CHAPTER 4

RESULTS

4.1 Population of Samples

In this study, about 10 subgingival plaque samples were successfully collected from Dental Polyclinic, Faculty of Dentistry USIM. A total of 176 isolated oral bacteria species have been successfully isolated from all collected samples. The subgingival plaque samples were obtained from patients representing both genders. Among 10 patients, there were eight males and two females.

4.2 Colony Forming Unit

Human oral microflora is complex due to the variety of environments accessible for colonization. The subgingival plaque samples were cultured on agar media, such as blood sheep agar to enhance the growth of aerobic, anaerobic, and facultative anaerobic and anaerobic bacteria. Figures 4.1 to 4.10 show the first bacteria culture on blood sheep agar from a subgingival plaque sample. Table 4.1 shows the number of viable colonies isolated on blood agar after performing serial dilution, counted, and calculated by using the colony forming unit formula: *CFU/mL = (number of colonies x dilution factor) / volume of the culture plate*. All samples were serially diluted in 10 mL 0of broth up to 10^{-4}. However, the first three plates cannot be counted because it is more than 300 colonies. Thus, it is classified as too numerous to count (TNTC).

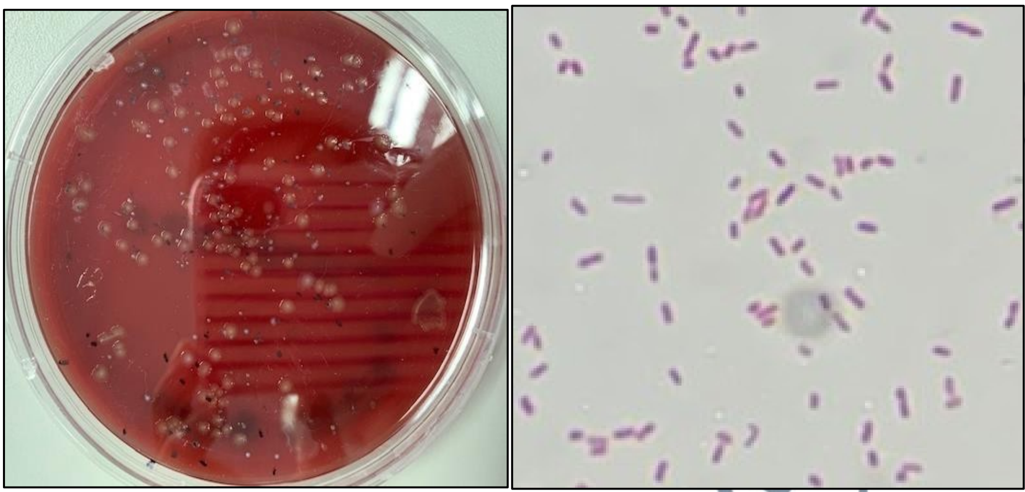

Figure 4.1: Bacteria colonies on blood sheep agar and microscopic analysis (rod-shaped) from subgingival plaque sample 1.

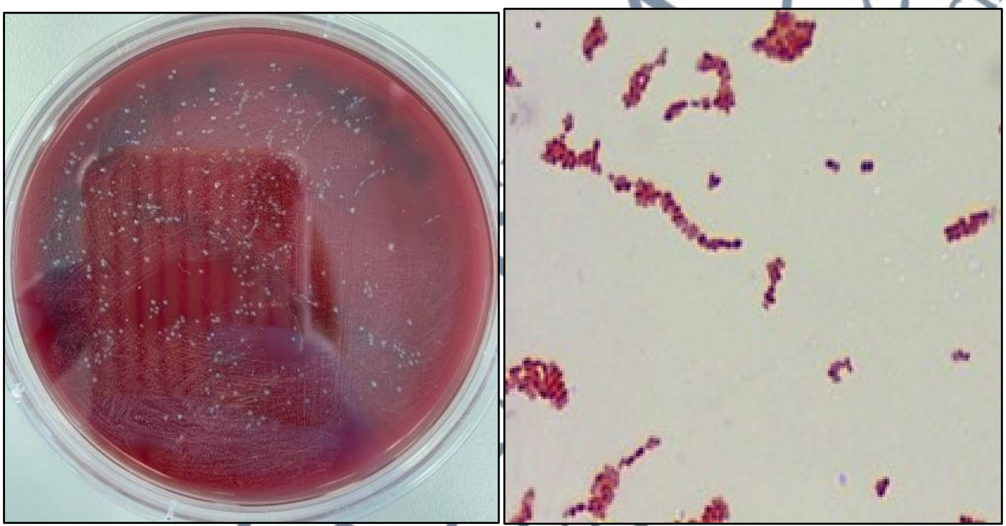

Figure 4.2: Bacteria colonies on blood sheep agar and microscopic analysis (cocci shaped and aggregates in chains) from subgingival plaque sample 2.

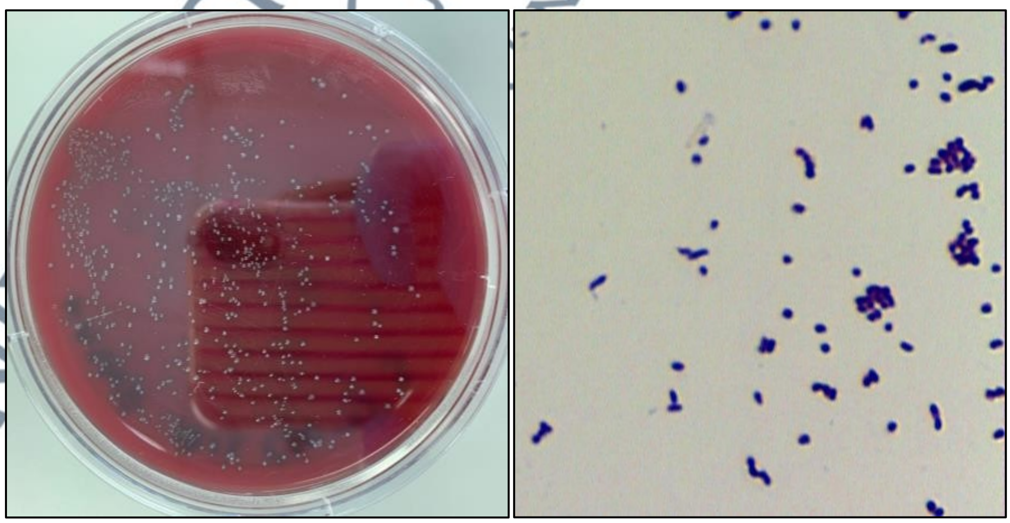

Figure 4.3: Bacteria colonies on blood sheep agar and microscopic analysis (cocci shaped) from subgingival plaque sample 3.

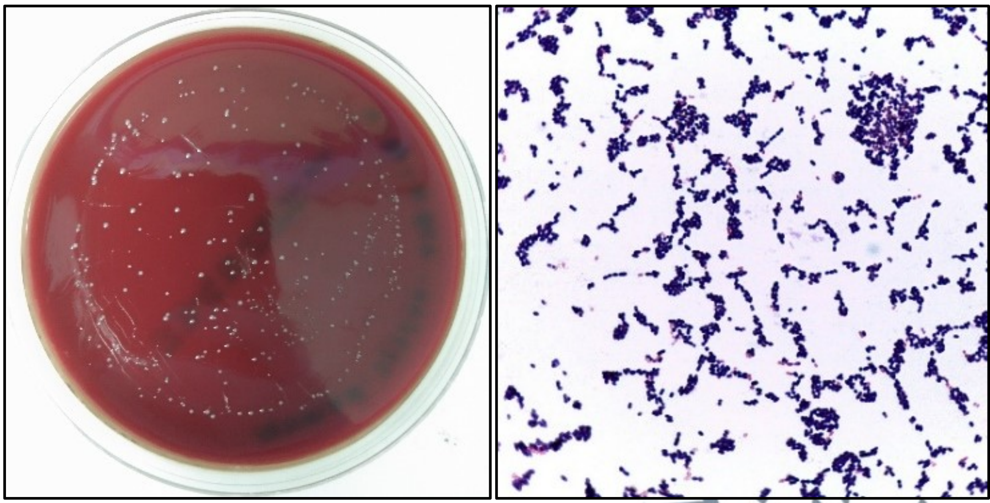

Figure 4.4: Bacteria colonies on blood sheep agar and microscopic analysis (cocci shaped and aggregates in chains) from subgingival plaque sample 4.

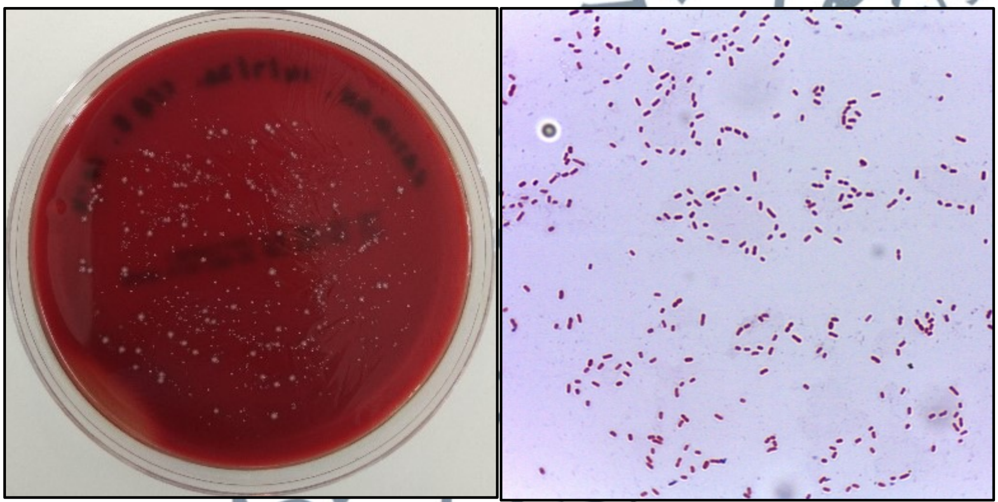

Figure 4.5: Bacteria colonies on blood sheep agar and microscopic analysis (rod-shaped) from subgingival plaque sample 5.

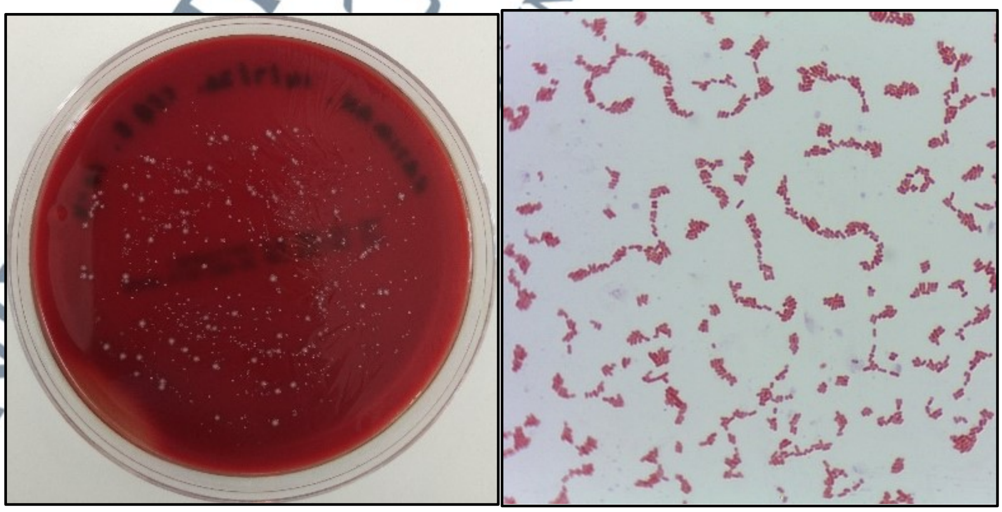

Figure 4.6: Bacteria colonies on blood sheep agar and microscopic analysis (rod-shaped and aggregates in chains) from subgingival plaque sample 6.

