitigating the risk of dental caries [8,9]. Conversely, prebiotics are substances that promote growth of beneficial bacteria in the oral cavity, can also exert a protective influence against dental caries [10]. For instance, xylitol, a common sugar alcohol found in chewing gum and dental products has been shown to promote the proliferation of non-cariogenic bacteria such as *Streptococcus salivarius* [11,12], thereby protecting against tooth decay and bolstering oral health. On the other hand, the inadvertent introduction of microorganisms may also occur during certain dental procedures such as scaling, dental surgeries or even when sharing toothbrushes [13–15]. In order to restore the natural balance of microbes in the oral cavity, the concept of oral microbiome transplant which involves the transfer of a donor's healthy oral microbiome to a recipient for the management of dental diseases has been suggested [16–18].

Oral diseases have a high prevalence worldwide, affecting approximately 50 % of the global population, or 3.5 billion people [19]. Oral infections are common when the normal oral bacteria become imbalanced, allowing harmful microorganisms to overgrow. This is often observed in individuals with weakened immune systems, like those with diabetes or cancer, making them more vulnerable to infections from oral bacteria such as *Streptococcus mutans* and *Porphyromonas gingivalis* which are responsible for dental caries and periodontal disease, respectively [20]. The emergence of antimicrobial resistance (AMR) has posed significant challenges in treating infections [21]. The World Health Organization (WHO) has identified AMR as one of the top 10 global public health concerns and emphasized the urgent need for research and development of new antimicrobial agents [22].

Plant-based agents have garnered significant attention in antimicrobial research due to their diverse array of bioactive compounds and potential to combat microbial infections effectively. Plant-based agents have a lot of potential for novel drug discovery [23] and possess several properties including broad-spectrum activity [24], reduced resistance risk [25], natural safety [26], and synergistic effect when combined with conventional treatments [27]. Many plant extracts can target a wide range of bacterial, fungal, and viral pathogens, due to their multiple bioactive compounds [24]. This multifaceted approach makes it harder for microbes to develop resistance [25]. Moreover, plant-based antimicrobial agents are renewable and environmentally friendly alternatives to synthetic drugs [26,28]. By exploring plant biodiversity, there is an opportunity to discover novel antimicrobial compounds as leads for new drug development.

Jatropha curcas, a perennial small tree belonging to the Euphorbiaceae possesses a wide range of pharmacological properties. Abdelgadir and Van Staden (2013) summarized the pharmacological properties of this plant in their review article, comprising the antioxidant, anti-inflammatory, cytotoxic, anticancer, antifungal, antimalarial, and antimicrobial effects [29]. It has been reported that *Jatropha curcas* exhibited prominent antimicrobial activities against pathogenic bacteria such as *Escherichia coli*, *Salmonella typhimurium*, *Proteus vulgar,* and *Enterobacter cloacae* [30–33]. In the context of oral health, *Jatropha curcas* has been traditionally used for treating toothaches [33,34], gum boils, oral infections, and strengthening of the gums [35]. While its traditional applications in oral

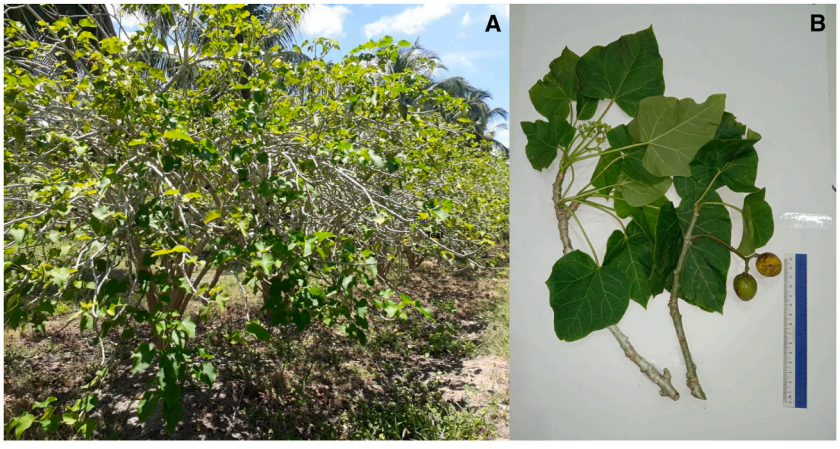

Fig. 1. (A) *Jatropha curcas* plant obtained from the Malaysian Agricultural Research and Development Institute (MARDI), Bagan Datuk, Perak, Malaysia. The figure emphasizes the (B) herbarium sample, including the stem, leaves, flowers and fruits of the plant.

health are clearly documented, its antimicrobial activity against oral microbes is lacking. Hence, the aim of this study is to investigate the antimicrobial effects of the ethanolic extract of the leaves and stem bark latex *Jatropha curcas* against common oral microorganims, representing the first such report.

2. Methodology

2.1. Plant samples

The plant samples of *Jatropha curcas* were collected from the Malaysian Agricultural Research and Development Institute (MARDI), Bagan Datuk, Perak, Malaysia. The plant was identified by Dr. Yong Kien Thai from the Institute of Biological Sciences, Faculty of Science, Universiti Malaya. A voucher specimen numbered KLU 50182 (Fig. 1B) was deposited at the Herbarium of the Institute of Biological Sciences, Faculty of Science, Universiti Malaya in 2022.

2.2. Preparation of plant extracts

The leaves of *Jatropha curcas* were separated, washed, and dried in an oven at a constant temperature of 45 °C for three days. The dried samples were ground into fine powder using a laboratory-grade grinder. The dried ground leaves (100 g) were extracted with ethanol: water (80:20) at room temperature for three days. The solution containing extract was filtered and evaporated under reduced pressure to obtain the ethanolic leaves extracts (ELJC). The extracts were kept at 4 °C until further use. The collected latex from the stem-bark of *Jatropha curcas* (LJC) were freeze-dried and stored in a centrifuge tube at 4 °C until further use. The concentration of samples was prepared according to the requirements for each assays prior to use. The ethanolic extract of the leaves (ELJC) of *Jatropha curcas* was fully dissolved with 0.5 % dimethyl sulfoxide (DMSO) (w/v). Whilst, the latex of *Jatropha curcas* (LJC) was fully dissolved in a sterile distilled water (DH_2O) (w/v).

2.3. Antimicrobial activity

2.3.1. Preparation of microbial suspension and control solutions

The frozen stocks of oral microorganisms namely *Streptococcus sanguinis* ATCC BAA1455, *Streptococcus mutans* ATCC 25175, *Streptococcus mitis* ATCC 49456, *Lactobacillus helveticus* ATCC 15009, *Candida albicans* ATCC 14053, and *Candida tropicalis* ATCC 13803 were obtained from the Balai Ungku Aziz Research Laboratory, Faculty of Dentistry, Universiti Malaya. Briefly, the microbial suspensions were prepared by transferring 1 µl of each strain of oral microorganism into 5 ml of brain heart infusion (BHI) broth, followed by an overnight incubation at 37 °C. Subsequently, each microbial sample was centrifuged at 10,000 rpm at a temperature of 10 °C for a duration of 5 min. The pellet obtained was washed twice with sterile saline (NaCl, 8.5 g/L) before being resuspended in 5 ml of BHI broth. The turbidity of the microbial suspension was adjusted using a spectrophotometer at a specific wavelength (nm) and optical density (OD) to obtain the desired bacterial cell concentration according to the experimental method. BHI broth was used as the blank [36]. An equal volume of adjusted microbial suspension was mixed to obtain the final mixture of microbial suspension. In this study, the positive control solution for the bacterial groups consisted of 0.12 % chlorhexidine gluconate (CHX) (w/v), while for the fungal groups, it was 250 mg/ml amphotericin B (w/v). Meanwhile, the negative control solution used was 0.5 % dimethyl sulfoxide (DMSO) (v/v) for ELJC and sterile distilled water (DH_2O) for LJC.

