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Phytochemical screening, antibacterial activity against periodontopathic bacteria – *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* and HPLC of *Opuntia ficus*-indica (prickly pear) fruit extracts

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ABSTRACT: The present study aimed to screen the phytochemicals, evaluate antibacterial efficacy of *Opuntia ficus* against periodontopathic bacteria, *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* using in-vitro assays, and conduct high-performance liquid chromatographic (HPLC) analysis. Using different solvents, like ethanol, ethyl acetate, n-hexane, and chloroform, extractions of the ficus powders were carried out using standard Soxhlet extraction methods. Phytochemical analysis, total phenolic contents, antibacterial activity, and high-performance liquid chromatographic (HPLC) analysis of *Opuntia ficus* extracts were investigated. Among the solvents, the most predominant phytoconstituents (flavonoids and phenols) were found in the ethanolic extract of *Opuntia ficus.* The total phenolic contents (TPC) in *Opuntia ficus* extracts varied, with the ethanol extract exhibiting the highest TPC at 63.3 mg GAE/g. Compared to standard antibiotics such as chloramphenicol (C), tetracycline (TE), and vancomycin (VA), the solvent extracts (n-hexane, ethyl acetate, and chloroform) from *Opuntia ficus* showed smaller inhibitory zone sizes against all tested bacteria. However, ethanol extract showed a significantly similar inhibitory zone when compared to the antibiotic ciprofloxacin ($p<0.05$). The zone size for ethanol was found to be significant in terms of sensitivity patterns, as per CLSI guidelines. High-performance liquid chromatographic profiles showed significant phenolic compounds, such as catechin, chlorogenic acid, gallic acid, hydroquinone, kaempferol, quercetin, resorcinol and syringic acid in the ethanol extracts.

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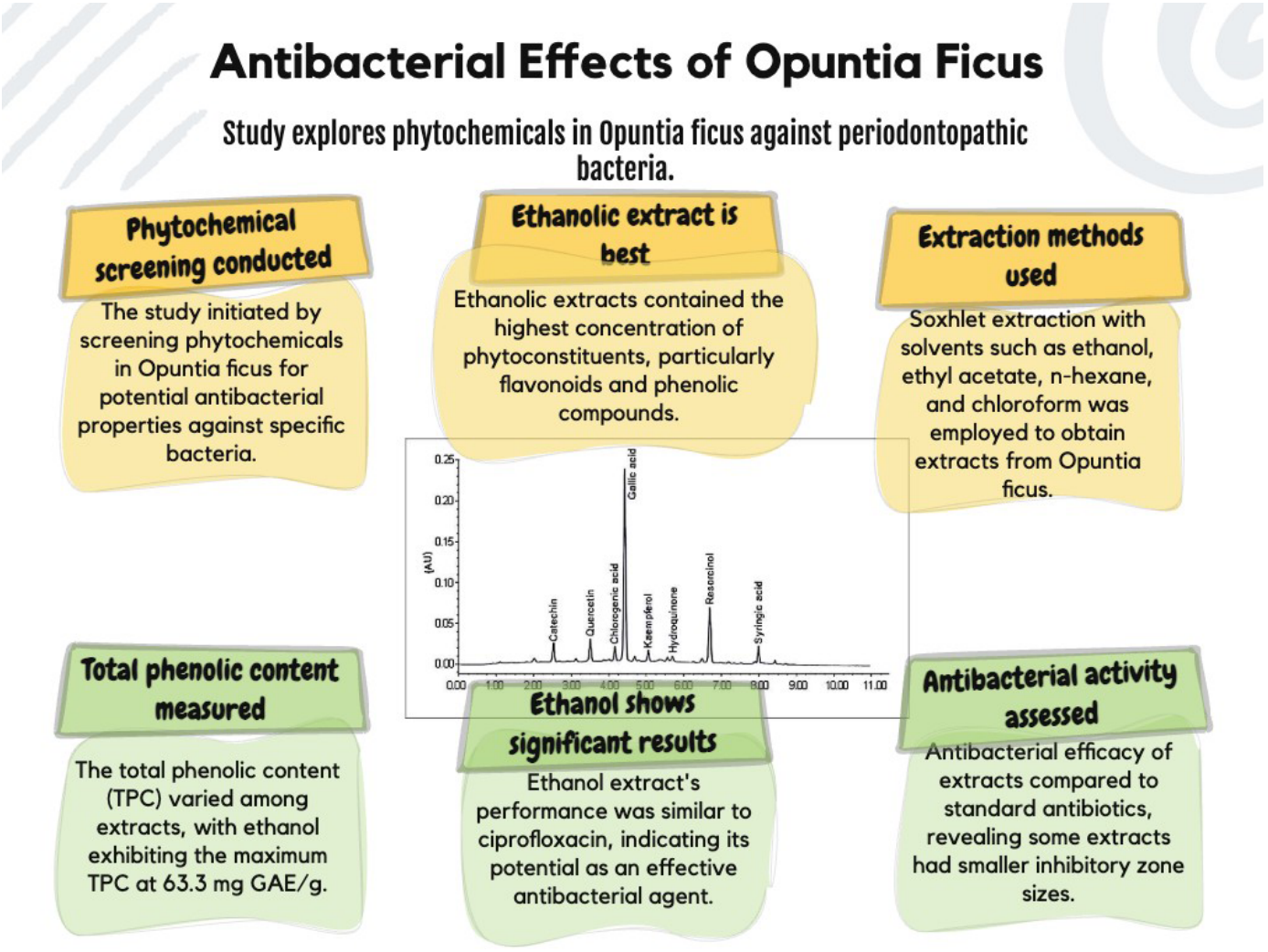
GRAPHICAL ABSTRACT



1. INTRODUCTION

Dental caries, gingivitis, periodontal disease and oral halitosis have their origins in dental biofilm bacteria. Periodontal disease begins with plaque-induced gingival inflammation (gingivitis), a reversible condition that, if unresolved, may progress to periodontitis with loss of connective tissue attachment and alveolar bone. This progression is sustained by dysbiotic microbial communities and dysregulated host responses that sustain inflammation and cause tissue destruction (Sedghi et al., 2021). The disease is not caused by a single pathogen but by complex microbial communities. A comprehensive study of the oral microbiome in gingivitis and periodontitis confirms a multispecies aetiology, lacking a singular "primary pathogen". Instead, changes in microbial consortia interact with host factors to influence disease progression (Lee et al., 2023). Available evidence emphasises that early, plaque-driven gingival inflammation is a precursor state with potential reversibility if managed effectively by reducing the bacterial load in dental biofilm (Sutthiboonyapan et al., 2020).