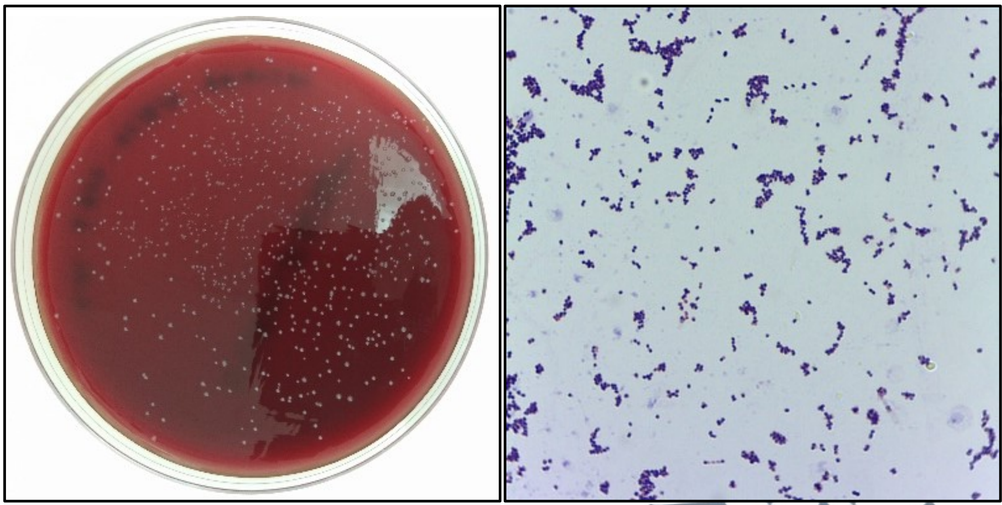

Figure 4.7: Bacteria colonies on blood sheep agar and microscopic analysis (cocci shaped) from subgingival plaque sample 7.

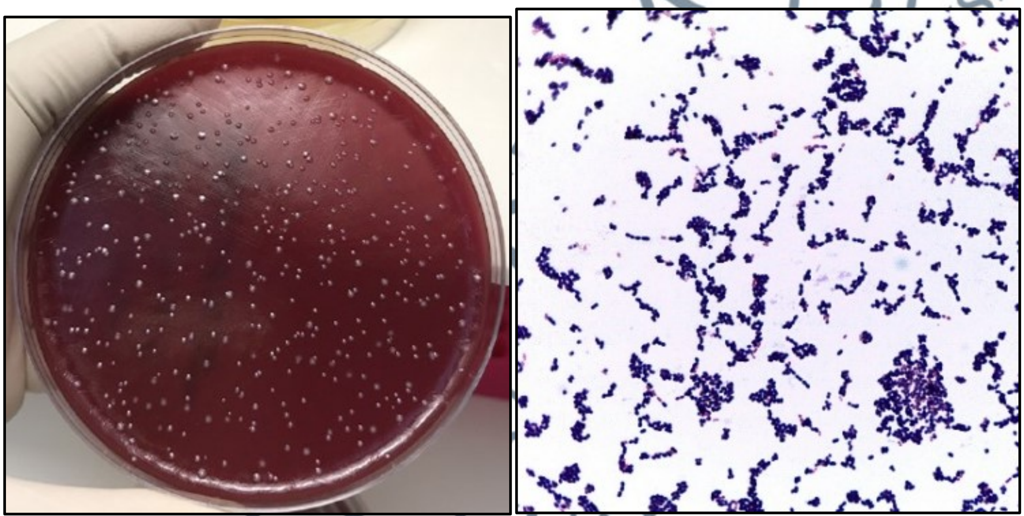

Figure 4.8: Bacteria colonies on blood sheep agar and microscopic analysis (cocci shaped) from subgingival plaque sample 8.

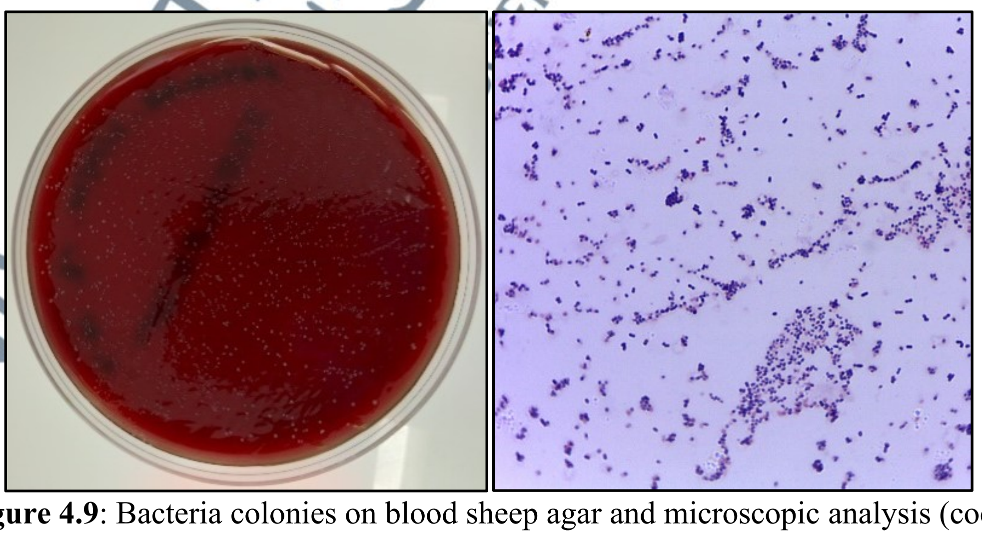

Figure 4.9: Bacteria colonies on blood sheep agar and microscopic analysis (cocci shaped) from subgingival plaque sample 9.

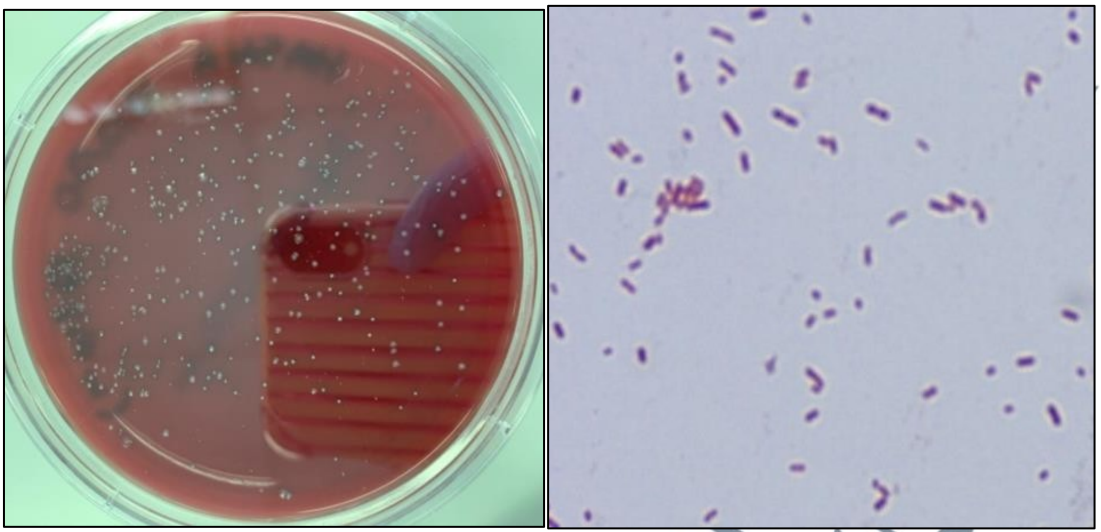

Figure 4.10: Bacteria colonies on blood sheep agar and microscopic analysis (rod-shaped) from subgingival plaque sample 10.

Table 4.1: Number of viable colonies for each subgingival plaque sample.

Samples	Colony forming unit (CFU/mL)
1	2.1×10^7
2	2.75×10^7
3	2.36×10^7
4	1.89×10^7
5	1.98×10^7
6	2.8×10^7
7	2.37×10^7
8	1.98×10^7
9	2.37×10^7
10	2.87×10^7

4.3 Morphology of Oral Bacteria

Oral bacteria form communities, including Gram-positive and Gram-negative bacteria. The streak plate method was used to obtain pure culture from subgingival plaque samples after growing on blood sheep agar. Figure 4.14 and Figure 4.15 showed the single colony of bacterial culture from sample 4 (BHI agar) and sample 5 (WCA agar). Table 4.2 shows the number of Gram-positive and Gram-negative oral bacteria after being isolated from blood agar. Based on the tabulated data, there were mixed colonies from the isolated subgingival plaque from each sample. A total of 97 and 79 bacterial species were for Gram-positive and Gram-negative bacteria respectively from the sample plaque. The morphology of the bacteria colony from sample 5 was white-pigmented and medium-sized with a circular shape. This single colony is pure culture obtained from blood agar culture. From the observation, most of the isolated bacteria from subgingival plaque samples were medium-sized, moist, and viscid.

The Gram-staining technique was performed after obtaining the colony from the media to determine if the colony is Gram-positive or Gram-negative bacteria as shown in Figure 4.11 to Figure 4.20. The Gram-staining method is important in this study before proceeding to molecular analysis due to the additional step for DNA extraction of Gram-positive bacteria. The morphology of Gram-negative bacterial sample 5 was rod-shaped and arranged as single cells with red colour under the microscope. Meanwhile, the morphology of Gram-positive bacterial sample 4 was coccus in chains. Table 4.3 shows the morphology of oral bacteria that were successfully isolated from subgingival plaque samples.

Figure 4.11: Bacteria colonies on WCA agar and microscopic analysis (rod-shaped) from subgingival plaque sample 1.

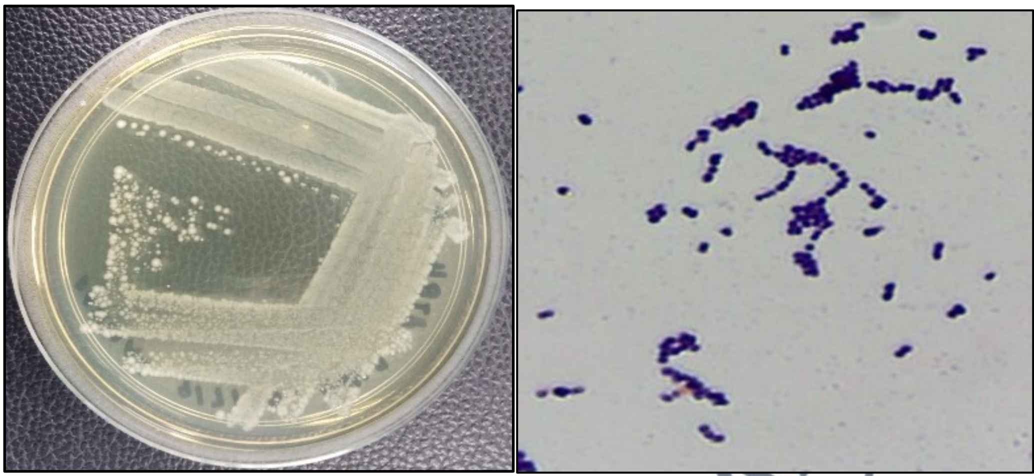

Figure 4.12: Bacteria colonies on BHI agar and microscopic analysis (cocci shaped) from subgingival plaque sample 2.

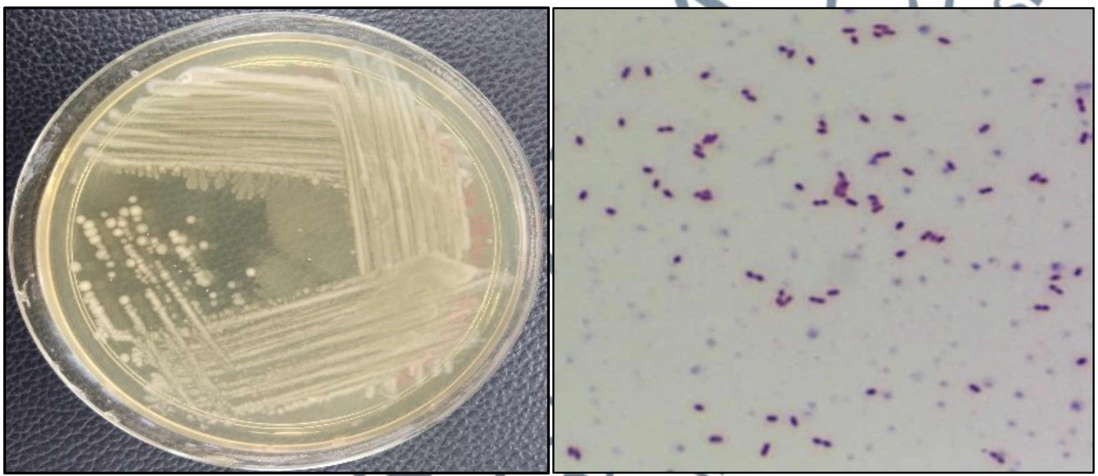

Figure 4.13: Bacteria colonies on WCA agar and microscopic analysis (rod-shaped) from subgingival plaque sample 3.