2.3.2. Agar well diffusion assay

The method was performed based on Boyanova et al. [37], with slight modification. A microbial suspension was prepared for *S. sanguinis*, *S. mutans*, *S. mitis, L. helveticus*, *C. albicans*, *C. tropicalis,* and a mixture of these microorganisms at an optical density (OD) of 0.144 measured at a wavelength of 600 nm. Then, 100 µl of each microbial suspension was evenly swabbed onto BHI agar plates using a sterile cotton swab. A sterile cork borer was used to create a well with a diameter of six mm on each plate. Subsequently, 20 µl of the test samples containing ELJC or LJC extracts at concentrations of 25, 50, and 100 mg/ml, as well as the positive and negative control solutions, were individually pipetted into the respective wells. The positive control for the bacterial groups consisted of 0.12 % CHX (w/v), while for the fungal groups it was 250 mg/ml amphotericin B (w/v). The negative control for the ELJC groups was 0.5 % DMSO (v/v), and for the LJC groups, it was DH_2O. All plates were incubated at 37 °C for 24 h. The inhibition zone diameter was measured in millimetres (mm) using an electronic digital vernier calliper. The experiments were performed in triplicate (n = 3). The measurement values were expressed in mean ± standard deviation.

2.3.3. Minimum inhibitory concentration (MIC)

The MIC values were determined for the selective oral microorganisms as described in Section 2.3.2. The ELJC was tested against *S. sanguinis*, while the LJC were tested against *S. mutans*. The MIC study followed the adaptation from Nyalo et al. [38]. A sterile 96-well microplate was divided into treatment, negative control, and positive control groups (0.12 % CHX) for each microorganism. Serial dilutions of 100 µl of the ELJC (100 mg/ml) and LJC (100 mg/ml) were prepared, respectively. Then, 20 µl of the microbial suspensions (adjusted to 0.144 OD at 600 nm) were added to the wells. The plates were incubated for 24 h at 37 °C. Following incubation, the turbidity of each well was measured at 600 nm using a microplate reader (Spectramax CRL M3 Multi-Mode Microplate Reader). The OD readings of the treatment groups were compared to the negative control groups for analysis. The MIC was determined as the lowest concentration at which the treatment group exhibited lower turbidity or OD reading compared to the negative control group. The

experiments were performed in triplicate (n = 3). The data values were expressed in mean ± standard deviation.

2.3.4. Minimum bactericidal concentration (MBC)

Following the MIC results, the MBC assay was conducted according to the modified method by Nyalo et al. [38]. Initially, the BHI agar plate was divided into three sections: treatment, positive control and negative control. Then, a sterile cotton swab was used to transfer the suspensions from the wells of the treatment groups at and above the determined MIC value, as well as the negative and positive control wells, onto the corresponding sections of the BHI agar plate. The plate was then incubated for 24 h at 37 °C. Following incubation, bacterial growth on the plate was observed, and the lowest concentration that showed no significant bacterial growth on BHI agar was determined as the MBC respectively. The experiments were performed in triplicate (n = 3).

2.3.5. Live-dead assay

The method was based on Ramalingam & Amaechi [39] with slight modifications. Microbial suspension of *S. sanguinis* (600 nm, 0.255 OD) and *S. mutans* (600 nm, 0.222 OD) were prepared in separate 1.5 ml microcentrifuge tubes, each containing 500 μl of the respective microbial suspension. These microbial suspensions were then treated with 500 μl of the respective MBC concentrations of the ELJC or LJC extracts. For the positive control, 500 μl of 0.12 % CHX was used, while the negative control received no treatment. The microbial suspensions were subjected to treatment at different time points specifically, at 5 min, 15 min, and 1 h after the initial treatment. Following each incubation period, 250 μl of the treatment suspensions with ELJC and LJC, respectively, as well as the positive and negative control suspensions, were transferred into a new 1.5 ml-microcentrifuge tube. To each tube, 1.25 μl of a mixture containing equal amounts of SYTO9 and propidium iodide dye was promptly added. The suspensions were then adequately mixed and incubated at room temperature for 15 min in the dark (wrapped in aluminium foil). Subsequently, 5 μl of each suspension was deposited onto an adhesive microscope slide and covered with a cover slip. The slide was then viewed using a fluorescence microscope (Nikon/Eclipse Fluorescence Microscope & Live Imaging). Initially, the slide was observed under phase contrast to confirm the presence of microorganisms, and a field with well-distributed microorganisms was selected. Following that, a fluorescein isothiocyanate (FITC) and tetramethyl rhodamine isothiocyanate (TRITC) filter was applied, and a fluorescence image was captured. Image analysis was conducted using the NIS-Elements Software to identify live (fluorescence green) and dead (fluorescence red) microorganisms in the treatment and control suspensions. The experiments were performed in triplicate (n = 3).

2.4. Field emission scanning electron microscopy (FE-SEM)

The bacterial suspensions (500 μl) of *S. mutans* and *S. sanguinis* (600 nm, 0.144 OD) were added into separate microcentrifuge tubes. Then, *S. mutans* and *S. sanguinis* suspensions were treated with the MBC of ELJC and LJC extracts (500 μl), respectively. Untreated bacterial suspensions of *S. mutans* and *S. sanguinis* served as negative controls for each respective experiment. All four tubes were incubated at 37 °C for 24 h. Following incubation, 100 μl of each suspension was dispensed onto a Nucleopore membrane filter (13 mm diameter, 0.2 μm pore size). This process was repeated five times for each suspension. The membrane filter was immediately immersed in 4 % glutaraldehyde for 3 days. Subsequently, the membrane filter was washed twice in a buffer solution for 10 min and then subjected to post-fixation in 1 % osmium tetraoxide for 1 h. The membrane filter was washed twice in filtered double distilled water (ddH_2O) for 10 min each, followed by dehydration using a series of ascending alcohol concentrations: 30 %, 50 %, 70 %, 80 %, 90 %, 95 %, and finally two rounds of 100 % ethanol (each for 15 min). This was followed by further dehydration using a mixture of ethanol and acetone at ratios 3:1, 1:1, and 1:3 ratios, each for 15 min, and two rounds of pure acetone for 20 min. The filter was then subjected to a critical point drying machine for 1 h. Once removed, it was mounted on a stub and coated with gold using a sputter coater. The stub, with the coated filter, was placed in a FE-SEM (FEI QUANTA FEG 650 S). The images of the microorganisms were captured and analysed. The method employed was adapted from the Central Unit For Advanced Research Imaging (CENTUARI), Faculty of Medicine, Universiti Malaya.

2.5. Liquid chromatography mass spectrometry quadrupole time of flight (LC-MS/QTOF)

2.5.1. Phytochemical analysis of ELJC

The ELJC analysis was conducted using the Agilent 1290 Infinity LC system coupled to the Agilent 6520 Accurate-Mass Q-TOF mass spectrometer featuring a dual electrospray ionization (ESI) source. The LC parameters included the use of an Agilent Eclipse XDB-C18 Narrow-bore (150 mm × 2.1 mm, 3.5-μm particle size) operated at a temperature of 25 °C. The autosampler temperature was set to 4 °C, and the flow rate was maintained at 0.5 mL/min. The mobile phase consisted of 0.1 % formic acid in water (solvent A) and 0.1 % formic acid in acetonitrile (Solvent B). A sample injection volume of 1.0 μL was employed. The sample analysis was performed in positive and negative mode. The total run time for each analysis was 25 min, followed by a post-run time of 5 min. The mass spectrometer operated in MS-only mode with a positive ion polarity set at Vcap 4000 V and a negative ion polarity at Vcap 3500 V. The fragmentor voltage was set to 125 V and the skimmer voltage was set to 65 V. The OCT 1 RF Vpp was 750 V. The drying gas flow rate was maintained at 10 L/min, and the gas temperature: was set to 300 °C. The nebulizer pressure was set at 45 psig. The mass range (m/z) analysis was set to a minimum of 100 and a maximum of 3200 m/z for both positive and negative polarities. Reference ions used for calibration were 121.0508–922.0097 for positive polarity and 119.03632 to 966.000725 for negative polarity. The acquisition rate (spectra/s) was set at 1.03, while the acquisition time (MS/spectrum) and transients/spectrum were set at 973 and 9632, respectively. Data analysis was performed using Agilent MassHunter Qualitative Analysis B.07.00. Raw data was processed with Molecular Feature Extraction (MFE) with specific settings. The extraction algorithm employed was for small molecules (chromatographic) and peak