Aggregatibacter actinomycetemcomitans (Aa) and *Porphyromonas gingivalis* (Pg) are recognised in the literature as major periodontopathogens. They are associated with various forms of periodontitis. This elucidates their significant role in the microbial aetiology and pathogenesis of periodontal disease (Hayakumo et al., 2014). The pathogens produce an array of virulence factors that enhance their pathogenic potential. For Aa, virulence factors such as leukotoxin, cytolethal distending toxin and the cell surface protein enhance

their pathogenicity by evading the immune response and promoting tissue damage (Fujita et al., 2021; Jacob et al., 2024). In the case of P. gingivalis, the presence of virulence factors, including capsules, fimbriae, protease-like gingipains, haemagglutinins, haemolysins, lipopolysaccharides, and outer membrane vesicles, allows for the degradation of host proteins and the impairment of immune functions, which further exacerbates periodontal tissue damage (Aleksijević et al., 2022). The intricate interplay between periodontopathic bacteria, including Aa and Pg along with other oral microbiota, highlights the multibacterial characteristics of periodontal disease (Chen et al., 2022).

Endophytes, fungi, lichens, plants and different aquatic sources, like seaweeds, corals and other microbes, are now the main focus of antimicrobial discovery because of their promising traits. These natural substances, particularly phytochemicals such as alkaloids, betalains, flavonoids, lignans, phenolic acids, tannins and triterpenoids demonstrate considerable antibacterial efficacy against diverse human pathogens (Rasoulpour et al., 2020), (Alves et al., 2022; Angelini, 2024; Elmowafy et al., 2022; Woo et al., 2023). Moreover, the advantages of using natural plant compounds and derivatives include accessibility, cost-efficiency, and negligible adverse effects. Further, the addition of these phytochemicals can augment the efficacy of conventional antibiotics and antimicrobials, presenting a viable approach to combat bacterial resistance. This strategy of twinning natural phytochemicals alongside conventional antibiotics can lower minimum inhibitory concentration (MIC) values and yield synergistic benefits (Alves et al., 2022; Angelini, 2024; Elmowafy et al., 2022; Woo et al., 2023).

A notable shift is occurring from the use of standard pharmaceutical products to the incorporation of plant-based and natural additives across the medical fields. This is also evident in the use of natural herbal additives in oral care products, especially for the prevention of dental caries (Tzimas et al., 2024). Phytochemicals derived from plants have anti-inflammatory properties that may serve as an alternate treatment for periodontal and peri-implant diseases. Recent research on many active plant phytochemical components shows substantial improvements in the periodontal clinical parameters (Abu Tamam et al., 2024; Ahmed et al., 2022). Moreover, various studies have shown *Aloe vera*, *C. officinalis*, curcumin, *Glycyrrhiza glabra*, green tea, guava leaf extract, *Momordica charantia, R. officinalis*, *Rosa damascena, Rosa rubiginosa, Sambucus williamsii* var. Coreana, Triphala, *Z. officinale* and many other plant extracts to have antimicrobial effects on periodontopathic bacteria (Abu Tamam et al., 2024; Ahmed et al., 2022; Peeran, Fageeh et al., 2024; Peeran, Murugan, Fageeh, Ibraheem, et al., 2024; Peeran, Murugan, Fageeh, Ibrahim, et al., 2024). Further research is required to propose them as alternatives to the non-herbal components.

Opuntia ficus-indica (OFI), a notable medicinal herb from the Cactaceae family, was chosen for this present study due to its diverse significant properties. OFI shows promise as a source of bioactive compounds with demonstrated antimicrobial and antibiofilm activities in various studies, including results from cladode-derived extracts (Blando et al., 2019; Dormousoglou et al., 2023). However, the scientific literature lacks any study about the effects of OFI extracts on key periodontal pathogens. However, an array of antimicrobial and antibiofilm studies on OFI extracts indicate that OFI could be effective in modulating periodontal biofilms and inflammatory responses (Conte et al., 2024; Dormousoglou et al., 2023; Matias et al., 2014).

Hence, this study aims to comprehensively evaluate the phytochemicals and antibacterial efficacy of OFI against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* using in-vitro assays. Phytochemical analysis, total phenolic contents, antibacterial activity and high-performance liquid chromatographic (HPLC) analysis of *OFI* fruit extracts were investigated.

2. MATERIALS AND METHODS

2.1. Collection and processing of *Opuntia ficus*

OFI fruits were collected from the local vegetable vendor, Medina Munawara, KSA. The OFI fruits (prickly pears) were washed, dried, and cut into small pieces. They were shade-dried for 5 to 10 days. The shade-dried ficus are presented in Figure 1. The dried ficus were milled into powder and sieved (Figure 2). Standard Soxhlet extraction methods(Soxhlet apparatus, ISKO Borosilicate, Gupta Scientific and Glass Works, India) were employed to extract fruit powders using several solvents, including ethanol, ethyl acetate, n-hexane, and chloroform. Aqueous extracts were obtained by applying the same extraction procedure. Following their individual transfer to petri dishes and oven drying at 50°C, the collected extracts were scraped and kept at room temperature in air-tight brown amber containers.

2.2. Procurement of test bacteria

Test organisms that had substantial etiological traits associated with periodontal disease were chosen for the investigation. *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* were the chosen bacteria. The testing facility for the present study, Gram Positive Lab in Coimbatore, India, provided the appropriate bacterial strains. The microbial strains were acquired from Himedia Labs in

Figure 1. Dried ficus or cladodes of *Opuntia ficus.*

Figure 2. Powdered *Opuntia ficus.*

Thane, India, which had already been in use at the testing facility.

2.3. Phytochemical analysis of *Opuntia ficus* extracts

The phytochemical analysis of ethanol, ethyl acetate, n-hexane, chloroform, and water extracts was carried out using standard tests, as described in the brief below.