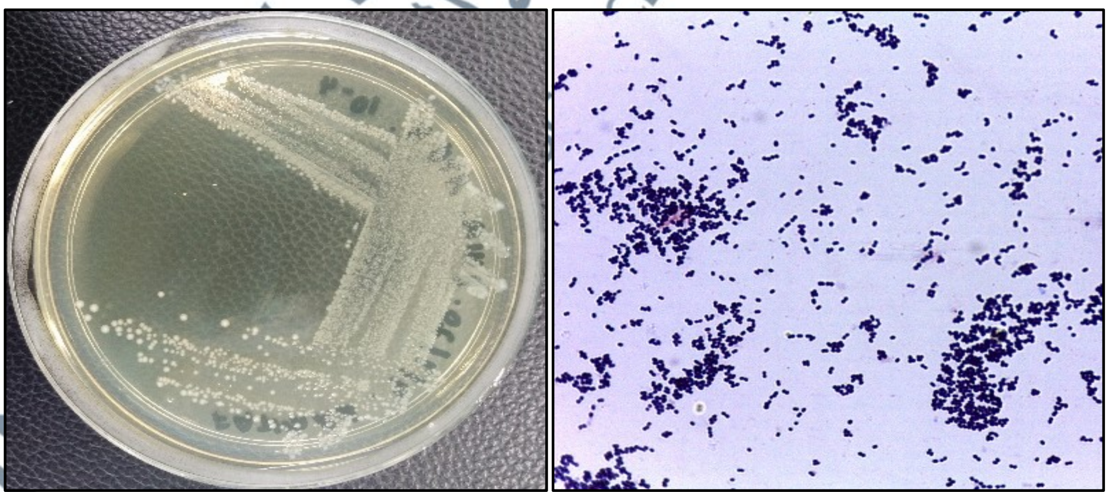

Figure 4.14: Bacteria colonies on BHI agar and microscopic analysis (cocci shaped) from subgingival plaque sample 4.

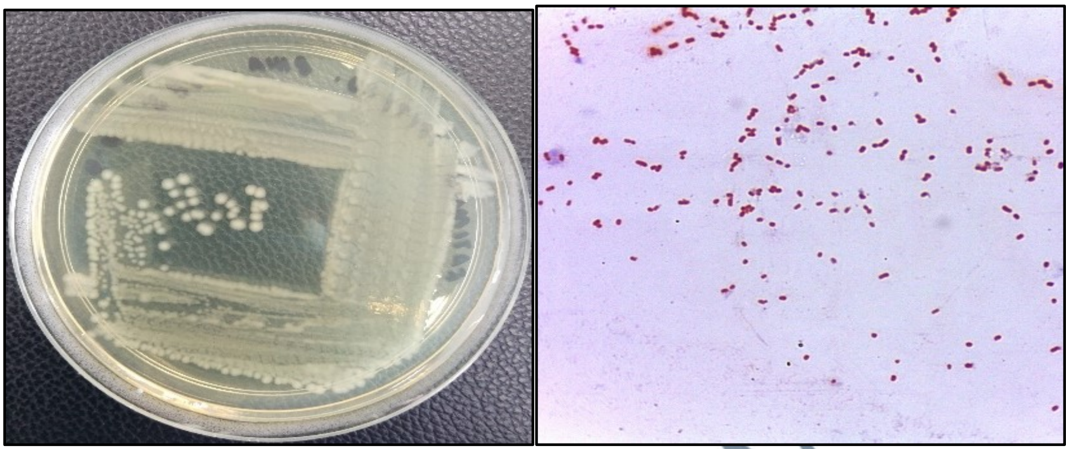

Figure 4.15: Bacteria colonies on WCA agar and microscopic analysis (cocci shaped) from subgingival plaque sample 5.

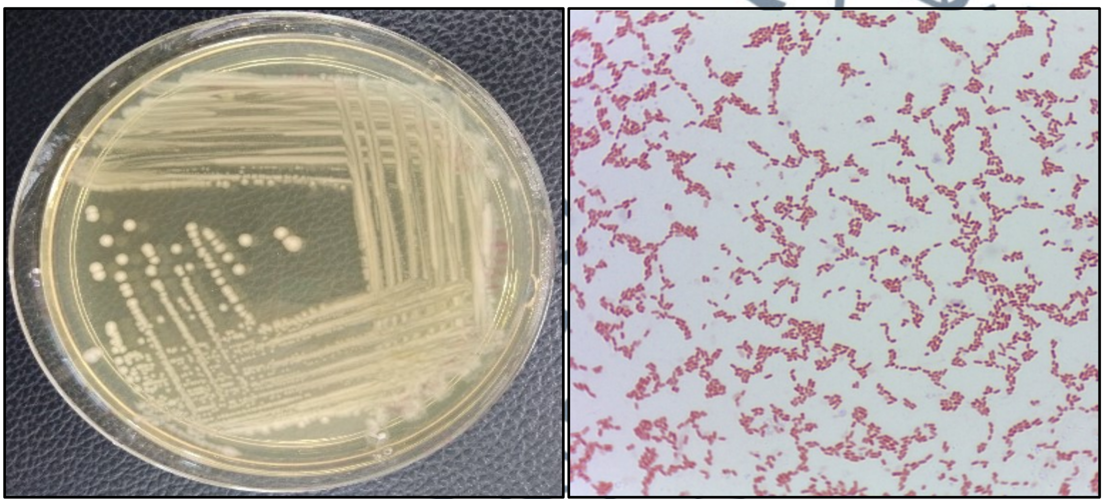

Figure 4.16: Bacteria colonies on WCA agar and microscopic analysis (rod-shaped) from subgingival plaque sample 6.

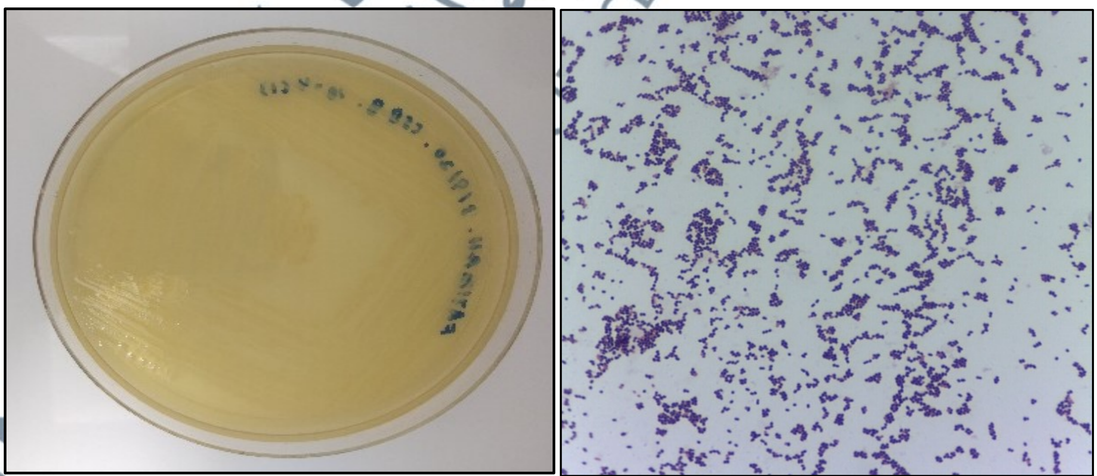

Figure 4.17: Bacteria colonies on BHI agar and microscopic analysis (cocci shaped) from subgingival plaque sample 7.

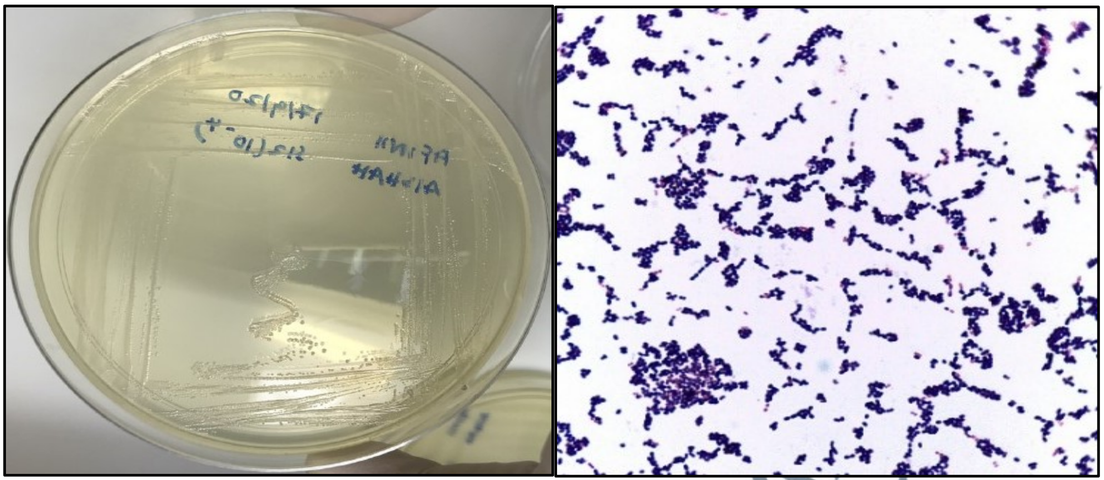

Figure 4.18: Bacteria colonies on WCA agar and microscopic analysis (cocci shaped) from subgingival plaque sample 8.

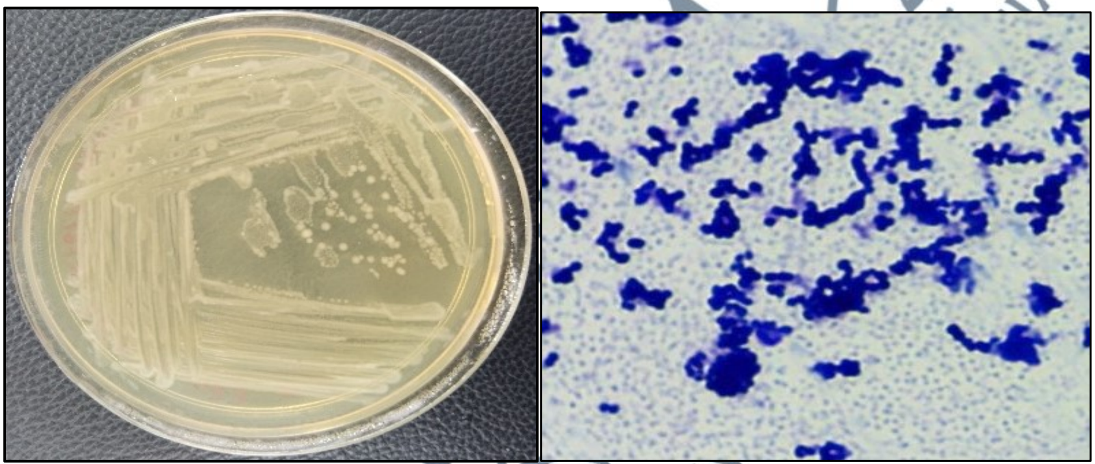

Figure 4.19: Bacteria colonies on BHI agar and microscopic analysis (cocci shaped) from subgingival plaque sample 9.