filters were set to include peaks with a height of ≥100 counts. The input data range was defined as 100–3200; Compound filters were set to consider only compounds with an absolute height of ≥5000 counts and a relative height of ≥2.5 %. Ion species considered were H^+, Na^+, K^+, NH_4^+ for positive ions and H^-, Cl^- for negative ions. Isotope grouping parameters included a peak spacing tolerance of 0.0025 *m/z* plus 7.0 ppm, with the isotope model set to common organic molecules. Charge states were limited to a maximum of 2. The identification of compounds was performed using the Metlin_AM_PCDL-N-170502.cdb search database. For compounds not available in the database, formulas were generated based on predefined parameters. An 80 % ethanol solution was used as a blank in the analysis.

2.5.2. Phytochemical analysis of LJC

The analytical system utilized for LJC analysis consisted of Agilent 1200 liquid chromatography system, comprising a binary pump, a vacuum degasser unit, an autosampler, and 6520 quadrupole time of flight mass spectrometers equipped with an electrospray ionization (ESI) source. The column used was Agilent ZORBAX EclipsePlus C18 Rapid Resolution HT (2.1 × 100 mm) 1.8 μm operated at a temperature of 40 °C. For positive ionization mode, the mobile phase employed was (A) 0.1 % formic acid in ddH_2O and (B) 0.1 % formic acid in acetonitrile. In negative ionization mode, the mobile phase was composed of (A) ddH_2O + 0.1 % ammonium formate and (B) 100 % acetonitrile. The gradient elution program was as follows 0.00–18.00 min, 5%–95 % (B); 18–23 min; 95 % (B); 23.10 min; 5 % (B). The total run time was 30 min. The LC condition was re-equilibrated for 2 min before each new injection. The sample injection volume was set at 5 μl, and the flow rate of the mobile phase was set at 0.25 ml/min. The mass spectrometer was operated in both positive and negative electrospray ionization (ESI) mode. The gas temperature was optimized at 325 °C, with a gas flow rate of 10 L/min for positive ESI mode and a nebulizer pressure of 30 psi for negative ESI mode. The identification of compounds was performed using the DBsearch database search tool.

2.6. Statistical analysis

The data from the MIC were analysed using Microsoft Excel Software. For the agar well diffusion assay, statistical analysis was conducted using the one-way Analysis of Variance (ANOVA) with post-hoc Tukey HSD test. These analyses were carried out in the Statistical Package for the Social Sciences (SPSS) software. The experiments were done in triplicates (n = 3) and the data values are expressed in mean ± standard deviation.

3. Results

3.1. Agar well diffusion assay

In order to evaluate the antimicrobial potential of *Jatropha curcas* extracts, the agar well diffusion susceptibility test was conducted against various microorganisms, namely *S. mitis*, *S. sanguinis*, *S. mutans*, *C. albicans, C. tropicalis* and *L. helveticus* (Table 1). In this assay, the size of the inhibition zone surrounding the well, caused by these extracts, served as a reliable indicator for their antibacterial potency against oral microorganisms. According to the data presented in Table 1, ELJC exhibited significant antimicrobial effects ($p < 0.05$) against *S. mitis*, *S. sanguinis*, *L. helveticus*, and *C. tropicalis*. However, ELJC was found to be inactive against *S. mutans*, *C. albicans*, and a mixture of the tested microorganisms. Conversely, LJC exhibited significant antibacterial effects against *S. mutans*. In contrast,

Table 1
Antibacterial activity of the ethanolic extract of the leaves (ELJC) and stem bark latex (LJC) of *Jatropha curcas* against oral microorganisms using agar well diffusion assay.

Extract	Diameter of inhibition zones (mm)[a]						
	Bacterial/Fungal strains						
	S. mitis	*S. mutans*	*S. sanguinis*	*L. helveticus*	*C. albicans*	*C. tropicalis*	Mixed species[b]
ELJC							
25 mg/ml	6.57 ± 0.02*	–	11.34 ± 0.57*	7.13 ± 0.12*	–	6.38 ± 0.14*	–
50 mg/ml	6.67 ± 0.27*	–	14.06 ± 0.87*	7.69 ± 0.58*	–	6.75 ± 0.15*	–
100 mg/ml	7.38 ± 0.29*	–	15.85 ± 0.06*	8.74 ± 0.33*	–	8.54 ± 0.23*	–
Positive control[c]	20.62 ± 0.15*	17.82 ± 0.56*	25.02 ± 0.59*	15.59 ± 0.94*	26.45 ± 1.15*	–	22.27 ± 0.67*
LJC							
25 mg/ml	–	10.72 ± 0.50*	–	–	–	–	–
50 mg/ml	–	14.28 ± 0.15*	–	–	–	–	–
100 mg/ml	–	18.86 ± 0.26*	–	–	–	–	–
Positive control[d]	18.84 ± 1.58*	17.75 ± 0.29*	26.79 ± 0.41*	16.31 ± 0.99*	24.55 ± 1.21*	–	22.39 ± 1.05*

[a] Diameter of inhibition zone include the diameter of the well (6 mm).
[b] Mixed species consist of *S. mitis, S. mutans, S. sanguinis, L. helveticus*, *C. albicans, and C. tropicalis*.
[c] Positive control for bacterial groups (*S. mitis, S. mutans, S. sanguinis* and *L. helveticus)* was 0.12 % chlorhexidine gluconate (CHX) (w/v), while for the fungal groups (*C. albicans, and C. tropicalis)* was 250 mg/ml amphotericin B (w/v); Negative control for ELJC was 0.5 % dimethyl sulfoxide (DMSO) (v/v) whereas for LJC was sterile distilled water (DH_2O); -: No zone of inhibition observed; Tabulated values are mean ± standard deviation (n = 3); *$p < 0.05$ indicates significant different from the negative control.

LJC displayed no activity against *S. mitis, S. sanguinis*, *L. helveticus*, *C. albicans*, *C. tropicalis*, and a mixture of the tested microorganisms.

3.2. Minimum inhibitory concentration (MIC)

The strongest antimicrobial effect of the ELJC and LJC extracts were observed against the oral bacterial species *S. sanguinis* and *S. mutans* respectively, as determined in section 3.1. Therefore, these bacterial species were selected for further investigations of their inhibitory effects, as indicated by the MIC values presented in Table 2. The MIC value of ELJC against *S. sanguinis* was determined to be 25 mg/ml, indicating considerable antibacterial activity. In contrast, LJC extract exhibited stronger activity against the tested *S. mutans*, with a lower MIC value of 6.25 mg/ml.

3.3. Minimum bactericidal concentration (MBC)

MBC was performed to further assess the bactericidal or killing activity of the *J. curcas* extracts. When tested against *S. sanguinis*, ELJC demonstrated no bacterial growth on BHI agar plates at concentrations of 25, 50, and 100 mg/ml. Consequently, the MBC for ELJC was determined to be 25 mg/ml. On the other hand, LJC exhibited bacterial growth on BHI agar plates at concentrations of 6.25 mg/ml and 12.5 mg/ml when tested against *S. mutans*. However, minimal bacterial growth was observed at concentrations 25, 50, and 100 mg/ml. Therefore, the MBC for LJC was also determined at 25 mg/ml.