1. *Alkaloids*
   Alkaloid was detected by combining 1 ml of extract with 10 ml of methanol. The suspension was filtered, and 2 ml of the resulting filtrate was mixed with 1% HCl. 1 ml of filtrate and 6 drops of Dragendorff reagent were added to this combination. A brownish-red precipitate demonstrated the existence of the alkaloids.
2. *Flavonoids*
   Flavonoid content was detected by mixing 1 ml of the extract with 10 ml of ethyl acetate and incubating in a water bath at 40°C for 5 minutes. The mixture was filtered, and 1 ml of ammonia was added to the filtrate. The mixture exhibited a deep, vivid brownish-yellow hue, signifying the presence of flavonoids.
3. *Tannin*
   Tannin detection was conducted using 1 ml of plant material in a solution of 10 ml of distilled water. The solution underwent filtration, and approximately 2 ml of the filtrate was combined with 2 ml of $FeCl_3$. The formation of a bluish-black precipitate signals the presence of tannins.
4. *Terpenoids*
   Terpenoids were identified by combining 2 ml of plant extract with 2 ml of acetic anhydride and a concentration of H_2SO_4. The emergence of a bluish-brown hue signifies the presence of terpenoids.
5. *Phenol*
   Approximately 1 ml of the plant extract was combined with 2 ml of a 2% $FeCl_3$ solution. The resultant mixture was agitated to achieve a stable and enduring froth. The solution was allowed to rest at ambient temperature, under conditions devoid of light, for a duration of 20 minutes, resulting in the appearance of a bluish-brown hue within the test tube.
6. Detection of saponin
   The presence of saponins was assessed using foam test. About 0.5 g of extract was mixed with 2 ml of water and agitated until a thick foam with small bubbles on top of the water layer formed.
7. Detection of glycosides
   To determine if glycosides are present, 0.5 g of extract and 1.5 ml of glacial acetic acid in a test tube were mixed. One drop of 5% ferric chloride was added to this mixture and shaken well. After settling, concentrated sulphuric acid was slowly added to the sides of the test tube until the solution turned brown.

2.4. Total phenolic contents of *Opuntia ficus* extracts

The total phenolic content was ascertained for the solvent and water extracts of OFI. In brief, the Folin–Ciocalteu reagent (100 µL, 50% v/v) was added to sodium carbonate (2 mL, 2% w/v) and mixed well in a rotor. To this mixture, the extract of OFI (100 µL) was added and allowed to develop in the absence of light at ambient temperature for a duration of 30 minutes. A blue colour develops after the incubation period and is read on a spectrophotometer at 750 nm, along with a blank. Gallic acid served as the benchmark for the comparative analysis of the results. The total phenolic content was quantified as mg/g in the extract of OFI.

2.5. Antibacterial activity of *Opuntia ficus* extracts

The antibacterial efficacy of OFI extracts was assessed against the test organisms (*Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*) via the well diffusion method. A sterile nutrient broth (HiMedia, Thane, India) (g/L) consisting of peptone: 5g yeast extract, 5g beef extract

and 3g sodium chloride: 5g, with a final pH of 7.0 ± 0.2, was utilised to inoculate all test cultures, which were subsequently allowed to proliferate for 24 to 48 hours. Sterile plates of Mueller-Hinton Agar (MHA) (HiMedia Labs, Thane, India) were prepared and permitted to solidify. In each case, swabs were used to evenly spread 0.1% inoculum suspensions of the test organism over the surface of the agar. Six-millimetre wells were excised from the agar surface of each plate while retaining sterility.

In the first set of four MHA plates inoculated with each test bacterium, four different antibiotics (ciprofloxacin, chloramphenicol, tetracycline and vancomycin) at a concentration of 4 µg/ml were loaded onto their respective wells.

In another set of four MHA plates inoculated with each test bacterium, we loaded five different extracts (ethanol, n-hexane, ethyl acetate, chloroform, and water) of OFI at a concentration of 0.5 mg/ml onto their respective wells.

Subsequently, all plates were incubated at 37°C for 24 hours to evaluate the presence of significant inhibitory zones following the incubation period. The sensitivity and resistance of each test bacterium to the tested antibiotics were measured and documented in accordance with the Clinical Laboratory Standards Institute (CLSI) recommendations (2017). Similarly, the zone of inhibition observed for each solvent extract was measured and compared with the zone size of each antibiotic tested.

2.6. High-performance liquid chromatographic (HPLC) analysis of *Opuntia ficus* extract

Extracts were examined for several phytochemical substances employing HPLC analysis. Five mg of extract were diluted in one millilitre of 95% ethanol and subsequently filtered using a 0.2 µm syringe filter. The filtrate is subsequently transferred to HPLC vials, sealed with a cap. Then it is positioned in the HPLC apparatus (Thermo Fisher Scientific, USA). HPLC analysis was conducted using the HPLC LC-10A series, which includes a liquid pump LS-10AS, a C-18 column (CLC-ODS 25 cm × 4.6 mm 5 µm), and a fluorescence UV-visible detector set at 280 nm. The column temperature was sustained at 25°C. A mobile phase of both ethanol and water in a 95:1 ratio was employed, with a flow rate of 1 mL per minute. The chemicals in HPLC were separated utilising isocratic elution methods. The retention duration, area, and concentration (mg/g DW) were quantified utilising the Internal Standard (ISTD) method. The identification of phenolic compounds was conducted by comparing their retention durations with those of reference standards from Sigma Chemicals Co., St. Louis, MO, USA.

3. RESULTS

3.1. Phytochemical analysis of different solvent extracts of *Opuntia ficus*

About four different solvent extracts and an aqueous extract of OFI were analysed to screen the phytochemical constituents. Among the solvents, the most predominant phytoconstituents (flavonoids and phenols) were found in the ethanolic extract of OFI (Figure 3). The n-hexane extracts, which contain alkaloids, flavonoids, and phenols, came next. Ethyl acetate extracts contain flavonoids and phenol; chloroform extracts contain terpenoids and phenol. Aqueous extract contains only alkaloids and glycosides. Saponin was absent in all the extracts. In Table 1 and Figure 3, the presence and absence of each type of phytochemical ingredient for solvent and aqueous extracts are presented.