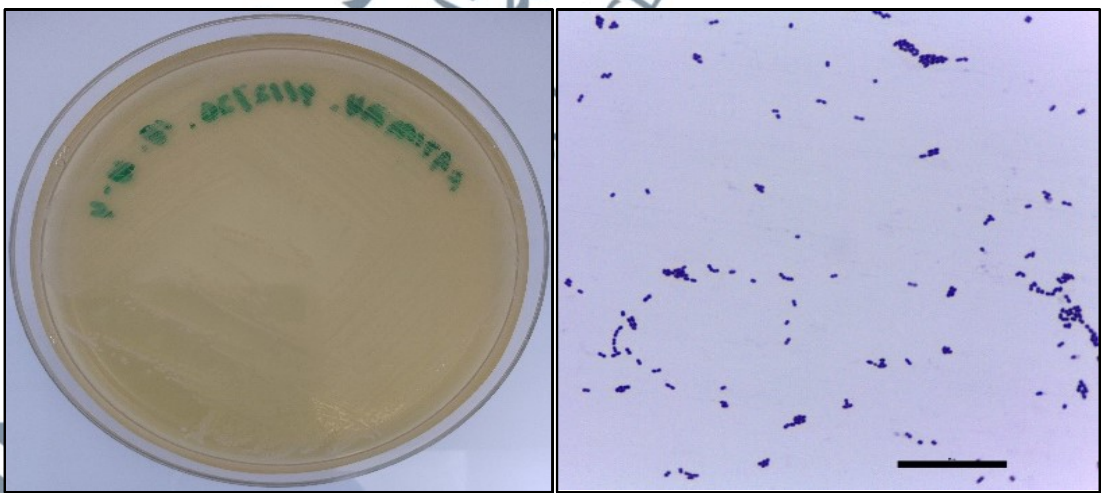

Figure 4.20: Bacteria colonies on BHI agar and microscopic analysis (rod-shaped) from subgingival plaque sample 10.

Table 4.2: The total number of Gram-positive and Gram-negative oral bacteria isolated from subgingival plaque samples.

Samples	Gram-positive	Gram-negative
1	6	9
2	10	13
3	7	4
4	12	5
5	8	20
6	11	6
7	24	7
8	6	2
9	8	3
10	5	10

4.4 Isolation and Identification Type of Oral Bacteria from Subgingival Plaque Samples

From 176 of isolated oral bacteria (Table 4.2), 17 bacteria were successfully identified in molecular analysis. The chosen number of bacteria is based on the similarity of shape and morphology. Molecular analysis was conducted to identify the type of oral bacteria from subgingival plaque samples, including DNA extraction, DNA purification, PCR, and gel electrophoresis. Figures 4.21 to Figure 4.25 show the band of PCR products after DNA purification obtained from sample 1, 2, 3 and 4. Based on the 16S rRNA gene sequencing analysis, a total of 17 oral bacteria species were identified from 10 subgingival plaque samples as shown in Table 4.4. These results were classified as Gram-positive and Gram-negative bacteria. Both types of oral bacteria play important mechanisms as early and late colonizers. These bacteria colonizers, include *S. gordonii, S. mutans, S. sanguinis, S. oralis, S. agalactiae, S. downei, S. lutetiensis, S. anginosus, S. salivarius, P. gingivalis, A. actinomycetemcomitans, K. pneumoniae, E. clocae, E. ludwigii, E. mori, E. tabacii* and

E. roggen kampii. Besides that, streptococci were among the earliest bacteria to colonize oral surfaces (Jenkinson, 1994).

PCR method was used to amplify the DNA extracts of bacteria from subgingival plaque samples. To ensure the quality of PCR results, the PCR products were analysed by running gel electrophoresis at 100 V for 60 minutes. A specific primer of 16S rRNA was used for Gram-positive and Gram-negative bacteria. Figure 4.21 to Figure 4.25 show the DNA band of gel electrophoresis of PCR using PCR products after DNA purification from all subgingival plaque samples.

To identify bacterial species of the amplified DNA gene, the purified PCR products were sent for sequencing. NCBI GenBank database was used to determine nucleotide sequence for bacterial species found in subgingival plaque samples using BLAST. Figure 4.26 and Figure 4.27 are the alignments of DNA sequencing analysis for sample 4 using the BLAST program. Appendix 1 includes all the results of the nucleotide sequence. Figure 4.28 shows the phylogenetic tree of oral bacteria species from subgingival plaque. The identification percentage of similar bacteria is between 95% to 100% for all samples. DNA sequencing analysis was successfully identified 17 oral bacteria species from 10 samples.

Table 4.3: Morphology of 176 isolated oral bacteria on media and under microscope.

Samples	Strain Id	Types of bacteria	Morphology of bacteria	
			Agar plate	Microscope
1	1(a)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	1(b)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
	1(c)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	1(d)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped
	1(e)	Gram negative	Large sized, mucoid, and white pigment	Bacilli, rod-shaped, single arrangement
	1(f)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
	1(g)	Gram negative	Large sized, mucoid, and white pigment	Bacilli, rod-shaped, single arrangement
	1(h)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	1(i)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	1(j)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped
	1(k)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	1(l)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped

Table 4.3: Continued.

	1(m)	Gram negative	Large sized, mucoid, and white pigment	Bacilli, rod-shaped, single arrangement
	1(n)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped
	1(o)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
2	2(a)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	2(b)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
	2(c)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	2(d)	Gram negative	Large sized, mucoid and white pigment	Bacilli, rod-shaped, single arrangement
	2(e)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	2(f)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	2(g)	Gram positive	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	2(h)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	2(i)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped
	2(j)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	2(k)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped

Table 4.3: Continued.

	2(l)	Gram negative	Large sized, mucoid and white pigment	Bacilli, rod-shaped, single arrangement
	2(m)	Gram negative	Large sized, mucoid and white pigment	Bacilli, rod-shaped, single arrangement
	2(n)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	2(o)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	2(p)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	2(q)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	2(r)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
	2(s)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	2(t)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	2(u)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	2(v)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	2(w)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
3	3(a)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	3(b)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain

Table 4.3: Continued.

	3(c)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	3(d)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
	3(e)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	3(f)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	3(g)	Gram negative	Large sized, mucoid and white pigment	Bacilli, rod-shaped, single arrangement
	3(h)	Gram negative	Large sized, mucoid and white pigment	Bacilli, rod-shaped, single arrangement
	3(i)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	3(j)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	3(k)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
4	4(a)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
	4(b)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	4(c)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	4(d)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	4(e)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain

Table 4.3: Continued.

	4(f)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	4(g)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	4(h)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	4(i)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
	4(j)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	4(k)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	4(l)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	4(m)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	4(n)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	4(o)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	4(p)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped
	4(q)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped
5	5(a)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
	5(b)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain

Table 4.3: Continued.

5(c)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
5(d)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
5(e)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
5(f)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
5(g)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
5(h)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
5(i)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped
5(j)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped
5(k)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
5(l)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
5(m)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
5(n)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
5(o)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
5(p)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped

Table 4.3: Continued.

	5(q)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped
	5(r)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped
	5(s)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	5(t)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	5(u)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	5(v)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped
	5(w)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	5(x)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped
	5(y)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	5(z)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	5(aa)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped
	5(ab)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
6	6(a)	Gram negative	Medium sized, raised colony, moist texture	Bacillus, rod-shaped,
	6(b)	Gram negative	Medium sized, raised colony, moist texture	Bacillus, rod-shaped,

Table 4.3: Continued.

6(c)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
6(d)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
6(e)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped
6(f)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
6(g)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
6(h)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
6(i)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
6(j)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
6(k)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
6(l)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
6(m)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
6(n)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
6(o)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
6(p)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain

Table 4.3: Continued.

	6(q)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
7	7(a)	Gram negative	Medium sized, raised colony, moist texture	Bacillus, rod-shaped,
	7(b)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped
	7(c)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
	7(d)	Gram negative	Large sized, mucoid and white pigment	Bacilli, rod-shaped, single arrangement
	7(e)	Gram negative	Raised and smooth white pigment	Rod-shaped, single arrangement
	7(f)	Gram negative	Raised and smooth white pigment	Rod-shaped, single arrangement
	7(g)	Gram negative	Raised and smooth white pigment	Rod-shaped, single arrangement
	7(h)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	7(i)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
	7(j)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
	7(k)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	7(l)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	7(m)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain

Table 4.3: Continued.

7(n)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
7(o)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
7(p)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
7(q)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
7(r)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
7(s)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
7(t)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
7(u)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
7(v)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
7(w)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
7(x)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
7(y)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
7(z)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
7(aa)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain

Table 4.3: Continued.

	7(ab)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	7(ac)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	7(ad)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	7(ae)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
8	8(a)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	8(b)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	8(c)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	8(d)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	8(e)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	8(f)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
	8(g)	Gram negative	Raised and smooth white pigment	Short rod-shaped, single arrangement
	8(h)	Gram negative	Raised and smooth white pigment	Rod-shaped, single arrangement
9	9(a)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
	9(b)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain

Table 4.3: Continued.

	9(c)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	9(d)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	9(e)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped
	9(f)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
	9(g)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	9(h)	Gram negative	Raised and smooth white pigment	Short rod-shaped, single arrangement
	9(i)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
	9(j)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	9(k)	Gram negative	Raised and smooth white pigment	Rod-shaped, single arrangement
10	10(a)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	10(b)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	10(c)	Gram positive	Circular shaped, small sized, white pigment	Cocci, rod-shaped, arranged in chain
	10(d)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain
	10(e)	Gram positive	Circular shaped, medium sized, white pigment	Cocci, rod-shaped, arranged in chain

Table 4.3: Continued.

10(f)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped
10(g)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped
10(h)	Gram negative	Circular shaped, small sized, shiny, and smooth	Coccobacilli, short rod-shaped
10(i)	Gram negative	Large sized, mucoid and white pigment	Bacilli, rod-shaped, single arrangement
10(j)	Gram negative	Raised and smooth white pigment	Rod-shaped, single arrangement
10(k)	Gram negative	Large sized, mucoid and white pigment	Bacilli, rod-shaped, single arrangement
10(l)	Gram negative	Raised and smooth white pigment	Short rod-shaped, single arrangement
10(m)	Gram negative	Raised and smooth white pigment	Rod-shaped, single arrangement
10(n)	Gram negative	Raised and smooth white pigment	Rod-shaped, single arrangement
10(o)	Gram negative	Circular shaped, medium sized, raised, and smooth	Coccobacilli, short rod-shaped

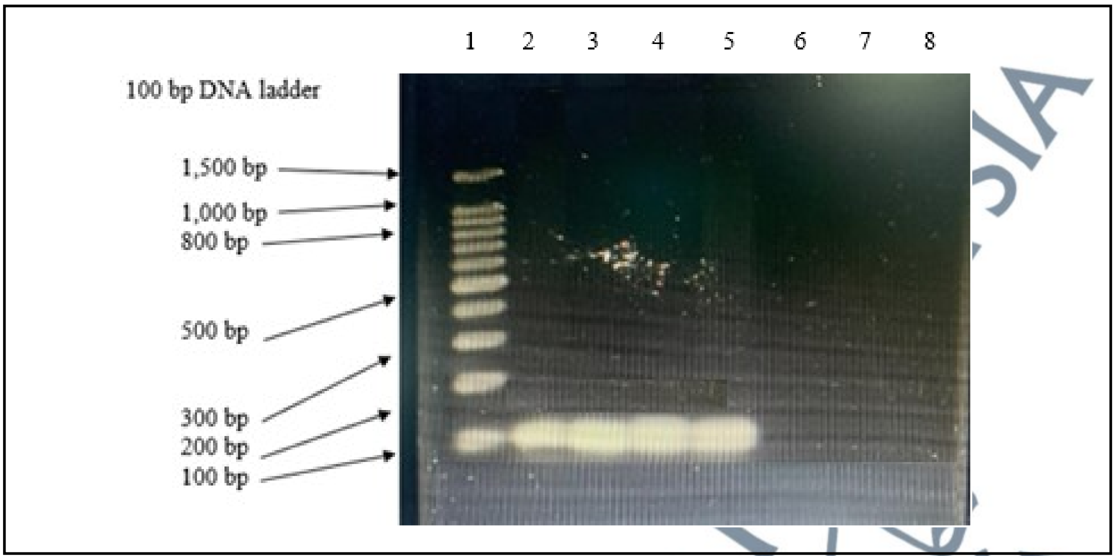

Figure 4.21: 1.5% agarose gel electrophoresis of PCR using PCR products after DNA purification obtained from sample 1, 2, 3 and 4 in 100 V for 60 minutes. Lane 1: 100 bp DNA ladder marker; Lane 2: Sample 1; Lane 3: Sample 2; Lane 4: Sample 3; Lane 5: Sample 4; Lane 6: No sample; Lane 7: No sample; Lane 8: No sample.