3.4. Bacterial viability using live/dead assay

The assay was performed to determine the viability and distribution of live and dead microorganisms treated with ELJC and LJC extracts. Initially, the presence and distribution of microorganisms in the microscope field were determined using phase contrast before applying the fluorescence filters. In this assay, the live bacteria cells are labelled with a green fluorescent dye (SYTO9). This dye can penetrate intact cell membranes and bind to nucleic acids, resulting in bright green fluorescence. While, dead bacteria with compromised membranes are labelled with a red fluorescent dye, emitting a red fluorescence. Fig. 2A shows oral bacterial cells (*S. sanguinis*) treated with ELJC exhibited a higher intensity of red fluorescence and a significantly diminished green fluorescence at different incubation times (5 min, 15 min, and 1 h) compared to the negative control groups. Additionally, the positive control groups, where *S. sanguinis* bacterial cells treated with 0.12 % CHX, displayed notably higher red fluorescence, particularly at the 1-h incubation time (Fig. 2A). CHX served as the positive control to conduct a comparative analysis between the selected species of oral bacteria and the mixtures of the selected species, leveraging the well-documented antiseptic attributes of CHX, which are commonly encountered in commercial mouthwash formulations. Similarly, the bacterial cells (*S. mutans*) treated with LJC extract at different incubation times (5 min, 15 min, and 1 h) exhibited a higher intensity of red fluorescence and a reduced intensity of green fluorescence compared to the negative control groups (Fig. 2B). Furthermore, the positive control groups on treated bacterial cells *S. mutans* with 0.12 % CHX displayed lower intensity of green fluorescence and higher intensity of red fluorescence when compared to the treatment groups (Fig. 2B). The results suggest that both ELJC and LJC extracts possess bactericidal properties, as they led to bacterial cell death with prolonged incubation time.

3.5. Morphological changes of bacterial cells treated with ELJC and LJC extracts

FE-SEM analysis was employed to examine the morphology of both untreated and treated bacterial cells (*S. sanguinis* and *S. mutans*) with ELJC and LJC extracts, respectively. The untreated bacterial cells, *S. sanguinis* (Fig. 3A) and *S. mutans* (Fig. 3B) displayed intact morphology without any observable damage or disruption. However, the cells of *S. sanguinis* treated with 25 mg/ml ELJC exhibited significant morphological changes, characterized by cell blebbing and cell rupture (Fig. 3A). In contrast, the bacterial cells of *S. mutans* treated with 25 mg/ml LJC showed a significant shrinkage (Fig. 3B) compared to the untreated cells. Importantly, no cell membrane leakage or rupture was observed in the cells treated with LJC extracts. These findings indicate that both ELJC and LJC extracts were able to disrupt the bacterial cells, leading to structural damage and cell membrane disruption. Conclusively, the ELJC extracts caused cell blebbing and rupture in *S. sanguinis*, while LJC extracts induced significant cell shrinkage in *S. mutans* without causing membrane leakage.

Table 2
Minimum inhibition concentrations (MIC) of ELJC and LJC extracts against selected oral microorganisms.

Sample	MIC (mg/ml)	
	Bacterial strains	
	S. sanguinis	*S. mutans*
ELJC	25	NA
LJC	NA	6.25
CHX[a]	0.78	0.78

[a] 0.12 % CHX: positive control; NA: not available.

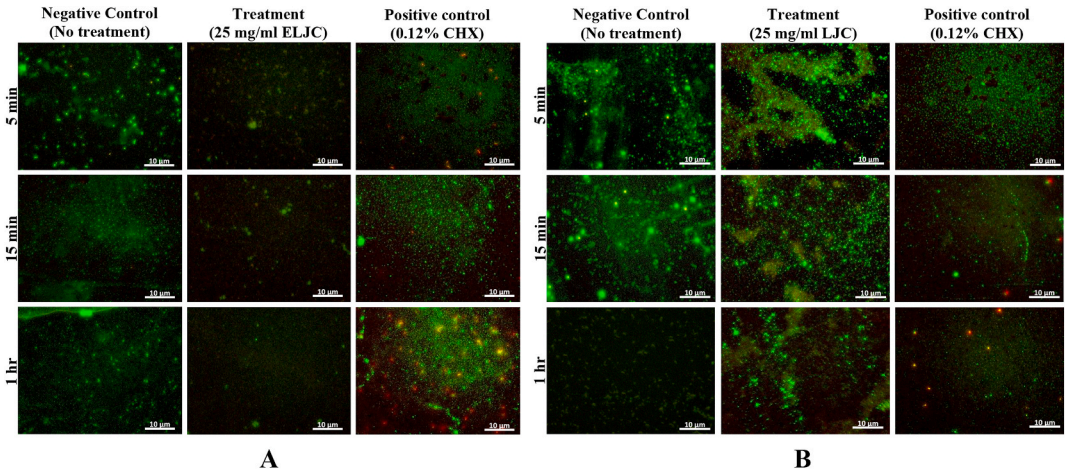

Fig. 2. Live/dead assay. Representative images of untreated and treated bacterial cells with (A) ELJC against *S. sanguinis*, and (B) LJC against *S. mutans*. In descending order, the treatment groups observed significantly more red fluorescence and significantly less green compared to the negative control groups at 5 min, 15 min, and 1 h. Positive control groups showed significant red fluorescence at 1 h (10× magnification, scale bar: 10 μm). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

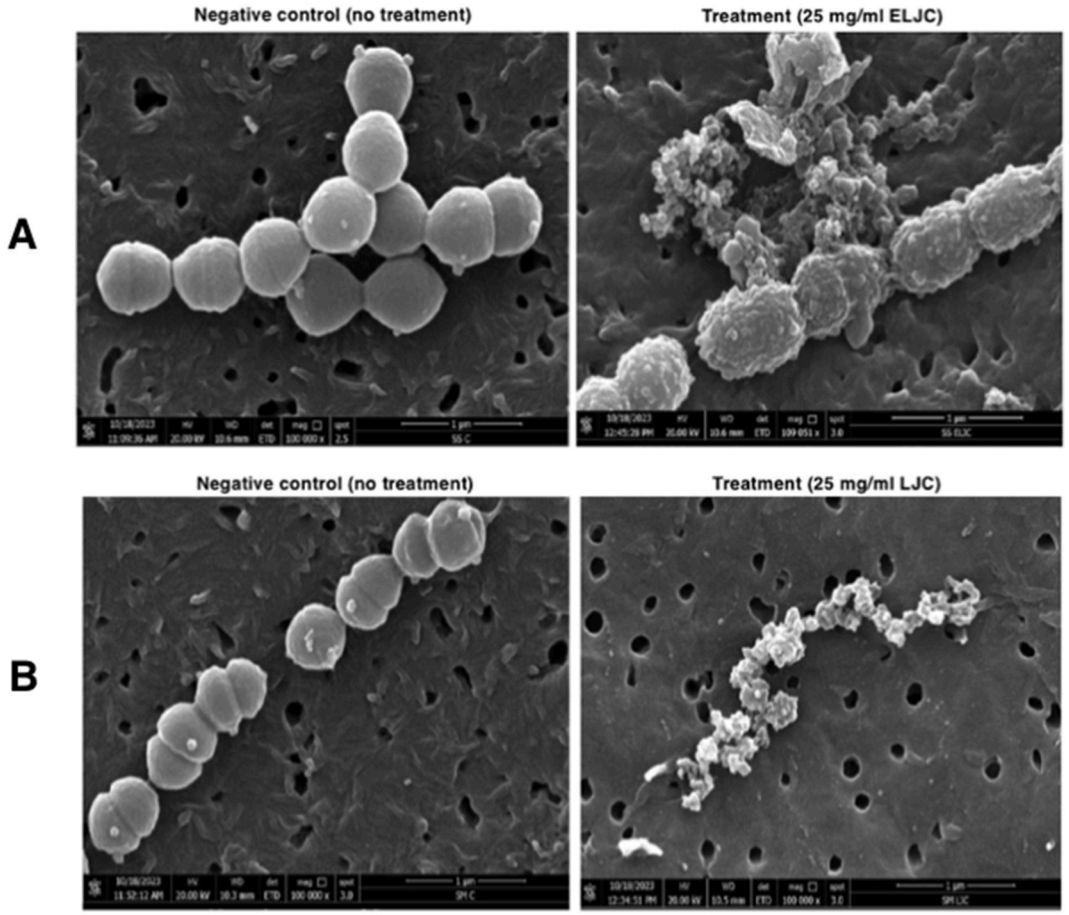

Fig. 3. FE-SEM scanning electron micrograph of untreated and treated oral bacterial cells with (A) 25 mg/ml of ELJC against *S. sanguinis;* and (B) 25 mg/ml of LJC against *S. mutans* (10,000× magnifications, scale bar: 1 μm).