3.2. Total Phenolic Content

The total phenolic content (TPC) of OFI extracts exhibited variability, as seen in Table 2. Ethanol extract has shown

Table 1
Phytochemical analysis of different solvent extracts of *Opuntia ficus.*

S. No.	Phytochemical constituents	Ethanol	n-hexane	Ethyl acetate	Chloroform	Water
1	Alkaloid	-	+	-	-	+
2	Flavonoid	++	+	+	-	-
3	Tannin	+	-	-	-	-
4	Terpenoid	+	-	-	+	-
5	Phenol	++	+	++	+	-
6	Saponin	-	-	-	-	-
7	Glycoside	+	+	-	-	+

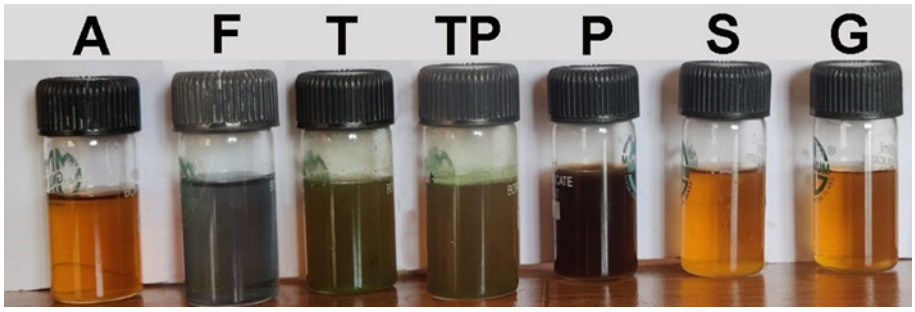


Figure 3. Phytochemical analysis of *Opuntia ficus* extract (Ethanol).
A: Alkaloid, F: Flavonoid, T: Tannin, TP: Terpenoid, P: Phenol, S: Saponin, G: Glycoside.

Table 2
Total phenolic content of different extracts of OFI extract.

S. No.	Extracts	Total phenolics (mg GAE/g)
1	Ethanol	63.3 ± 1.05
2	n-hexane	32.6 ± 0.75
3	Ethyl acetate	59.3 ± 1.05
4	Chloroform	36.9 ± 0.57
5	Water	16.3 ± 1.05

higher TPC (63.3 mg/g of GAE), followed by n-hexane, ethyl acetate and chloroform extract with respective TPCs of about 32.6 mg/g of GAE, 59.3 mg/g of GAE and 36.9 mg/g of GAE. The aqueous extract exhibited a relatively low total phenolic content (TPC) of 16.3 mg/g of gallic acid equivalents (GAE).

3.3. Antibacterial activity

The antibacterial efficacy of several solvent extracts and aqueous extracts of OFI was assessed using the usual agar well diffusion method. The inhibitory zones were analysed alongside standard antibiotics to determine the sensitivity and resistance profiles of the test bacteria. The zone of inhibition adhered to the Clinical Laboratory Standards Institute's recommendations (CLSI, 2017) for antibacterial testing in the current study.

Porphyromonas gingivalis exerted inhibitory zones of about 13.6 ± 0.75 mm, 14.6 ± 0.75 mm and 22.3 ± 1.05 mm for their respective ethyl acetate, n-hexane, and ethanol extracts. Chloroform and aqueous extracts did not exert inhibitory zones. Ethanol extract values were found to be similar to standard antibiotic ciprofloxacin (22.6 ± 0.75 mm), and other solvent extracts were slightly lower when compared to other antibiotics such as tetracycline and chloramphenicol (15.9 ± 0.57 mm and 18.3 ± 1.05 mm).

Aggregatibacter actinomycetemcomitans showed inhibitory zones of about 12.9 ± 0.57 mm, 16.3 ± 1.05 mm, and 22.3 ± 1.05 mm for their respective ethyl acetate, n-hexane, and ethanol extracts. Chloroform and aqueous extracts did not exert inhibitory zones. Ethanol extract values were found to be similar to standard antibiotic ciprofloxacin (22.9 ± 0.57mm) and other solvent extracts were slightly lower when compared to other antibiotics such as tetracycline and chloramphenicol (13.6 ± 0.75 mm and 15.9 ± 0.57 mm).

All the zone sizes recorded for the standard antibiotic, ciprofloxacin, against all test bacteria were inferred as sensitive as per CLSI guidelines. As per CLSI guidelines, a zone size ≥ 21 mm was obtained for CIP against all test organisms (Table 4; Figure 4). Based on these CLSI guidelines, ethanol extract exhibited greater than 21 mm of inhibitory zones against all the test organisms and hence confirmed that organisms were sensitive to ethanol extracts of OFI.

Ethanol extracts showed 22.3 ±1.05 and 22.3 ±1.05 for *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*, respectively. The zone of inhibition was determined according to the guidelines from the Clinical Laboratory Standards Institute (CLSI, 2017) for antibacterial testing.

Zone size with diameter ≥ 21 mm was recorded sensitive for Ciprofloxacin and Chloramphenicol and zone size < 21 recorded resistant. Zone size with diameter ≥ 23 and ≥ 17 mm was recorded sensitive for Tetracycline and Vancomycin (Clinical Laboratory Standards Institute's guidelines – CLSI, 2017).

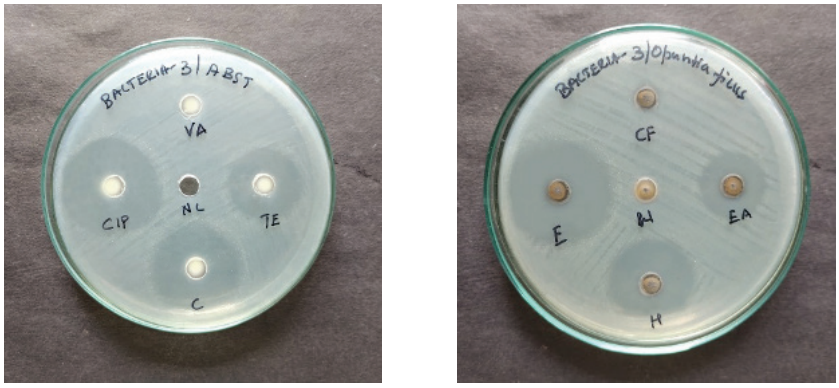
3.4. High-performance liquid chromatographic analysis of *Opuntia ficus* extract

High-performance liquid chromatographic profiles of ethanolic extracts of OFI showed significant phenolic and flavonoid compounds, such as catechin, quercetin, chlorogenic acid, gallic acid, kaempferol, hydroquinone, resorcinol, and syringic acid. A major peak was obtained for the phenolic compound, gallic acid, at the retention time of 4.61 min and

Table 3
Antibacterial activity of *Opuntia ficus* extracts against test organisms.