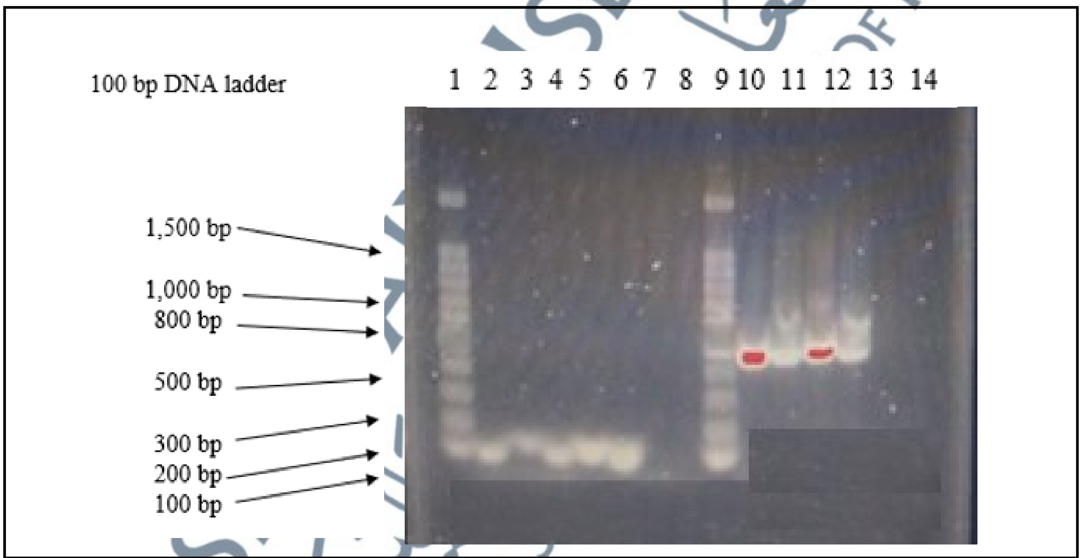

Figure 4.22: 1.5% agarose gel electrophoresis of PCR using PCR products after DNA purification obtained from sample 4, 6 and 7 in 100 V for 60 minutes. Lane 1: 100 bp DNA ladder marker; Lane 2: Sample 4a; Lane 3: Sample 4b; Lane 4: Sample 4c; Lane 5: Sample 7a; Lane 6: Sample 7b; Lane 7: No sample; Lane 8: Negative control; Lane 9: 100 bp DNA ladder; Lane 10: *F. nucleatum*; Lane 11: Sample 6a; Lane 12: Sample 6b; Lane 13: Sample 6c; Lane 14: Negative control.

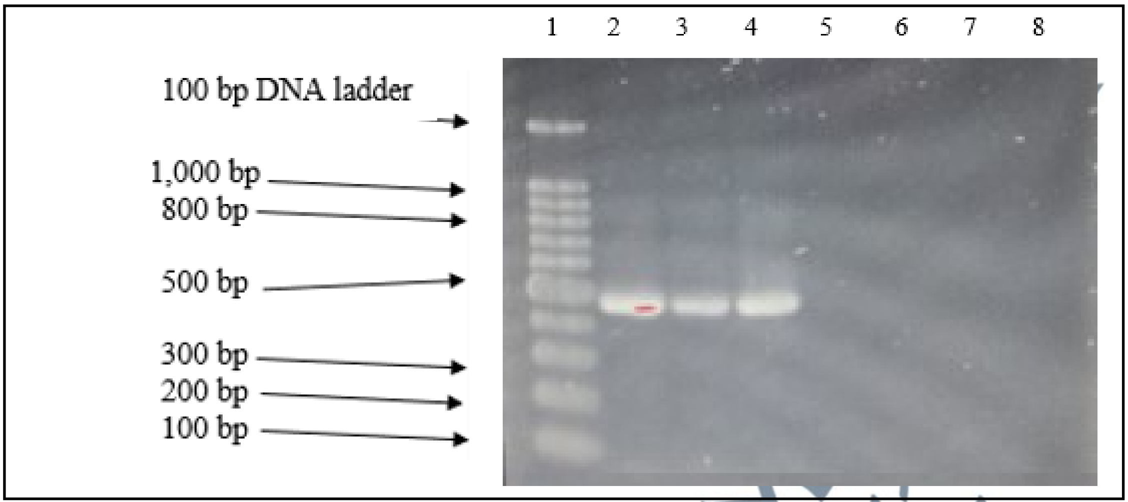

Figure 4.23: 1.5% agarose gel electrophoresis of PCR using PCR products after DNA purification obtained from sample 5 in 100 V for 60 minutes. Lane 1: 100 bp DNA ladder marker; Lane 2: *A. actinomycetemcomitans*; Lane 3: Positive control (*P. gingavalis*); Lane 4: Sample 5; Lane 5: No sample; Lane 6: No sample; Lane 7: No sample; Lane 8: No sample.

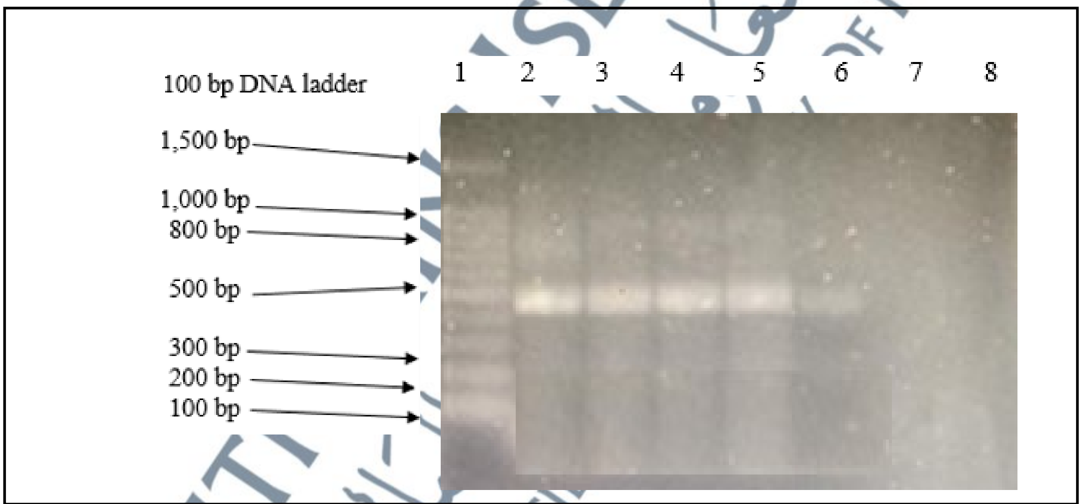

Figure 4.24: 1.5% agarose gel electrophoresis of PCR using PCR products after DNA purification obtained from sample 8 in 100 V for 60 minutes. Lane 1: 100 bp DNA ladder marker; Lane 2: Sample 8a; Lane 3: Sample 8b; Lane 4: Sample 8c; Lane 5: Sample 8d; Lane 6: Negative control.

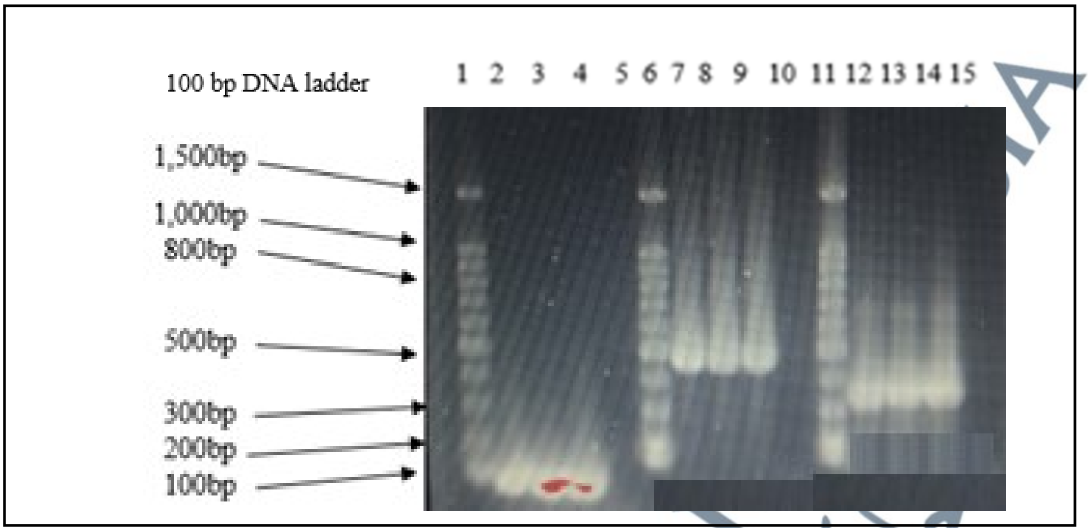

Figure 4.25: 1.5% agarose gel electrophoresis of PCR using PCR products after DNA purification obtained from sample 10 in 100 V for 60 minutes. Lane 1: 100 bp DNA ladder marker; Lane 2: Sample 10a (positive primer); Lane 3: Sample 10b (positive primer); Lane 4: Sample 10c (positive primer) ; Lane 5: Negative control; Lane 6: 100bp DNA ladder; Lane 7: Sample 10a (negative primer); Lane 8: Sample 10b (negative primer) ; Lane 9: Sample 10c (negative primer) ; Lane 10: Negative control ; Lane 11: 100bp DNA ladder ; Lane 12: Sample 10a (mix primer) ; Lane 13: Sample 10b (mix primer); Lane 14: Sample 10c (mix primer) Lane 15: Negative control.

Table 4.4: Oral bacteria species which are mostly found in the subgingival plaque samples.

Types of oral bacteria	Number of strains	Identification (%)
Gram-positive		
Streptococcus gordonii	6	100
Streptococcus mutans	31	100
Streptococcus sanguinis	8	100
Streptococcus oralis	23	100
Streptococcus agalactiae	5	97
Streptococcus downei	3	97
Streptococcus lutetiensis	5	91
Streptococcus salivarius	12	99
Streptococcus anginosus	4	99
Gram-negative		
Porphyromonas gingivalis	34	96
Aggregatibacter actinomycetemcomitans	20	95
Klebsiella pneumoniae	14	99
Enterobacter clocae	2	99
Enterobacter ludwigii	3	99
Enterobacter mori	3	99
Enterobacter tabacii	1	99
Enterobacter roggen kampii	2	99

```
Streptococcus mutans strain KCOM 1075 16S ribosomal RNA gene, partial sequence
Sequence ID: MT233343.1  Length: 1530  Number of Matches: 1

Range 1: 390 to 447  GenBank  Graphics            ▼ Next Match  ▲ Previous Match

Score            Expect   Identities     Gaps        Strand
108 bits(58)     1e-19    58/58(100%)    0/58(0%)    Plus/Plus

Query  15   AGTCTGACCGAGCACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTG  72
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  390  AGTCTGACCGAGCACGCCGCGTGAGTGAAGAAGGTTTTCGGATCGTAAAGCTCTGTTG  447
```