3.6. HPLC-ESI-MS/MS analysis

The chromatographic profiles of the *Jatropha curcas* extracts of ELJC and LJC were obtained, respectively. The identified compounds in both extracts are outlined in Tables 3–7.

3.6.1. Phytochemical analysis of ELJC

As shown in Table 3, the LC-MS/MS analysis of the ELJC extract revealed the presence of 60 bioactive compounds with positive polarity compounds. The relative abundance of these compounds ranged from 0.14 % to 8.31 % of the total volume. Additionally, the ELJC extract contained 25 unknown positive polarity bioactive compounds, with volume percentage between 10.97 % and 0.20 %. Furthermore, the ELJC extract was found to have 280 known negative polarity bioactive compounds, with volume percentages ranging

Table 3
Bioactive compounds identified by LC-MS/MS analysis.

J. curcas extract	Identified bioactive compounds							
	Positive polarity compounds				Negative polarity compounds			
	Known	Vol %	Unknown	Vol %	Known	Vol %	Unknown	Vol %
ELJC	60	8.31–0.14	25	10.97–0.20	280	16.40–0.22	22	3.61–0.22
LJC	>500	2.04–0.01	>1000	3.18–0.01	143	20.77–0.01	145	2.71–0.01

Table 4
Bioactive compounds of the ethanolic leaves extract of *J. curcas* (ELJC) with their known antimicrobial properties.

J. curcas extract	Polarity	Identified compound	Formula	Classification	Vol (%)	Ref.
ELJC	Positive	Isovitexin	$C_{21}H_{20}O_{10}$	Flavonoids	8.31	[43]
		Coumermic Acid	$C_{27}H_{21}N_3O_{10}$	Organic Acids	5.34	[44]
		Isovitexin 7-O-rhamnoside	$C_{27}H_{30}O_{14}$	Flavonoids	3.06	[43]
		Hexadecyl Acetyl Glycerol	$C_{21}H_{42}O_4$	Lipids	2.48	[45]
		Nigerose (Sakebiose)	$C_{12}H_{22}O_{11}$	Carbohydrates	1.66	[46]
		6-Hydroxyluteolin 5-rhamnoside	$C_{21}H_{20}O_{11}$	Flavonoids	1.51	[43]
		Phaeophorbide b	$C_{35}H_{34}N_4O_6$	Chlorophyll Derivatives	1.37	[47]
		Pheophorbide a	$C_{35}H_{36}N_4O_5$	Chlorophyll Derivatives	1.29	[47]
		Monomethyl Glutaric Acid	$C_6H_{10}O_4$	Organic Acids	1.21	[48]
		L-Glutamate	$C_5H_9NO_4$	Organic Acids	0.16	[48]
	Negative	Isovitexin	$C_{21}H_{20}O_{10}$	Flavonoids	16.40	[43]
		Isovitexin 7-O-rhamnoside	$C_{27}H_{30}O_{14}$	Flavonoids	5.75	[43]
		Isovitexin 2″-O-xyloside	$C_{26}H_{28}O_{14}$	Flavonoids	12.14	[43]
		Theobromine	$C_7H_8N_4O_2$	Alkaloids	3.54	[49]
		Nigerose (Sakebiose)	$C_{12}H_{22}O_{11}$	Carbohydrates	2.19	[46]
		Luteolin 7-rhamnosyl(1->6)galactoside	$C_{27}H_{30}O_{15}$	Flavonoids	1.60	[43]
		Pheophorbide a	$C_{35}H_{36}N_4O_5$	Chlorophyll Derivatives	0.97	[47]
		Citric Acid	$C_6H_8O_7$	Organic Acids	0.65	[48]
		alpha-D-Mannoheptulopyranose	$C_7H_{14}O_7$	Carbohydrates	0.38	[50]

Table 5
Bioactive compounds of the stem bark latex of *J. curcas* (LJC) with their known antimicrobial properties.

Plant Sample	Polarity	Identified compound	Formula	Classification	Vol (%)	Ref.
LJC	Positive	Docosanedioic acid	$C_{22}H_{42}O_4$	Fatty Acids	2.04	[51]
		3α,12α-Dihydroxy-5β-chol-8(14)-en-24-oic Acid	$C_{24}H_{38}O_4$	Terpenoid	0.97	[52]
		Terbutaline	$C_{12}H_{19}N\ O_3$	Alkaloids	0.85	[53]
		Muconic dialdehyde	$C_6H_6O_2$	Aldehydes	0.58	[54]
		2,3-Butanediol glucoside	$C_{10}H_{20}O_7$	Nil	0.40	[55]
	Negative	2-hexyl-decanoic acid	$C_{16}H_{32}O_2$	Fatty acid	20.77	[34]
		2,4,6-Trihydroxybenzoic acid	$C_7H_6O_5$	Phenolic	14.43	[56]
		Ent-afzelechin-7-O-beta-D-glucopyranoside	$C_{21}H_{24}O_{10}$	Flavonoids	3.56	[43]
		Squamosinin A	$C_{36}H_{62}O_8$	Flavonoids	2.28	[43]
		Gallic acid	$C_7H_6O_5$	Phenolic	0.75	[57]
		beta-Sitosterol 3-O-beta-D-galactopyranoside	$C_{35}H_{60}O_6$	Steroids	0.51	[58]
		Rhamnocitrin 3-(5‴-*p*-coumarylapiosyl)-(1->2)-glucoside	$C_{36}H_{36}O_{17}$	Flavonoids	0.31	[43]
		Ambroxol	$C_{13}H_{18}Br_2\ N_2O$	Alkaloids	0.21	[59]
		4-Methyl-3-oxoadipate	$C_7H_{10}O_5$	Aldehydes	0.20	[60]
		Gingerol	$C_{17}H_{26}O_4$	Alkaloids	0.17	[61]
		Epifisetinidol-4alpha-ol	$C_{15}H_{14}O_6$	Flavanoids	0.16	[43]
		Isorhamnetin 3-O-beta-D-2″,3″,4″-triacetylglucopyranoside	$C_{28}H_{28}O_{15}$	Flavanoids	0.14	[43]
		Platycarpanetin 7-O-glucoside	$C_{24}H_{24}O_{12}$	Flavanoids	0.12	[43]

Table 6
Bioactive compounds of ELJC and LJC extracts with their unknown antimicrobial properties.

J. curcas extract	Polarity	Name	Formula	Classification	Vol (%)
ELJC	Negative	1,9-Dimethyluric acid	$C_7 H_8 N_4 O_3$	Xanthine Derivatives	11.11
		6-Hydroxyluteolin 5-rhamnoside	$C_{21} H_{20} O_{11}$	Flavonoid Glycoside	2.98
		1-Methylxanthine	$C_6 H_6 N_4 O_2$	Xanthine Derivatives	2.30
	Positive	N-Acryloylglycine	$C_5 H_7 N O_3$	Glycine Derivative	2.56
		Emmotin A	$C_{16} H_{22} O_4$	Nil	2.42
LJC	Negative	ent-Catechin 7-O-beta-D-glucopyranoside	$C_{21} H_{24} O_{11}$	Flavonoid Glycoside	8.40

*The unknown compounds are present abundantly in the samples of ELJC and LJC extracts of *J. curcas*.