S. No.	Test organism	Zone of inhibition (millimetres)									
		VA	TE	C	CIP*	NC	CF	EA	H	E*	W
3	*Porphyromonas gingivalis*	0	15.9 ± 0.57	18.3 ± 1.05	22.6 ± 0.75	0	0	13.6 ± 0.75	14.6 ± 0.75	22.3 ± 1.05	0
4	*Aggregatibacter actinomycetemcomitans*	0	13.6 ± 0.75	15.9 ± 0.57	22.9 ± 0.57	0	0	12.9 ± 0.57	16.3 ± 1.05	22.3 ± 1.05	0

E: Ethanol, H: n-hexane, EA: ethyl acetate, Cf: Chloroform, W: Aqueous, VA: Vancomycin, TE: Tetracycline, C: Chloramphenicol, CIP: Ciprofloxacin, NC: DMSO (Negative control).
*Inhibitory zone size for ethanol was found statistically significant, similar to the zone size for Ciprofloxacin ($p<0.05$).



Porphyromonas gingivalis

Aggregatibacter actinomycetemcomitans

Figure 4. Antibacterial activity of *Opuntia ficus* extracts against test organisms. CIP: Ciprofloxacin, C: Chloramphenicol, TE: Tetracycline, VA: Vancomycin NC: DMSO (Negative control), E: Ethanol, H: n-hexane, EA: ethyl acetate, CF: Chloroform, W: Aqueous.

the lowest peak was recorded for hydroquinone at 5.74 min retention time. In Figure 5 and Table 4, the HPLC profile of the identified compounds with their respective major peaks eluted at their retention time, area in percentage and quantity obtained (in mg/g DW) was presented.

4. DISCUSSION

OFI, a species of cactus plant used in traditional medicine, is currently the subject of several recent studies. This study aimed to conduct a preliminary phytochemical screening of

all OFI extracts and assess their bioactive potential via antibacterial assays. However, only ethanolic extract was used to conduct quantitative HPLC analyses because it showed the highest antimicrobial potential. The antibacterial efficacy of different solvent extract concentrations in vitro was evaluated against *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans*.

Different solvent extracts, which include ethanol, n-hexane, ethyl acetate, chloroform and water extracts of OFI were analysed to screen the phytochemical constituents alkaloids, flavonoids, tannins, terpenoids, phenols, saponins, and glycosides. Flavonoids, tannins, terpenoids, and phenols were found in ethanol extracts while alkaloids and glycosides were found in water and n-hexane extracts. Saponin was not found in any extract. The presence of these phytochemicals explains the antibacterial, anti-inflammatory, anti-cancer, and antioxidant qualities of OFI extracts (Ullah et al., 2020).

Table 4
HPLC analysis of *Opuntia ficus.*

S. No.	Compounds identified	Retention time (min)	Area (%)	Quantity (mg/g DW)
1	Catechin	2.52	1.54	2.6 ± 0.02
2	Quercetin	3.61	2.91	3.1 ± 0.01
3	Chlorogenic acid	4.25	0.98	2.8 ± 0.02
4	Gallic acid	4.61	3.23	8.4 ± 0.01
5	Kaempferol	5.18	4.88	2.3 ± 0.01
6	Hydroquinone	5.74	2.96	1.8 ± 0.02
7	Resorcinol	6.83	5.11	5.7 ± 0.01
8	Syringic acid	8.06	3.84	2.5 ± 0.01

The presence of phenols is also attributed to its antioxidant and antimicrobial potential.(Aruwa et al., 2019) Polyphenols of OFI were also reported to be associated with antioxidative and antimicrobial properties (Kadda et al., 2023). The results of our present study are similar to that of Adam et al. (2017) that showed cladode extracts of OFI possessed significant quantities of flavonoids, tannins, and phenols while exhibiting minimal levels of saponins (Adam et al., 2017).

The antibacterial activity of OFI fruit volatiles against dental organisms, *Staphylococcus aureus* and *Enterococcus faecalis*, was studied by Karadag et al. (2018) using a microdilution assay.(Karadağ et al., 2018) The minimal inhibitory concentration of OFI cladode extracts against both test organisms was determined to be 500 µg/mL. Blando et al. (2019) evaluated the minimum inhibitory concentration (MIC) of OFI cladode extracts against Escherichia coli, Salmonella typhimurium, and Enterobacter aerogenes, finding that there was inhibition at a concentration of 2000 µg/mL.The other two biofilm producers, *Staphylococcus aureus* and *Enterococcus faecalis,* exhibited the MIC value of 1500 µg/mL (Blando et al., 2019). Sitenda et al. (2024) exhibited antibacterial inhibitory zones of about 27 mm for fruit extracts and 21 mm for stem extracts against *Escherichia coli* for the OFI extracts; the obtained zone size (28 mm) was slightly less when compared to standard ampicillin (control) (Sitenda et al., 2024). Similar results were seen in our present study, where OFI fruit extracts showed excellent antibacterial activity.

Bargougui et al., (2019) studied the antimicrobial activity of OFI extracts (Bargougui et al., 2019). The authors revealed that methanol extracts exhibited broader antimicrobial activity against *Staphylococcus aureus* and other test bacteria compared to ethyl acetate extracts. The study recorded significant