Figure 4.26: Alignments of the nucleotide sequence for sample 4 after the BLAST program in NCBI data for *S. mutans*.

```
Porphyromonas gingivalis ATCC 33277 16S ribosomal RNA, partial sequence
Sequence ID: NR_074234.1  Length: 1475  Number of Matches: 1

Range 1: 381 to 791  GenBank  Graphics            ▼ Next Match  ▲ Previous Match

Score            Expect   Identities       Gaps        Strand
671 bits(363)    0.0      397/413(96%)     4/413(0%)   Plus/Plus

Query  2    GGGCGAAGA-CCTGAA-CAGCCAAGTCGCGTGAAGGAAGACTGTCCTAAGGATTGTAAAC  59
            ||||| ||| |||||| ||||||||||||||||||||||||||||||||||||||||||
Sbjct  381  GGGCG-AGAGCCTGAACCAGCCAAGTCGCGTGAAGGAAGACTGTCCTAAGGATTGTAAAC  439

Query  60   TTCTTTTATACGGGAATAACGGGCGATACGAGTATTGCATTGAATGTACCGTAAGAATAA  119
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  440  TTCTTTTATACGGGAATAACGGGCGATACGAGTATTGCATTGAATGTACCGTAAGAATAA  499

Query  120  GCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCGAGCGTTATCCGGA  179
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  500  GCATCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCGAGCGTTATCCGGA  559

Query  180  TTTATTGGGTTTCAAGGGTGCGTACGTTGTTCGGTAAATCAGCGGTGAAACCTGAGCGCT  239
            |||||||||||| ||||||||||| |||||||||||| ||||||||||||||||||||||
Sbjct  560  TTTATTGGGTTTAAAGGGTGCGTAGGTTGTTCGGTAAGTCAGCGGTGAAACCTGAGCGCT  619

Query  240  CAACGTTCTGCCTGCCGTTGAAACTGCCGGCTTGATTTCAGCGGCGGCAGGCGGAATTC  299
            |||||||| |||||||||||||||||||||||||| ||||||||||||||||||||||||
Sbjct  620  CAACGTTCAGCCTGCCGTTGAAACTGCCGGCTTGAGTTCAGCGGCGGCAGGCGGAATTC  679

Query  300  TCGGTGTAGCGGTGATATGCATATATATCACGAGGAACTCCGATTGCGAAGGCAGCTTGC  359
              ||||||||||||| ||||||| ||||||||||||||||||||||||||||||||||||
Sbjct  680  GTGGTGTAGCGGTGAAATGCATAGATATCACGAGGAACTCCGATTGCGAAGGCAGCTTGC  739

Query  360  CATACTGTGACTGACACTGAAGCACGAACGCGTGGGTATCAAACACTGATTAG  412
            ||||||| |||||||||||||||||||| ||||||||||||||||  ||||||
Sbjct  740  CATACTGCGACTGACACTGAAGCACGAAGGCGTGGGTATCAAACAG-GATTAG  791
```

Figure 4.27: Alignments of the nucleotide sequence for sample 5 after the BLAST program in NCBI data for *P. gingivalis*.

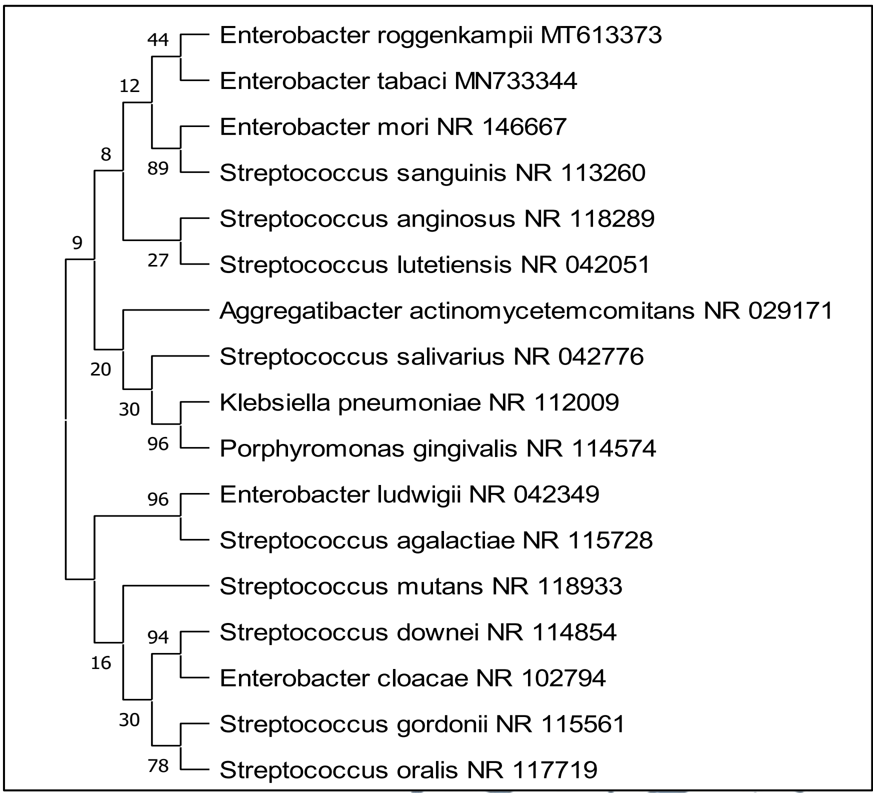

Figure 4.28: Phylogenetic tree of oral bacteria species from subgingival plaque.

4.5 Antibacterial Activities of *Salvadora persica* Stem Extracts (SPSE) Against Selected Oral Bacteria from Subgingival Plaque

4.5.1 Extraction of *Salvadora persica*

The yield of SPSE from five types of solvent were shown in Table 4.5. SPSE-methanol obtained was 1.362 g which is 26.37% of the original amount of powdered *S. persica*. The weight of the SPSE was obtained by dividing the weight of SPSE by the total weight of powdered *S. persica* to get the percentage of SPSE.

Table 4.5: Total yield of five types of different types of SPSE.

SPSE	*S. persica*	
	Yield (g)	Percentage (%)
SPSE-*n*-hexane	1.276	24.70
SPSE-DCM	0.871	16.86
SPSE-acetone	0.708	13.71
SPSE-ethanol	0.948	18.35
SPSE-methanol	1.362	26.37

4.5.2 Selected Oral Bacteria

Two types of oral bacteria were chosen from bacteria isolation as representatives for antibacterial activity, including *S. mutans* (Gram-positive) and *P. gingivalis* (Gram-negative).

4.5.2.1 Disc Diffusion Assay (DDA)

The antibacterial activity of SPSE-*n*-hexane, SPSE-DCM, SPSE-acetone, SPSE-ethanol, and SPSE-methanol against *S. mutans* and *P. gingivalis* are listed in Table 4.6 and Table 4.7 respectively. Appendix 2 includes the plate of inhibition zone of *S. mutans* and *P. gingivalis* when exposed to SPSE. The statistical test used in this study for DDA analysis was One-Way ANOVA because there were more than two groups for the independent and dependent variables. The collected data were analysed in SPSS Statistic software. From the DDA results, there is a significant difference between the types of solvents in SPSE and the diameter of the inhibition zone for both *S. mutans* and *P. gingivalis* ($p < 0.001$).

Table 4.6: Inhibition zone of DDA for SPSE against *S. mutans.*

SPSE	Mean ± s.d. inhibition zone
SPSE-*n*-hexane	7.96 ± 0.78
SPSE-DCM	7.63 ± 0.36
SPSE-acetone*	9.04 ± 0.23
SPSE-ethanol	7.56 ± 0.42
SPSE-methanol	7.07 ± 0.19
Chlorhexidine (Control)	16.26 ± 1.10
Dimethyl sulfoxide (Control)	6.00 ± 0.00

Value represented as mean ± s.d.* shows significant difference from negative control (p<0.001)

Table 4.7: Inhibition zone of DDA for SPSE against *P. gingivalis*.

SPSE	Mean ± s.d. inhibition zone
SPSE-*n*-hexane	8.26 ± 0.65
SPSE-DCM	8.74 ± 0.10
SPSE-acetone*	9.85 ± 0.34
SPSE-ethanol	7.15 ± 0.14
SPSE-methanol	6.82 ± 0.21
Chlorohexidine (Control)	15.85 ± 0.13
Dimethyl sulfoxide (Control)	6.00 ± 0.00

Value represented as mean ± s.d.* shows significant difference from negative control (p<0.001)

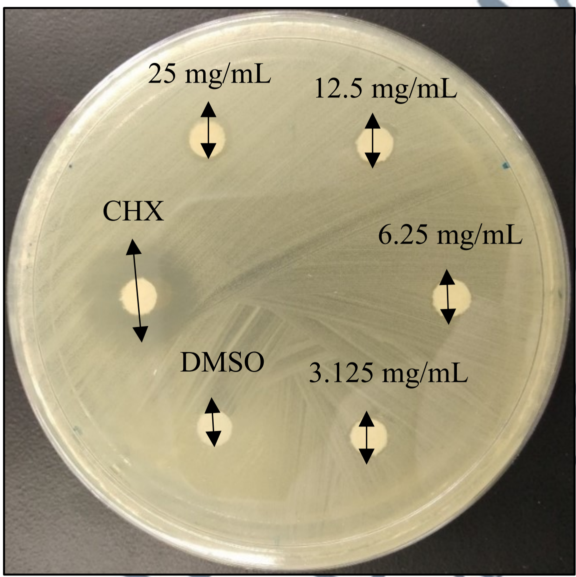

Figure 4.29: Inhibition zone of *S. mutans* when exposed to different concentrations of SPSE-methanol after 24 hours.

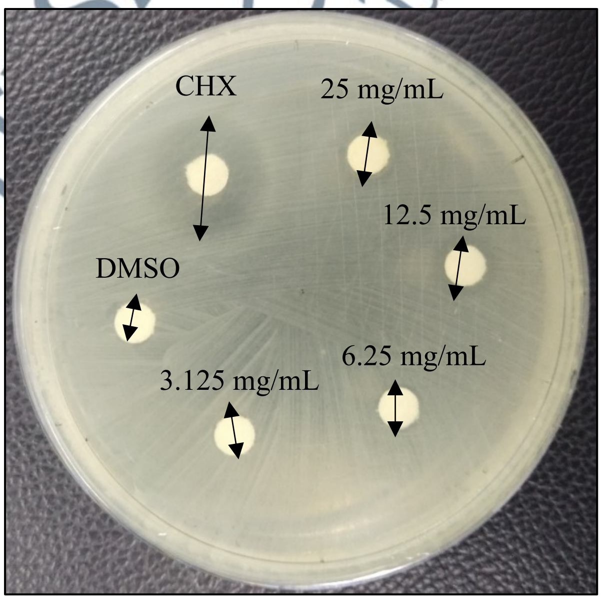

Figure 4.30: Inhibition zone of *P. gingivalis* when exposed to different concentrations of SPSE-methanol after 24 hours.

4.5.2.2 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Figure 4 .31 shows the 96-well plate containing *S. mutans* treated with *S. persica* extracts for MIC. SPSE against *S. mutans* for MIC was included in Appendix 3. Table 4.8 shows the tabulated data for MIC and MBC of SPSE against *S. mutans* and *P. gingivalis*. SPSE-*n*-hexane has the lowest concentration of MIC to inhibit the growth of *S. mutans* at 3.13 mg/mL. Thus, the MBC concentration of SPSE-*n*-hexane is as lowest as 6.25 mg/mL to kill *S. mutans*. In contrast, SPSE-*n*-hexane and SPSE-DCM inhibited *P. gingivalis* with 0.78 mg/mL, respectively, but the MBC value for both extracts are 0.78 mg/mL and 1.56 mg/mL, respectively. Figure 4.32 includes the growth of *S. mutans* on BHI agar, whereas Figure 4.33 shows the colonies of *P. gingivalis* colony on WCA agar for MBC. Appendix 4 includes the plate of bacteria colonies on BHI and WCA agar for the MBC method.

Table 4.8: MIC and MBC of SPSE against *S. mutans* and *P. gingivalis*.

SPSE	*S. mutans*		*P. gingivalis*	
	MIC (mg/mL)	MBC (mg/mL)	MIC (mg/mL)	MBC (mg/mL)
SPSE-*n*-hexane*	3.13	6.25	0.78	0.78
SPSE-DCM	12.50	12.50	0.78	1.56
SPSE-acetone	25.00	25.00	1.56	1.56
SPSE-ethanol	25.00	25.00	1.56	1.56
SPSE-methanol	25.00	25.00	1.56	1.56
Chlorhexidine	1.56	3.13	1.56	3.13
Dimethyl sulfoxide	0.00	0.00	0.00	0.00

*Indicate the most effective SPSE against *S. mutans* and *P. gingivalis*.