Table 7
The unknown bioactive compounds present in the ELJC and LJC extracts of *J. curcas*.

Plant Sample	Polarity	Label	Formula	*m/z*	Mass	Vol (%)
ELJC	Positive	Cpd 75: $C_{36} H_{32} N_8 O$	$C_{36} H_{32} N_8 O$	593.2793	592.2711	10.97
		Cpd 2: $C_5 H_{13} N O$	$C_5 H_{13} N O$	104.1063	103.0990	5.59
		Cpd 81: $C_{42} H_{40} N_2 O_3$	$C_{42} H_{40} N_2 O_3$	621.3093	620.3027	2.64
	Negative	Cpd 14: $C_7 H_{12} O_6$	$C_7 H_{12} O_6$	191.0574	192.0646	3.61
LJC	Positive	Cpd 2094: 14.801	N/A	767.5021	766.4948	3.18
		Cpd 1223: 8.286	N/A	679.5116	678.5043	3.16
		Cpd 403: 2.114	N/A	239.1481	238.1408	2.12
	Negative	Cpd 270: 25.794	N/A	1033.9881	1034.9954	2.71

*The unknown compounds are present abundantly in the samples of ELJC and LJC extracts of *J. curcas*.

from 16.40 % to 0.22 %. Alongside the known negative polarity compounds, the extract also contained 22 unknown negative polarity bioactive compounds, with volume percentages of 3.61 % to 0.22 %.

The specific bioactive compounds identified in the ELJC are outlined in Table 4. This analysis revealed the presence of several compounds known to possess antimicrobial properties, namely isovitexin, coumermic acid, isovitexin 7-O-rhamnoside, hexadecyl acetyl glycerol, nigerose (Sakebiose), 6-hydroxyluteolin 5-rhamnoside, phaeophorbide b, pheophorbide a, monomethyl glutaric acid, L-glutamate, isovitexin, isovitexin 7-O-rhamnoside, isovitexin 2″-O-xyloside, theobromine, nigerose (Sakebiose), luteolin 7-rhamnosyl (1->6)galactoside, pheophorbide a, citric acid, and alpha-D-mannoheptulopyranose. The volume percentage of these bioactive compound in the ELJC extract ranged from 16.40 % to 0.16 %.

3.6.2. Phytochemical analysis of LJC

The LC-MS/MS analysis of the LJC extract revealed the presence of more than 500 known positive polarity bioactive compounds, with volume percentages ranging between 0.01 % and 2.04 % (Table 4). Additionally, the LJC extract contained more than 1000 unknown positive polarity bioactive compounds, with volume percentages between 3.18 % and 0.01 %. Moreover, the LJC extract was found to have 143 known negative polarity bioactive compounds, with volume percentage between 20.77 % and 0.01 % and 145 unknown types with a volume of 2.71 % to 0.01 % (Table 3).

Table 5 provides an overview of the identified compounds found in LJC. This analysis revealed the presence of several bioactive compounds that were known to possess antimicrobial properties, namely docosanedioic acid, 3α,12α-dihydroxy-5β-chol-8(14)-en-24-oic acid, terbutaline, muconic dialdehyde, 2,3-butanediol glucoside, 2-hexyl-decanoic acid, 2,4,6-trihydroxybenzoic acid, ent-afzelechin-7-O-beta-D-glucopyranoside, squamosin a, gallic acid, beta-sitosterol 3-O-beta-D-galactopyranoside, rhamnocitrin 3-(5‴-*p*-coumarylapiosyl)-(1->2)-glucoside, ambroxol, 4-methyl-3-oxoadipate, gingerol, epifisetinidol-4alpha-ol, isorhamnetin 3-O-beta-D-2″,3″,4″-triacetylglucopyranoside, and platycarpanetin 7-O-glucoside. The volume percentages of these bioactive compound in the LJC extract ranged between 20.77 % and 0.11 %. Furthermore, both the ELJC and LJC extracts were found to contain several abundant but uncharacterized bioactive compounds (specifically for antimicrobial properties), as outlined in Tables 6 and 7. These compounds, including ent-catechin 7-O-beta-D-glucopyranoside, 1,9-dimethyluric acid, 6-hydroxyluteolin 5-rhamnoside, 1-methylxanthine, N-acryloylglycine and emmotin A (Table 6). Furthermore, both ELJC and LJC exhibited the presence of unidentified bioactive compounds (Table 7), merit further investigation to determine their specific identities and potential biological activities.

4. Discussion

Plants are renowned for producing secondary metabolites that exhibit antimicrobial effects while displaying fewer adverse effects compared to conventional antimicrobial agents [40]. Though the *Jatropha curcas* plant has been traditionally used as an antibacterial agent [29,41,42], scientific validation of its efficacy against oral microbiome remains limited. Therefore, the findings of this study demonstrate the *in vitro* antimicrobial effects of ethanolic extract leaves (ELJC) and stem bark latex of *Jatropha curcas* (LJC) against selected oral microbiomes, namely *S. mitis*, *S. mutans*, *S. sanguinis*, *L. helveticus*, *C. tropicalis*, *C. albicans* and a mixture of these microorganisms. The agar well diffusion assay revealed that ELJC possessed notable antimicrobial effects against *S. mitis*, *S. sanguinis*, *C. tropicalis*, and *L. helveticus,* while, LJC showed antimicrobial effects solely against *S. mutans*. Interestingly, the ELJC extract

demonstrated significant antifungal activity against *C. tropicalis*, even outperforming the standard antifungal drug amphotericin B, is contrary to previous research on the resistance profile of this fungal species [43,44] suggesting the potential of ELJC as an alternative antifungal agent. Previous studies have reported that resistance to amphotericin B in *C. tropicalis* is generally infrequent, and only a few strains consistently exhibit a significant level of resistance to this antifungal agent [45]. Hence, the observation of the *C. tropicalis* strain in the current study exhibiting resistance to amphotericin B warrants further investigation to comprehend the reasons behind this unusual resistance pattern.

Notably, the LJC extract at a concentration of 100 mg/ml showed potent inhibitory effects specifically against *S. mutans,* a primary causative agent of dental caries. Importantly, the inhibition zone diameter of LJC against *S. mutans* was comparable to that of 0.12 % CHX, a widely used antimicrobial ingredient in commercial mouthwashes. While CHX is effective against oral pathogens, long-term use can lead to undesirable side effects like tooth staining and altered taste perception. Therefore, the finding that the LJC extract exhibited similar inhibitory effects on *S. mutans* as CHX indicates that it could serve as a viable alternative or complementary ingredient in oral care products.

However, when the antimicrobial efficacy of ELJC and LJC extracts was tested against a mixed culture of the oral microorganisms, the activity was reduced and not as potent against the individual species. This observation may be attributed to the ability of *S. sanguinis* to produce hydrogen peroxide, which can exhibit antimicrobial properties against other oral microorganisms, thereby influencing the overall microbial dynamics [4].

The study reported a significant difference in the inhibition zone diameter observed for the ELJC (100 mg/ml) against the *L. helveticus*, compared to the negative control. Lactobacillus species are known to be an important members of the oral microbiome, contributing to the maintenance of a balanced of microbial ecosystem. Therefore, the finding that the ELJC extract exhibited antimicrobial activity against *L. helveticus* is noteworthy, as it suggests the extract may have broad-spectrum effects, potentially impacting both beneficial and pathogenic oral microbial species. This observation raises concerns about the potential disruption of the delicate balance within the oral microbiome, which is crucial for maintaining overall oral health.

Further investigation into the selective antimicrobial properties of the ELJC and LJC extracts is warranted to elucidate their impact on the complex microbial community within the oral cavity. The ideal goal for any potential oral care application of these natural extracts would be to selectively target pathogenic species while preserving the beneficial commensal bacteria. Additional studies are needed to elucidate the specific mechanisms by which the ELJC extract inhibits *L. helveticus* and other oral microorganisms, as well as to optimize the extract formulation and dosage to achieve a more targeted and selective antimicrobial effect. This would help maximize the potential of the ELJC and LJC extracts as a natural alternative for maintaining oral health without disrupting the overall balance of the oral microbiome.