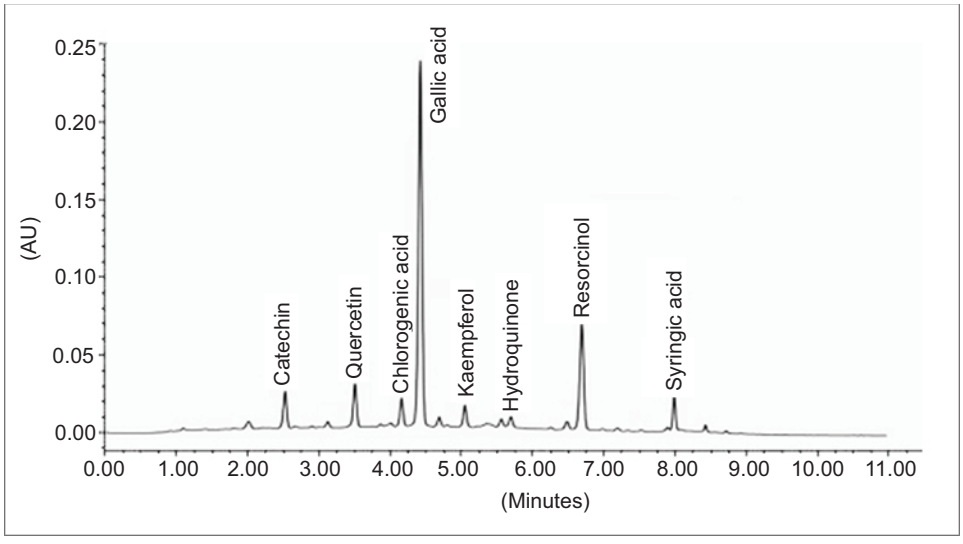


Figure 5. High-performance liquid chromatogram of *Opuntia ficus.*

inhibitory zone sizes of about 27.0 ± 2.47, 24.0 ± 1.16, 29.0 ± 2.06, and 25.0 ± 1.66 mm against all the test organisms. (Bargougui et al., 2019) In our present study, ethanol extract of OFI showed good inhibitory zones ranging from 22.3 ± 1.05 mm for both the test organisms.

Welegerima et al. (2018) discovered that cladode extracts of OFI had significant antibacterial efficacy against both gram-positive and gram-negative bacteria (Welegerima et al., 2018). El Feghali et al. (2018) utilised extracts from OFI cladodes to examine their inhibitory effects on *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* (El Feghali et al., 2018). In the present study, we found similar results for OFI fruit extracts.

Sanchez et al. (2014) evaluated the antibacterial activity of cladode extracts of OFI against *Staphylococcus aureus* and *Escherichia coli* and found it to be promising (Sánchez et al., 2014). In our present study, ethanol extracts produced similar inhibition zones when compared to ciprofloxacin. But ethanal extracts showed better inhibition zones than chloramphenicol and tetracycline. Moreover, inhibitory zones exerted on ethanol extracts were found to be significant in terms of sensitivity patterns as per CLSI guidelines. As per CLSI guidelines, zone size ≥ 21 mm is considered significant. (Table 3; Figure 4). Based on these CLSI guidelines, ethanol extract exhibited greater than 22.3 ±1.05 mm of inhibitory zones against the test organisms, and hence it is confirmed that both the organisms were sensitive to ethanol extracts of OFI.

A number of studies have shown promising antibacterial activity of OFI similar to our findings (Bargougui et al., 2019; Sánchez et al., 2014; Welegerima et al., 2018). The promising and potent antibacterial activity of OFI extracts alludes to the presence of secondary plant metabolites, such as phenols, terpenoids, flavonoids, tannins, and alkaloids in the solvent extracts, which are directly responsible for the antimicrobial properties. Our literature search found numerous studies that support this concept, in particular the importance of presence of various phytochemical compounds in OFI. Hernandez et al. (2022) identified phenolic acids, betalains, and lignans as significant phytochemical constituents from the fruit pulp of OFI (Hernández et al., 2022). The obtained polyphenolic compounds were related to phenylalanine metabolism and hence attributable to antioxidant activity, anti-inflammatory activity and antiviral activity. The mode of action of flavonoids and tannins was also explained: it could cause cell death by stopping the synthesis of bacterial nucleic acids and by inhibiting the bacterial extracellular enzyme production (Cushnie & Lamb, 2005).

The present findings highlight that the HPLC analysis performed in our study represents a valuable tool for identifying significant phenolic and flavonoid compounds present in OFI fruit extracts. The study identified several important compounds, including kaempferol, quercetin, gallic acid, chlorogenic acid, syringic acid, and catechin, which were found to have various properties, including antibacterial, antioxidant, anti-inflammatory, and anticancer effects.

4. LIMITATIONS OF THE STUDY

Our present study did not validate the biofilm-producing capability of the test bacteria, *Porphyromonas gingivalis and Aggregatibacter actinomycetemcomitans*. Scanning Electron Microscopy (SEM) analysis should be conducted to examine biofilm formation by these bacteria and to evaluate the inhibitory effects of *Opuntia ficus*-indica extract on biofilms. Furthermore, comprehensive molecular docking studies are advised for the future to investigate the spectrum of activity of OFI constituent compounds against oral pathogens and to assess their safety and efficacy in clinical applications. Moreover, variation in chemical composition due to plant origin, harvest time, and extraction method poses a regular challenge for reproducibility and regulatory approval when dealing with plant-derived natural products (Estarriaga-Navarro et al., 2025). Additional research is necessary to examine the extract's efficacy against various oral pathogens and to assess its safety in clinical environments.

5. CONCLUSION

Opuntia ficus belongs to the Cactaceae family and has many significant pharmacological and medicinal properties. The current study conducted phytochemical screening, total phenolic content assessment, antibacterial activity evaluation, and high-performance liquid chromatography (HPLC) analysis of Ficus extracts from OFI. This is the first study, to the best of our knowledge, to study the inhibitory effects of OFI fruit extracts on *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* and to carry out HPLCC of OFI ficus. Phytochemical analysis in our study showed flavonoids, tannins, terpenoids, and phenols that were found in ethanol extracts, while alkaloids and glycosides were found in water and n-hexane extracts. In our present study, ethanol extract of OFI ficus showed excellent inhibitory zones ranging from 22.3 ±1.05 mm for both the test organisms, *Aggregatibacter actinomycetemcomitans* and *Porphyromonas gingivalis*. The HPLCC study carried over in our study identified gallic acid as the main constituent of the entire OFI ficus, followed by resorcinol, quercetin, chlorogenic acid, catechin, syringic acid, kaempferol, and hydroquinone. Thus, this present study shows promising potential for use of OFI Ficus extract as an antibacterial additive in oral hygiene products. However,

further research and clinical validation are mandatory before medicinal use.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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DATA AVAILABILITY

All datasets generated or analysed during this study are included in the manuscript.

ETHICS STATEMENT

Not applicable.