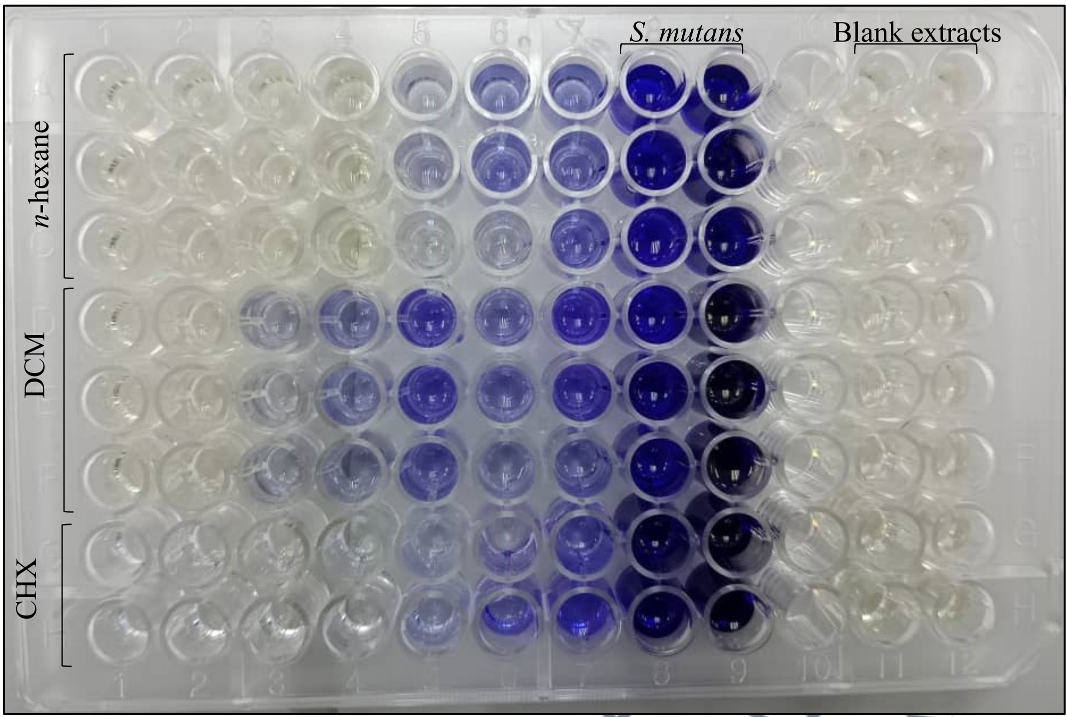

Figure 4.31: The colour of MTT was change after *S. mutans* treated with SPSE (MIC).

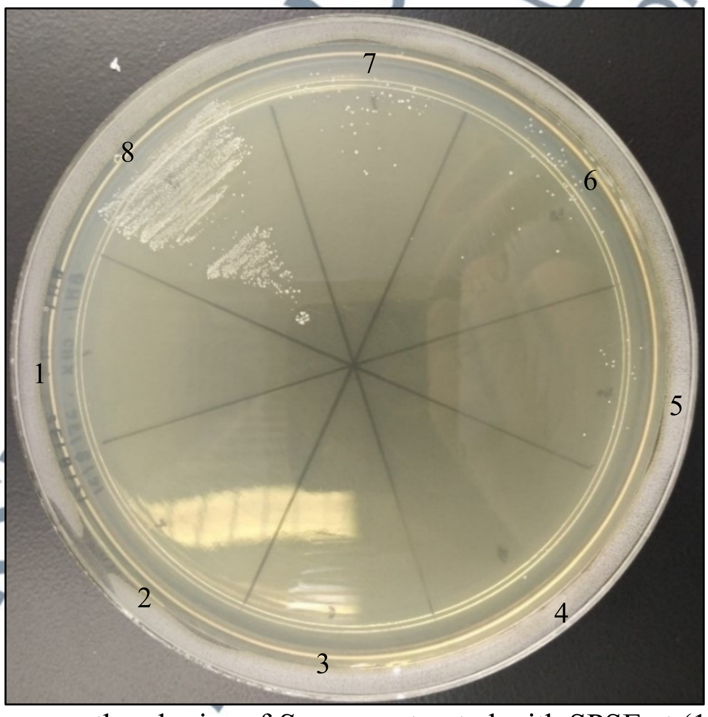

Figure 4.32: The growth colonies of *S. mutans* treated with SPSE at (1) 25.00 mg/mL; (2) 12.50 mg/mL; (3) 6.25 mg/mL; (4) 3.13 mg/mL; (5) 1.56 mg/mL; (6) 0.78 mg/mL; (7) 0.39 mg/mL; (8) 0.20 mg/mL on BHIA agar (MBC).

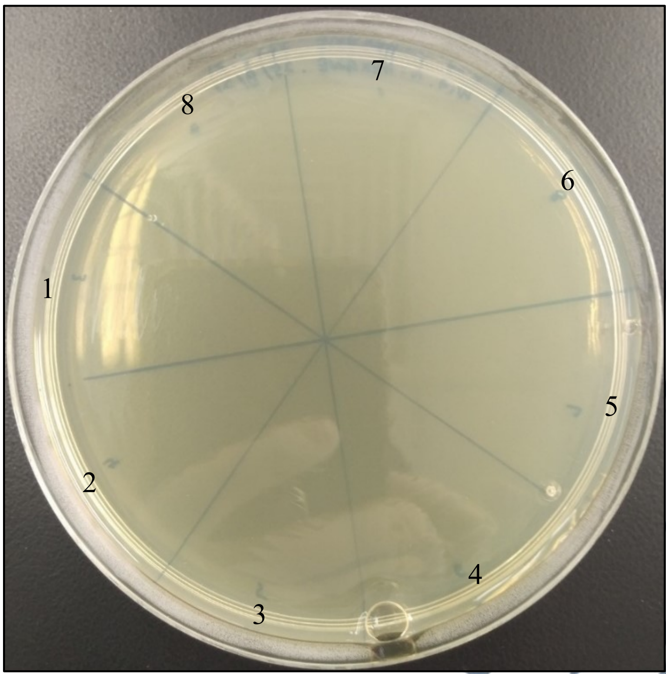

Figure 4.33: The growth colonies of *P. gingivalis* treated with SPSE at (1) 25.00 mg/mL; (2) 12.50 mg/mL; (3) 6.25 mg/mL; (4) 3.13 mg/mL; (5) 1.56 mg/mL; (6) 0.78 mg/mL; (7) 0.39 mg/mL; (8) 0.20 mg/mL on WCA agar (MBC).

4.6 Gas Chromatography Mass Spectrometry (GCMS)

Phytochemical analysis of SPSE from five different types of solvents (*n*-hexane, DCM, acetone, ethanol, and methanol) was performed using GCMS analysis. The identified chemical constituents of SPSE are showing a chromatogram as shown in Figure 4.34 to Figure 4.38 according to their order of elution through GCMS column and time peak for SPSE-*n*-hexane, SPSE-DCM, SPSE-acetone, SPSE-ethanol, and SPSE-methanol. The purpose of this analysis was to identify the active compounds iSPSE which can inhibit oral bacteria activity.

Tabulated GCMS data from (Table 4.9) represented the comparison between the analysis of SPSE-*n*-hexane, SPSE-DCM, SPSE-acetone, SPSE-ethanol, and SPSE-methanol. The main compounds selected were based on the percentage of abundance. Results from the analysis of GCMS data also found that the SPSE-*n*-hexane, SPSE-

DCM, SPSE-acetone, SPSE-ethanol, and SPSE-methanol showed the presence of major components of hexadecanoic acid and octadecanoic acid respectively.

Hexadecanoic and octadecanoic acids are from the group of fatty acid derivatives. Hexadecanoic acid also known as palmitic acid, is the most common saturated fatty acid found in plants. Meanwhile, another name for octadecanoic acid is stearic acid, which exists as glycerol in plants. Benzoic acid was the first compound found in SPSE-DCM, SPSE-acetone, SPSE-ethanol, and SPSE-methanol when the retention time started.

Seven main compounds found in SPSE-*n*-hexane were hexadecanoic acid, isopropyl palmitate, vaccenic acid, octadecanoic acid, oleic acid, isopropyl linoleate, and octadecenoate. Meanwhile, SPSE-DCM indicated the presence of nine major compounds number, whereas the main compounds found in SPSE-acetone were benzoic acid, dimethylethyl, hexadecanoic acid, benzylbenzamide, vaccenic acid, octadecanoic acid, and oleic acid. Six main compounds present in SPSE-ethanol included benzoic acid, dimethylethyl, hexadecenoic acid, tridecanoic acid, vaccenic acid, and octadecanoic acid. There are six main compounds found in SPSE-methanol.

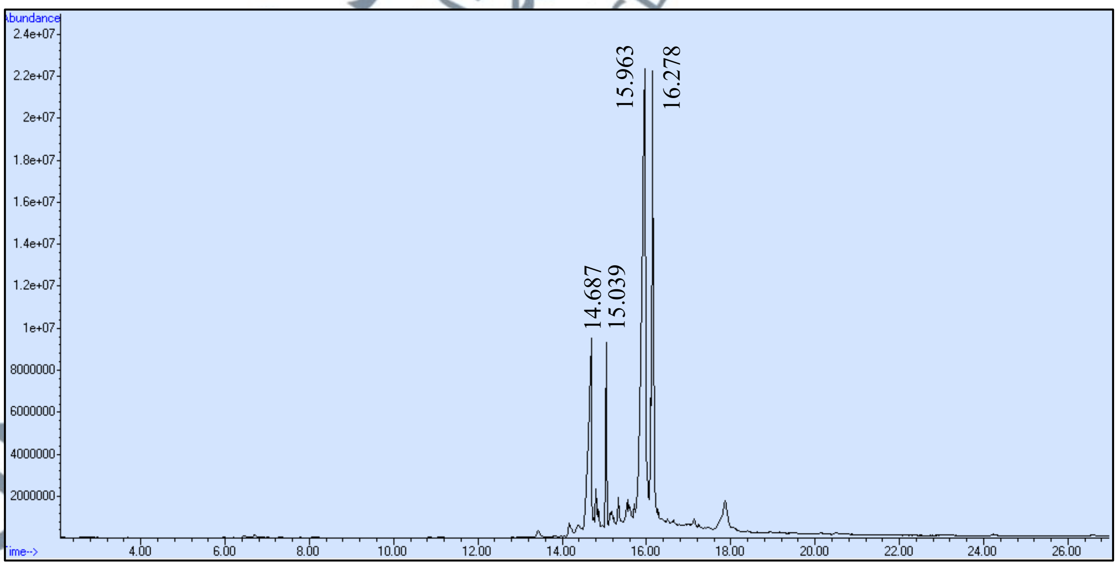

Figure 4.34: Chromatogram for SPSE-*n*-hexane from GCMS analysis.

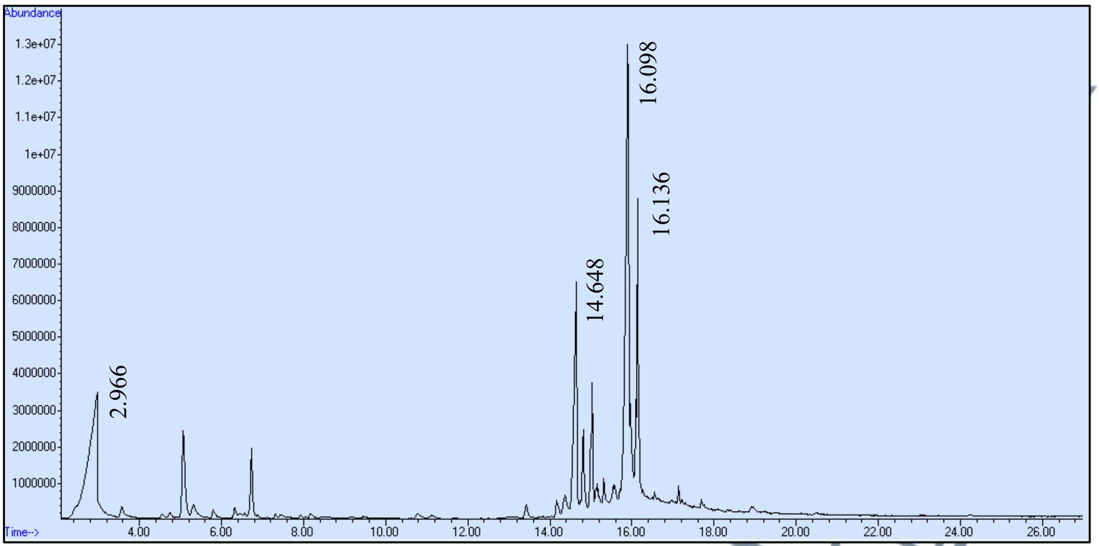

Figure 4.35: Chromatogram for SPSE-DCM from GCMS analysis.