The MIC and MBC analyses revealed the strong antimicrobial effect of these *J. curcas* extracts. In particular, the LJC exhibited a low MIC against *S. mutans* compared to the MIC of ELJC against *S. sanguinis*, indicating the extract's ability to effectively inhibit the growth of this cariogenic bacterium, which is a major contributor to dental caries. However, both ELJC and LJC extracts demonstrated the same MBC value against *S. mutans* and *S. sanguinis*, respectively. Microorganism growth is linked to cells' ability to proliferate, governed by complex regulatory networks [46]. At this stage, it may be premature to fully explore the clinical implications of the determined concentrations regarding potential therapeutic applications. However, these results will be valuable when conducting subsequent pre-clinical or *in vivo* studies, which are critical precursors to clinical research.

The live-dead assay using double staining (SYTO9 and propidium iodide (PI)), provided tangible evidence of the antimicrobial efficacy of the plant extracts of *J. curcas*. This assay revealed the ability of the extracts to inhibit the growth of the bacterial cultures in a time-dependent manner. SYTO9 stains bacteria with intact cell membranes in green fluorescence. Whereas PI stains bacteria with damaged cell membranes in red fluorescence. The employment of these different colours helps to distinguish between live and dead bacterial cells after exposure to *J. curcas* extracts. The treated bacterial cells of *S. sanguinis* and *S. mutans* with 25 mg/ml of ELJC and LJC respectively, demonstrated reduced green fluorescence (live cells) and increased red fluorescence (dead cells) compared to the negative control. Additionally, the antimicrobial extracts was shown to impede the growth of the bacterial cultures in a time-dependent manner, as observed in the live-dead assay. The strategic or selection of specific time points (5 min, 15 min and 1 h) in this assay mimicked the optimal treatment durations and application modalities for effective oral health management which allowed a more comprehensive understanding of the antimicrobial efficacy of ELJC and LJC extracts; providing insights into the kinetics and duration of antimicrobial activity. Notably, the positive control (0.12 % CHX) exhibited a significantly higher intensity of red fluorescence, especially in *S. sanguinis*, indicating more substantial membrane damage experienced by the cells. In this study, chlorhexidine (CHX) was used as the positive control to facilitate a comparative examination of the antimicrobial effects between the *Jatropha curcas* extracts and a widely recognised oral antiseptic. CHX is commonly employed in commercial mouthwashes and is renowned for its effectiveness in preventing and combating oral infections. Whilst, in fungal groups, the antifungal amphotericin B were used. Both therapeutic approaches were meticulously incorporated. In contrast, antibiotics are formulated to selectively target particular bacterial or fungal classes. By juxtaposing the antimicrobial activity of *Jatropha curcas* with that of CHX, facilitated a comprehensive evaluation of *Jatropha curcas*'s potential as an oral antimicrobial agent in relation to a commonly used antiseptic in commercial oral care products. This comparison allowed for a more holistic assessment of the antimicrobial efficacy of ELJC and LJC extracts and their potential for incorporation into oral health management strategies alongside or as alternative to commonly used antiseptic like CHX.

The FE-SEM analysis revealed distinct effects of ELJC and LJC extracts on the cell membranes of the oral bacteria. Specifically, the ELJC extracts exhibited more pronounced damage to the cell membranes of *S. sanguinis*, while the LJC extract had a greater impact on the cell membranes of *S. mutans.* This suggest that the different extracts of *J. curcas* may harbour distinct antimicrobial compounds with selective activity against specific oral microbiome members. However, the extent of cell membrane damage observed in the FE-SEM analysis was not as prominent in the live/dead assay, which assessed the antimicrobial activity at shorter time points of 5 min, 15

min and 1 h. This discrepancy may be attributed to the disparate treatment durations, with the FE-SEM analysis conducted at 24 h of exposure, compared to the shorter time in the live/dead assay. Furthermore, the study also revealed that 0.12 % CHX exhibited a stronger antimicrobial effect than 25 mg/ml LJC but was slightly weaker than 25 mg/ml ELJC, as indicated by the fluorescence intensity comparison. This suggests that the ELJC extract may possess antimicrobial potency comparable to or even greater than the commonly used CHX. Additionally, the extent of cell membrane damage caused by the *J. curcas* extracts was directly proportional to the duration of exposure. This could be due to several factors, including the accumulation of treatment effects over time leading to increased damage; accumulated stress on membrane integrity with continued exposure; increased interaction time between the extracts and cell membrane components; accumulation of treatment by-products or metabolites in the cellular environment, affecting membrane integrity and potential threshold effects, where prolonged exposure triggers accelerated membrane damage. Further investigation is needed to determine the exact reasons for this relationship between exposure duration and cell membrane damage. This study also highlighted the value of FE-SEM analysis in visualising the morphological and structural alterations induced by plant extracts on bacterial cells. Understanding these structural modifications could aid in the development of potent antimicrobial agents for improving oral health. The observed structural changes, such as cell shrinkage in *S. mutans* observed in the FE-SEM could be due to starvation and water loss within the cells and this has also been reported previously [47]. On the other hand, *S. sanguinis* exhibited blebbing, which involves protrusions in the cell membrane and is a common cellular response to various stimuli that protects against cell breakdown [48]. It was proposed that blebbing occurs because of the turnover of peptidoglycan in the cell wall causing the outer membrane to bulge and eventually forming blebs [49]. Moreover, blebbing is strongly associated with the typical apoptotic morphological changes. Cells undergoing apoptosis do not suffer cell membrane leakage. Instead, they exhibit blebbing, forming vesicles that eventually break down the cell [50]. Other changes linked to apoptosis include cell shrinkage, condensed chromatin, fragmented DNA, and generation of vesicles [51]. Overall, these findings suggest that the *J. curcas* plant may harbour antimicrobial compounds with selective activity against certain oral microbiome members. The differential effects observed between the leaf and stem bark extracts could indicate the presence of distinct bioactive compounds or varying concentrations of antimicrobial constituents in the different parts of the plant. Further investigation into the specific antimicrobial mechanisms and the identification of the active compounds in these *Jatropha curcas* extracts could provide valuable insights. Additionally, evaluating their efficacy in more complex *in vitro* or *in vivo* models, as well as assessing their safety and tolerability, would be crucial in exploring the potential of these natural antimicrobials in oral health management.

Phytochemical analysis of the ELJC and LJC conducted using the HPLC-ESI-MS/MS revealed a rich composition of bioactive compounds in both extracts. ELJC contained over 300 bioactive compounds, while LJC exhibited an even more extensive array with over 1500 bioactive compounds, respectively. Given the abundant bioactive profiles observed in both ELJC and LJC, this study provided an overview of the HPLC-ESI-MS/MS results, with a particular emphasis on identifying compounds known for their antimicrobial properties.

Delving further into the matter, the HPLC-ESI-MS/MS analysis of ELJC extract elucidated various antimicrobial bioactive compounds comprising of flavonoids, organic acids, lipids, carbohydrates, chlorophyll derivatives, and alkaloids. Among these, isovitexin, a flavonoid, was identified as the most abundant compound, collectively accounting for 42.60 % of the ELJC extract. Flavonoids are known for their antimicrobial properties, primarily damaging the cell membrane, leading to cellular leakage and breakdown [59]. Additionally, isovitexin and other flavonoids may exhibit antibacterial mechanisms such by inhibiting alpha-glucosidase (an enzyme involved in carbohydrate breakdown) [59], which can disrupt the energy metabolism of bacterial cells and lead to reduced growth and proliferation of certain bacterial species. Moreover, isovitexin and other flavonoids such as vitexin possesses antioxidant properties [60], which can interfere with the normal redox balance in bacterial cells and subsequently impair their ability to function properly, leading to cellular damage. FE-SEM images of *S. sanguinis* treated with ELJC demonstrated formation of cell blebbing and vesicles, ultimately resulting in cell membrane rupture and cell death. The FE-SEM images show the typical apoptotic morphology of bacterial cells, such as blebbing and vesicle formation, a process preceding cellular decomposition and demise. Therefore, it can be inferred that the compound isovitexin, found abundantly in ELJC extract, may potentially contribute to the observed antibacterial effect against *S. sanguinis*.