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REFERENCES

Abu Tamam, A.N., Kukreja, B.J., Ramachandra, S.S., Reddy, M.S., Souza, J.L.D., & Abdelmagyd, H.A.E. (2024). Herbal medicine as an adjunct in the treatment of periodontal diseases - A systematic review. *The Open Dentistry Journal*, *18*(1). https://doi.org/10.2174/0118742106295311240419074231

Adam, I.K., Unogu, O.I., & Yusuf, M.B. (2017). Studies on Phytochemical and Antioxidant Properties of Opuntia ficus-indica. *Dutse Journal of Pure and Applied Sciences*, *3*, 134–141. https://fud.edu.ng/journals/dujopas/2017.JUNE.Vol3.1/Ibrahim_AK1%20134%20-%20141.pdf

Ahmed, N., Karobari, M.I., Yousaf, A., Mohamed, R.N., Arshad, S., Basheer, S.N., Peeran, S.W., Noorani, T.Y., Assiry, A.A., Alharbi, A.S., & Yean, C.Y. (2022). The Antimicrobial Efficacy Against Selective Oral Microbes, Antioxidant Activity and Preliminary Phytochemical Screening of. *Infection and Drug Resistance*, *15*, 2773–2785. https://doi.org/10.2147/IDR.S364175

Aleksijević, L.H., Aleksijević, M., Škrlec, I., Šram, M., Šram, M., & Talapko, J. (2022). Porphyromonas gingivalis Virulence Factors and Clinical Significance in Periodontal Disease and Coronary Artery Diseases. *Pathogens (Basel, Switzerland)*, *11*(10). https://doi.org/10.3390/pathogens11101173

Alves, P. e. S., de Figuerêdo, J.S., Santos, F.P.S., Furtado, P.V.O.S., Andrade, T.J.A.S., Júnior, J.S.C., Lima, N.M., & Feitosa, C.M. (2022). Natural products from plants with antimicrobial action. In *Promising Antimicrobials from Natural Products* (pp. 183–198). Springer International Publishing. https://doi.org/10.1007/978-3-030-83504-0_9

Angelini, P. (2024). Plant-Derived Antimicrobials and Their Crucial Role in Combating Antimicrobial Resistance. *Antibiotics (Basel, Switzerland)*, *13*(8). https://doi.org/10.3390/antibiotics13080746

Aruwa, C.E., Amoo, S., & Kudanga, T. (2019). Phenolic compound profile and biological activities of Southern African Opuntia ficus-indica fruit pulp and peels. *Lebensmittel-Wissenschaft Und Technologie [Food Science and Technology]*, *111*, 337–344. https://doi.org/10.1016/j.lwt.2019.05.028

Bargougui, A., Maatoug Tag, H., Bouaziz, M., & Triki, S. (2019). Antimicrobial, antioxidant, total phenols and flavonoids content of four cactus (Opuntia ficus-indica) cultivars. *Biomedical & Pharmacology Journal*, *12*(3), 1353–1368. https://doi.org/10.13005/bpj/1764

Blando, F., Russo, R., Negro, C., De Bellis, L., & Frassinetti, S. (2019). Antimicrobial and Antibiofilm Activity against of (L.) Mill. Cladode Polyphenolic Extracts. *Antioxidants (Basel, Switzerland)*, *8*(5). https://doi.org/10.3390/antiox8050117

Chen, S., Tang, L., Xu, M., Chen, T., Zhao, S., Liu, M., & Liu, S. (2022). Light-emitting-diode-based antimicrobial photodynamic therapies in the treatment of periodontitis. *Photodermatology, Photoimmunology & Photomedicine*, *38*(4), 311–321. https://doi.org/10.1111/phpp.12759

Conte, R., Valentino, A., De Luca, I., Soares Pontes, G., Calarco, A., & Cerruti, P. (2024). Thermo-Responsive Hydrogel Containing Microfluidic Chitosan Nanoparticles Loaded with Extract for Periodontitis Treatment. *International Journal of Molecular Sciences*, *25*(17). https://doi.org/10.3390/ijms25179374

Cushnie, T.P.T., & Lamb, A.J. (2005). Antimicrobial activity of flavonoids. *International Journal of Antimicrobial Agents*, *26*(5), 343–356. https://doi.org/10.1016/j.ijantimicag.2005.09.002

Dormousoglou, M., Efthimiou, I., Antonopoulou, M., Dailianis, S., Herbst, G., & Vlastos, D. (2023). Phytochemical Analysis and Genotoxicological Evaluation of Prickly Pear Peel Extracts. *Plants (Basel, Switzerland)*, *12*(7). https://doi.org/10.3390/plants12071537

El Feghali, P.A., Ibrahim, R., & Nawas, T. (2018). Antibacterial activity of Curcuma longa, Opuntia ficusindica and Linum usitatissimum. *MOJ Toxicology*, *4*(3), 214–220. http://medcraveonline.com/MOJT/MOJT-04-00102.pdf

Elmowafy, E., El-Marakby, E.M., Gad, H.A., & Gad, H.A. (2022). Delivery systems of plant-derived antimicrobials. In *Promising Antimicrobials from Natural Products* (pp. 397–442). Springer International Publishing. https://doi.org/10.1007/978-3-030-83504-0_16

Estarriaga-Navarro, S., Valls, T., Plano, D., Sanmartín, C., & Goicoechea, N. (2025). Potential Application of Plant By-Products in Biomedicine: From Current Knowledge to Future Opportunities. *Antioxidants (Basel, Switzerland)*, *14*(8). https://doi.org/10.3390/antiox14080942

Fujita, A., Oogai, Y., Kawada-Matsuo, M., Nakata, M., Noguchi, K., & Komatsuzawa, H. (2021). Expression of virulence factors under different environmental conditions in Aggregatibacter actinomycetemcomitans. *Microbiology and Immunology*, *65*(3), 101–114. https://doi.org/10.1111/1348-0421.12864

Hayakumo, S., Arakawa, S., Takahashi, M., Kondo, K., Mano, Y., & Izumi, Y. (2014). Effects of ozone nano-bubble water on periodontopathic bacteria and oral cells – studies. *Science and Technology of Advanced Materials*, *15*(5), 055003. https://doi.org/10.1088/1468-6996/15/5/055003

Hernández, F., Andreu-Coll, L., Bento-Silva, A., Serra, A.T., Mena, P., Legua, P., & Bronze, M.R. (2022). Phytochemical Profile of (L.) Mill Fruits (cv. "Orito") Stored at Different Conditions. *Foods (Basel, Switzerland)*, *11*(2). https://doi.org/10.3390/foods11020160