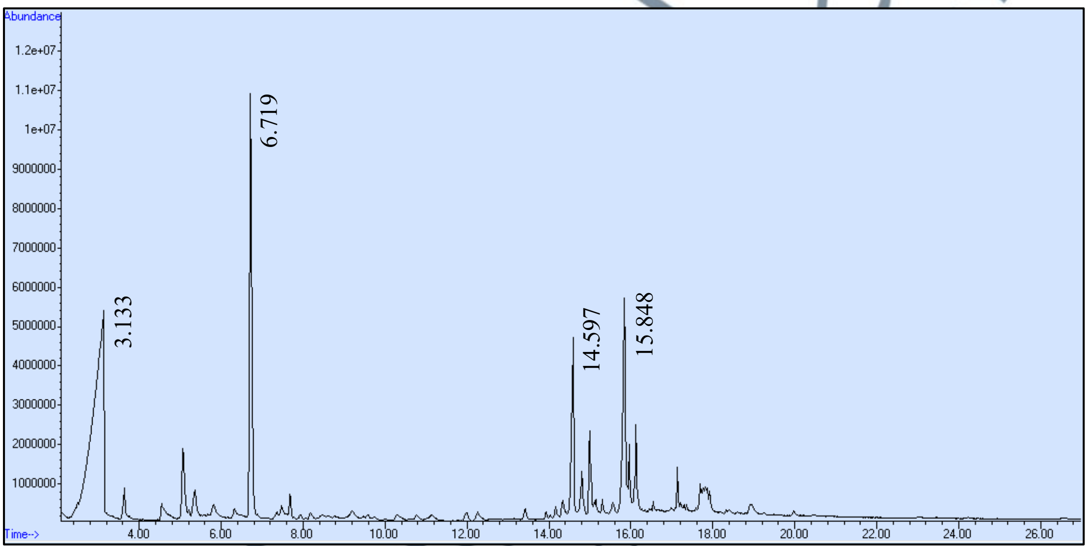

Figure 4.36: Chromatogram for SPSE-acetone from GCMS analysis.

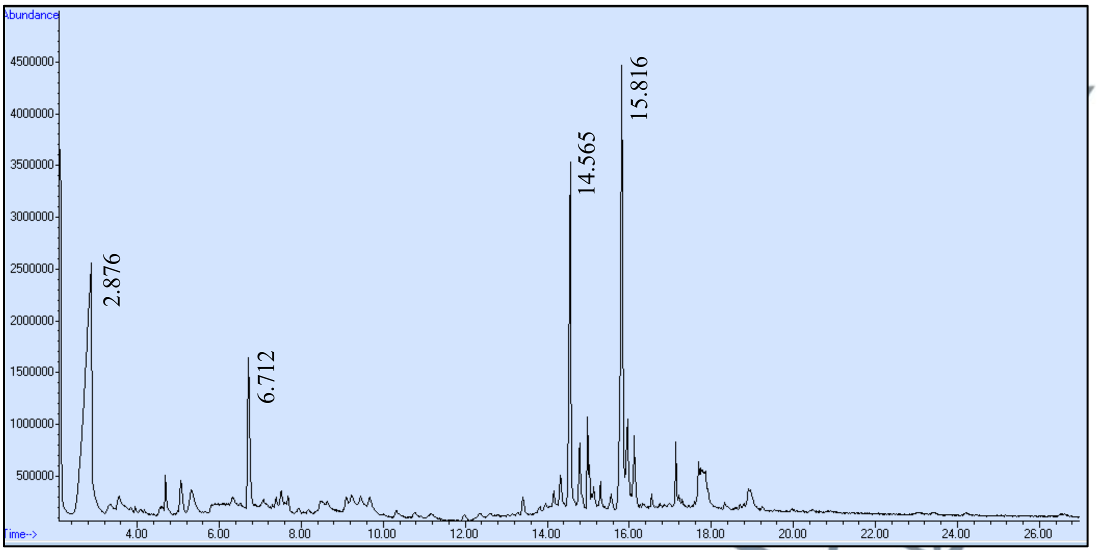

Figure 4.37: Chromatogram for SPSE-ethanol from GCMS analysis.

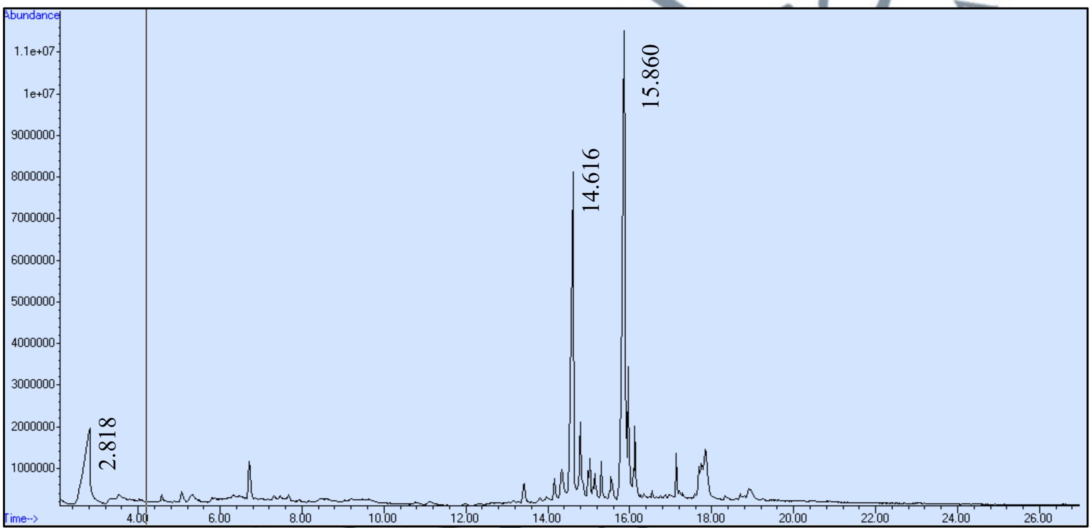

Figure 4.38: Chromatogram for SPSE-methanol from GCMS analysis.

Table 4.9: Phytochemical compounds found in SPSE using GCMS analysis.

SPSE	Compounds	RT (min)	Area percentage (%)	Molecular formula	Molecular weight (g/mol)
n-hexane	*n*-hexadecanoic acid*	14.687	11.72	$C_{16}H_{32}O_2$	256.42
	Isopropyl palmitate	15.039	3.94	$C_{19}H_{38}O_2$	298.5
	n-hexadecanoic acid			$C_{16}H_{32}O_2$	256.42
	cis-Vaccenic acid	15.963	34.42	$C_{18}H_{34}O_2$	282.5
	trans-13-Octadecenoic acid*			$C_{16}H_{32}O_2$	256.42
	Oleic Acid			$C_{16}H_{32}O_2$	256.42
	Isopropyl linoleate	16.104	3.53	$C_{21}H_{38}O_2$	322.5
	9,12-Octadecadienoic acid (Z,Z)-*			$C_{18}H_{32}O_2$	280.4
	n-Propyl 11-octadecenoate	16.149	9.75	$C_{21}H_{40}O_2$	324.5
	i-Propyl 9-octadecenoate			$C_{21}H_{40}O_2$	324.5
	cis-13-Octadecenoic acid*	16.175	3.83	$C_{18}H_{34}O_2$	282.5
	cis-Vaccenic acid			$C_{18}H_{34}O_2$	282.5
	Oleic Acid			$C_{18}H_{34}O_2$	282.5

Table 4.9: Continued.

	trans-13-Octadecenoic acid*			$C_{18}H_{34}O_2$	282.5
	cis-Vaccenic acid	16.278	2.45	$C_{18}H_{34}O_2$	282.5
	Oleic Acid			$C_{18}H_{34}O_2$	282.5
DCM	Benzoic acid*	2.966	17.71	$C_7H_6O_2$	122.12
	n-hexadecanoic acid*	14.648	10.09	$C_{16}H_{32}O_2$	256.42
	N-benzylbenzamide	14.994	1.71	$C_{14}H_{13}NO$	211.26
	Isopropyl palmitate	15.033	2.69	$C_{19}H_{38}O_2$	298.5
	n-hexadecanoic acid*			$C_{16}H_{32}O_2$	256.42
	cis-13-Octadecenoic acid*			$C_{18}H_{34}O_2$	282.5
	cis-Vaccenic acid	15.899	22.86	$C_{18}H_{34}O_2$	282.5
	trans-13-Octadecenoic acid*			$C_{18}H_{34}O_2$	282.5
	Cyclododecyne	15.963	3.83	$C_{12}H_{20}$	164.29
	Isopropyl linoleate	16.098	2.23	$C_{21}H_{38}O_2$	322.5
	9,12-Octadecadienoic acid (Z,Z)-*			$C_{18}H_{34}O_2$	282.5

Table 4.9: Continued.

	cis-13-Octadecenoic acid*			$C_{18}H_{34}O_2$	282.5
	n-propyl 11-octadecenoate	16.136	5.09	$C_{21}H_{40}O_2$	324.5
	cis-Vaccenic acid			$C_{18}H_{34}O_2$	282.5
Acetone	Benzoic acid*	3.133	29.96	$C_7H_6O_2$	122.12
	Phenol, 2,4-bis(1,1-dimethylethyl)	6.719	13.54	$C_{22}H_{30}O_3S$	374.5
	n-hexadecanoic acid*	14.597	6.03	$C_{16}H_{32}O_2$	256.42
	N-benzylbenzamide	14.988	1.87	$C_{14}H_{13}NO$	211.26
	cis-Vaccenic acid			$C_{18}H_{34}O_2$	282.5
	cis-13-Octadecenoic acid*	15.848	8.35	$C_{18}H_{34}O_2$	282.5
	trans-13-Octadecenoic acid*			$C_{18}H_{34}O_2$	282.5
	Cyclopropaneoctanal, 2-octyl-			$C_{19}H_{36}O$	280.5
	trans-13-Octadecenoic acid*	16.130	2.94	$C_{18}H_{34}O_2$	282.5
	Oleic Acid			$C_{18}H_{34}O_2$	282.5
Ethanol	Benzoic acid*	2.876	22.28	$C_7H_6O_2$	122.12

Table 4.9: Continued.

	Phenol, 2,4-bis(1,1-dimethylethyl)	6.712	5.08	$C_{22}H_{30}O_3S$	374.5
	n-hexadecanoic acid*	14.565	8.80	$C_{16}H_{32}O_2$	256.42
	Tridecanoic acid			$C_{13}H_{26}O_2$	214.34
	cis-Vaccenic acid	15.816	12.67	$C_{18}H_{34}O_2$	282.5
	cis-13-Octadecenoic acid*			$C_{18}H_{34}O_2$	282.5
	trans-13-Octadecenoic acid*			$C_{18}H_{34}O_2$	282.5
Methanol	Benzoic acid*	2.818	9.43	$C_7H_6O_2$	122.12
	n-hexadecanoic acid*	14.616	15.68	$C_{16}H_{32}O_2$	256.42
	Tetradecanoic acid			$C_{14}H_{28}O_2$	228.37
	Tridecanoic acid			$C_{13}H_{26}O_2$	214.34
	cis-13-Octadecenoic acid*	15.860	25.14	$C_{18}H_{34}O_2$	282.5
	cis-Vaccenic acid			$C_{18}H_{34}O_2$	282.5
	9-Octadecenoic acid, (E)-*	16.868	0.34	$C_{18}H_{34}O_2$	282.5

Compounds* shows the major phytochemical of all SPSE