The HPLC-ESI-MS/MS analysis of LJC revealed a different profile of antimicrobial bioactive compounds, encompassing fatty acids, terpenoids, alkaloids, aldehydes, phenolic compounds, flavonoids, and steroids. Notably, 2-hexyl-decanoic acid (20.77 %), a fatty acid, and 2,4,6-trihydroxybenzoic acid (14.43 %), a flavonoid within the phenolic compound, were found in substantial quantities in LJC. Hence, these bioactive compounds could possibly be attributed to the observed antimicrobial effects of LJC against *S. mutans*. Fatty acids have been demonstrated to have the following features; a significant antibacterial effect against Gram-positive bacteria [52], antimicrobial effects against *S. mutans* [53], the ability to disrupt the stability of bacterial cell membranes, and cause growth inhibition [54]. Fatty acids were found to quickly induce membrane depolarization and disturb macromolecular synthesis [55]. Similarly, flavonoids and phenolic compounds are known to have antimicrobial effects against multiple species of microorganisms [56], where they cause cell membrane damage [57]. In addition, 2,4,6-trihydroxybenzoic acid has been identified as an inhibitor of cyclin-dependent kinase and cell proliferation [58]. This evidence substantiates the observed morphological alteration of *S. mutans* when treated with LJC, demonstrating a significant reduction in size as indicated by the FE-SEM.

Previous study using *in situ* Raman analyses [61] has revealed the antagonistic dynamics between the oral bacterial *S. sanguinis* and *S. mutans* within biofilms. Through the Raman quantification of glucan metabolites within live biofilms, it was observed that *S. sanguinis* adopts a passive strategy to assert its competitive edge by releasing oxygen radicals into the biofilm environment. These radicals disrupt the network of water-insoluble α−1,3−glucans through glycosidic bond cleavage and subsequent oxidation of resulting oligomers, thereby inducing leakage within the biofilm matrix and facilitating external ingress. Conversely, *S. mutans* employ an active competitive approach by secreting mutacins, which directly impedes the growth of *S. sanguinis* [61]. Hence, this

demonstrates how the oral bacterial species may utilize different mechanisms of oxidative stress or by secretion of certain antimicrobial peptides that may affect the composition of the polymicrobial oral biofilm and its properties.

The phytochemical analysis has identified the most abundant bioactive compounds present in ELJC and LJC extracts. These compounds may contribute to the observed antimicrobial effects against the oral bacteria *S. sanguinis* and *S. mutans*. To further understand the underlying antimicrobial effects, it is crucial to study the isolated bioactive compounds, such as the major components found in ELJC and LJC extracts: isovitexin against *S. mutans*, and 2-hexyl-decanoic acid and 2,4,6-trihydroxybenzoic acid against *S. sanguinis*. Additionally, it is important to consider the microanatomical structure and physiological characteristics of the target microorganisms, but also the specific actions of the identified bioactive compounds. Given the inherent variability in microbial traits, it is crucial to acknowledge that the efficacy of bioactive compounds may vary accordingly.

The major bioactive compounds found in the ELJC and LJC extracts include the flavonoid, isovitexin, the phenolic compound, 2,4,6-trihydroxybenzoic acid, and the fatty acid 2-hexyl-decanoic acid. These compounds are known to exhibit distinct yet potentially overlapping mechanisms of antimicrobial action. It has been suggested that these compounds share a hydrophilic structure capable of interacting with the phospholipid bilayer of microbial cells, thereby inducing destabilization, disruption, cellular leakage, and eventual microbial demise [62,63].

Isovitexin has been reported to induce membrane disruption, impede nucleic acid synthesis, counteract bacterial virulence factors, and suppress quorum sensing to disrupt biofilm formation [64]. Flavonoids like isovitexin can also inhibit cell envelope synthesis, by inhibiting fatty acid synthase and hindering peptidoglycan cross-linking, and suppress efflux pumps to reverse antimicrobial resistance. Furthermore, they can inhibit essential cellular process such as the respiratory chain and ATP synthesis [64–66]. The phenolic compound 2-hexyl-decanoic acid can act as antioxidant, generating reactive oxygen species that damage crucial cellular constituents. It may also chelate essential metal ions, depriving microbial cells of nutrients required for growth and virulence [52]. Additionally, trihydroxybenzoic acid has been shown to disrupt quorum sensing, a critical mechanism for coordinating microbial behaviour and gene expression [67] and consequently inhibiting the expression of virulence factors and the formation of biofilms [68]. Taken together, the antimicrobial efficacy of *Jatropha curcas* extracts may be attributed to the synergistic actions of these bioactive compounds. Based on previous studies, they appear to target multiple aspects of microbial aspects of microbial physiology, including cell membrane integrity, enzymatic activities, oxidative stress, nutrient availability, and intercellular communication.

In the broader context of oral health, the findings of this current study provide compelling support for the traditional use of *Jatropha curcas* as an antimicrobial agent in the treatment and prevention of oral diseases, thereby validating its historical medicinal efficacy. Conclusively, the findings demonstrated that *Jatropha curcas* extracts have significant antimicrobial effects against oral microorganisms, particularly the latex and leaves ethanolic extract, in eradicating oral microorganisms such as *S. mutans*, *S. sanguinis*, *L. helveticus,* and *C. tropicalis* with a potential to foster a balanced oral microbiota, thereby offering a preventive strategy against oral inflammation, periodontal diseases and dental caries. This is further supported by the identification of key bioactive compounds in ELJC and LJC that have shown broad antimicrobial activity in previous studies. These results provide a strong basis for further exploration of *Jatropha curcas* in oral health care. The study highlights the potential of *Jatropha curcas* as a valuable source of alternative antimicrobial agents with fewer side effects, particularly in addressing the global challenge of antimicrobial resistance. Thus, *Jatropha curcas* has the potential to be developed as an oral medication or oral health product such as mouthwashes, or disinfectants in oral prophylaxis procedures.

While these findings provide compelling evidence for the antimicrobial potential of *Jatropha curcas*, further investigation, is needed to fully elucidate the precise mechanisms of action against specific oral pathogens. In addition, conducting *in vivo* studies is crucial to validate their efficacy and safety profiles. Ultimately, the translation of these research findings into clinical applications holds promise for effectively managing oral infections in a clinical setting.

5. Conclusion

The leaves and stem bark latex of the *J. curcas* plant demonstrate promising potential as natural oral antimicrobial agents. The leaf extract exhibited specific efficacy against the bacteria *S. sanguinis*, while the stem bark latex was effective against *S. mutans*. The antimicrobial effects are likely attributed to the phytochemical compounds isovitexin in the leaves and 2-hexyl-decanoic acid and 2,4,6-trihydroxybenzoic acid in the stem bark latex. These findings suggest that *J. curcas* may be a valuable natural source for the development of oral care products targeting the bacteria associated with dental plaque and tooth decay.

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Data availability

The data supporting the findings of this study maybe provided by writing to the corresponding author stating the relevant justification.

CRediT authorship contribution statement

Anita Kamaruddin: Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Wan Himratul**

Aznita Wan Harun: Supervision, Resources, Project administration. **Marina Mohd Bakri:** Validation, Supervision, Project administration, Formal analysis. **Syafiq Asnawi Zainal Abidin:** Visualization, Resources, Project administration, Formal analysis. **Nelli Giribabu:** Writing – review & editing, Supervision, Formal analysis. **Syarifah Nur Syed Abdul Rahman:** Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

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