Jacob, S., Gusmao, L., Godboley, D., Velusamy, S.K., George, N., Schreiner, H., Cugini, C., & Fine, D.H. (2024). Molecular Analysis of ApiA, a Multi-Functional Protein. *Pathogens (Basel, Switzerland)*, *13*(11). https://doi.org/10.3390/pathogens13111011

Kadda, S., Belabed, A., Conte, R., Ouahhoud, S., Hamdaoui, H., Mechchate, H., & Elarbi, Z. (2023). Phytochemical analysis for the residues of Opuntia ficus indica l seed oil of eastern region of Morocco. *Materials Today: Proceedings*, *72*, 3662–3668. https://doi.org/10.1016/j.matpr.2022.08.533

Karadağ, A.E., Demirci, B., Polat, D. Ç., & Okur, M.E. (2018). Characterization of Opuntia ficus-indica (L.) Mill. fruit volatiles and antibacterial evaluation. *NVEO - NATURAL VOLATILES & ESSENTIAL OILS Journal | NVEO*, 35–38. https://nveo.org/index.php/journal/article/view/68

Lee, Y.-H., Park, H.J., Jeong, S.-J., Auh, Q.-S., Jung, J., Lee, G.-J., Shin, S.-I., & Hong, J.-Y. (2023). Oral microbiome profile of gingivitis and periodontitis by next-generation sequencing. In *Research Square*. https://doi.org/10.21203/rs.3.rs-3530768/v1

Matias, A., Nunes, S.L., Poejo, J., Mecha, E., Serra, A.T., Madeira, P.J.A., Bronze, M.R., & Duarte, C.M.M. (2014). Antioxidant and anti-inflammatory activity of a flavonoid-rich concentrate recovered from Opuntia ficus-indica juice. *Food & Function*, *5*(12), 3269–3280. https://doi.org/10.1039/c4fo00071d

Peeran, S.W., Fageeh, H., Ibraheem, W., Fageeh, H.I., Al-Ak'hali, M.S., Doggalli, N., Alam, M.N., & Basheer, S.N. (2024). Antibacterial Activity, Antiadherence Activity, Antioxidant Activity, and Molecular Docking of Sweetbrair Rose () Extract on Periodontopathogens: and – An Study. *Journal of Pharmacy & Bioallied Sciences*, *16*(Suppl 5), S4667–S4677. https://doi.org/10.4103/jpbs.jpbs_697_24

Peeran, S.W., Murugan, M., Fageeh, H., Ibraheem, W., Fageeh, H.I., & Basheer, S.N. (2024). Antibacterial Activity, Antiadherence Activity, Antioxidant Activity, and Molecular Docking Analysis of Arab Rose Extract on Periodontopathic Bacteria: and An Study. *Journal of Pharmacy & Bioallied Sciences*, *16*(Suppl 5), S4678–S4687. https://doi.org/10.4103/jpbs.jpbs_1300_24

Peeran, S.W., Murugan, M., Fageeh, H., Ibrahim, W., Al-Ak'hali, M.S., Basheer, S.N., & Mohamed, A.M.B. (2024). Evaluation of Wild Bitter Melon () Extracts against Periodontopathic Bacteria (and) using Antibacterial, Anti-Inflammatory and Molecular Docking Analysis. *Journal of Pharmacy & Bioallied Sciences*, *16*(Suppl 2), S1554–S1564. https://doi.org/10.4103/jpbs.jpbs_512_23

Rasoulpour, R., Izadpanah, K., & Afsharifar, A. (2020). Opuntin B, the antiviral protein isolated from prickly pear (Opuntia ficus-indica (L.) Miller) cladode exhibits ribonuclease activity. *Microbial Pathogenesis*, *140*, 103929. https://doi.org/10.1016/j.micpath.2019.103929

Sánchez, E., Dávila-Aviña, J., Castillo, S.L., Heredia, N., Vázquez-Alvarado, R., & García, S. (2014). Antibacterial and antioxidant activities in extracts of fully grown cladodes of 8 cultivars of cactus pear. *Journal of Food Science*, *79*(4), M659–M664. https://doi.org/10.1111/1750-3841.12416

Sedghi, L.M., Bacino, M., & Kapila, Y.L. (2021). Periodontal Disease: The Good, The Bad, and The Unknown. *Frontiers in Cellular and Infection Microbiology*, *11*, 766944. https://doi.org/10.3389/fcimb.2021.766944

Sitenda, D., Anywar, G., & Kakudidi, E.K. (2024). Traditional uses and anti-bacterial activity of Opuntia ficus-indica stem and fruit extracts in Kasese District, Western- Uganda. In *Research Square*. https://doi.org/10.21203/rs.3.rs-5106710/v1

Sutthiboonyapan, P., Wang, H.-L., & Charatkulangkun, O. (2020). Flowcharts for Easy Periodontal Diagnosis Based on the 2018 New Periodontal Classification. *Clinical Advances in Periodontics*, *10*(3), 155–160. https://doi.org/10.1002/cap.10095

Tzimas, K., Antoniadou, M., Varzakas, T., & Voidarou, C.C. (2024). Plant-Derived Compounds: A Promising Tool for Dental Caries Prevention. *Current Issues in Molecular Biology*, *46*(6), 5257–5290. https://doi.org/10.3390/cimb46060315

Ullah, A., Munir, S., Badshah, S.L., Khan, N., Ghani, L., Poulson, B.G., Emwas, A.-H., & Jaremko, M. (2020). Important Flavonoids and Their Role as a Therapeutic Agent. *Molecules (Basel, Switzerland)*, *25*(22). https://doi.org/10.3390/molecules25225243

Welegerima, G., Zemene, A., & Tilahun. (2018). Phytochemical composition and antibacterial activity of Opuntia Ficus Indica cladodes extracts. *Journal of Medicinal Plants Studies*, *6*(2), 243–246. https://www.plantsjournal.com/archives/2018/vol6issue2/PartD/6-2-35-824.pdf

Woo, S., Marquez, L., Crandall, W.J., Risener, C.J., & Quave, C.L. (2023). Recent advances in the discovery of plant-derived antimicrobial natural products to combat antimicrobial resistant pathogens: insights from 2018-2022. *Natural Product Reports*, *40*(7), 1271–1290. https://doi.org/10.1039/d2np00090c