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Effect of khat chewing on periodontal pathogens in subgingival biofilm from chronic periodontitis patients

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ABSTRACT

Aims: Existing in vitro and in vivo data suggest that khat may have a favorable effect on periodontal microbiota. The purpose of this study was to assess the effect of khat chewing on major periodontal pathogens in subgingival plaque samples from subjects with chronic periodontitis.

Materials and methods: 40 subgingival plaque samples were obtained from periodontitis and healthy sites of 10 khat chewers (40 y median age) and 10 khat non-chewers (37.5 y median age) with chronic periodontitis. Absolute and relative counts of 6 periodontal pathogens were determined in each sample using highly sensitive and specific Taqman real-time PCR assays. Data were analyzed using an ordinal regression model.

Results: Significantly more total bacteria were detected in samples from the periodontitis sites of the khat chewers (OR = 20). *Treponema denticola* was present at significantly higher absolute counts at the healthy as well as periodontitis sites of the khat chewers (OR = 3.13 and 13, respectively). However, the khat chewers harbored significantly lower absolute counts of *Porphyromonas gingivalis* at the healthy sites (OR = 0.07). Furthermore, khat chewing was significantly associated with lower relative counts of *Porphyromonas gingivalis*, fusobacterium ssp., prevotella ssp. and *Parvimonas micra*-like species in subgingival plaque samples from both healthy and periodontitis sites (OR = 0.11–0.33). Only *Treponema denticola* was found in higher relative counts at the healthy sites of the khat chewers (OR = 2.98).

Conclusions: Overall, there was a lower burden of pathogens in the khat chewers. Findings from the current study are suggestive of a potential prebiotic effect for khat on periodontal microbiota.

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1. Introduction

Khat, qat or miraa are common names for *Catha edulis*, an evergreen plant of family Celastraceae that endemically grows in South-West Arabia and East Africa, where millions of local people habitually chew its fresh leaves and twigs for their stimulating, amphetamine-like effects. Khat has legally or illegally found its way to many Western countries where practicing the habit by immigrants is becoming an increasing phenomenon (Al-hebshi and Skaug, 2005b). Cathinone, the so-called natural amphetamine, and to a lesser extent, cathine are the psychoactive components of khat (Kalix, 1996). Various other compounds are found in khat including a complex group of at least 62 alkaloids, carbohydrates, tannins, flavonoids, terpenoids, sterols, glycosides, vitamins (particularly vitamin C), non-toxic metals, and amino acids (Al-hebshi and Skaug, 2005b). Khat has complex pharmacological effects centrally and peripherally; detailed information on these can be found elsewhere (Kalix and Braenden, 1985; Graziani et al., 2008).

The literature is full of reports linking khat chewing to so many adverse health effects (Halbach, 1972; Al-Habori, 2005; Hassan et al., 2007). However, many of these reports are anecdotal or, at the best, based on small-scale, cross-sectional studies that frequently have questionable research design and fail to control for bias and confounders; these concerns are fortunately raised by some critical reviewers (Kennedy, 1987a; Al-hebshi and Skaug, 2005b; Fitzgerald, 2009). In other words, while khat chewing is likely to have some adverse health effects, the level of existing evidence is generally inadequate for making incautious conclusions.

Abbreviations: OR, odds ratio; PCR, polymerase chain reaction; PD, pocket depth; PI, plaque index.

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What seem to be ignored in the literature, on the other hand, are the potential positive effects or medicinal aspects of khat. In fact, khat was first mentioned in the 11th century as a medicinal plant in Turkistan and Afghanistan (Kennedy, 1987b). In Ethiopia, the processed leaves and roots of the plant are used in the treatment of various chest problems (Lemessa, 2001). Recently, khat has been shown to lower serum glucose, triglycerides and cholesterol levels (Al-Habori and Al-Mamary, 2004), to possibly booster sperm activity (Adeoya-Osiguwa and Fraser, 2005), and to induce apoptosis in leukemia and prostate cancer cell lines (Elhag et al., 1999; Dimba et al., 2003).

Microbiologically, isolated compounds from khat were found to possess more potent anti-mycobacterial activity than streptomycin (Elhag et al., 1999). Recently, the effect of khat on oral bacteria has been assessed in a series of studies. In vitro, crude khat extracts were shown to interfere with biofilm formation by *Streptococcus mutans*, suggesting anticariogenic properties (Al-hebshi et al., 2005). In another study the extracts showed selective anti-microbial properties against major periodontal pathogens (Al-hebshi et al., 2006), and was found to foster growth of some health-compatible species (Al-hebshi, 2005). Among young healthy adults, khat chewing was found to produce bacterial shifts that are compatible with periodontal health (Al-hebshi and Skaug, 2005a). The current study takes this further by exploring effects of khat chewing on periodontal bacteria in subjects with chronic periodontitis. Specifically, the objective was to assess and compare levels of members of the pathogenic red and orange complexes described by Socransky et al. (1998) in subgingival biofilm samples from khat chewers and non-chewers with chronic periodontitis.

2. Materials and methods

2.1. Study subjects

Ten khat chewers and 10 khat non-chewers with chronic periodontitis (having at least 1 site per quadrant with pocket depth ≥5), were recruited from among patients attending dental clinics at Al-thawra hospital, Sana'a, Yemen. A history of khat chewing for 5 or more years at a frequency of at least 2 days per week was used to define a khat chewer. Exclusion criteria included smoking, periodontal treatment or antibiotic/oral antiseptic use in the previous 6 months, pregnancy/breast feeding, and any systemic disease or medication intake known to modify periodontal inflammation.

Subjects were screened for periodontal status using the community periodontal index (CPI; World Health Organization, 1997) by a single, well-trained and precalibrated examiner (Shuga-aldin HM). In eligible subjects, a Williams probe was used to establish pocket depth (PD) for the deepest pocket in each quadrant in millimeters. The plaque index (PI; Silness and Loee, 1964), measured on the labial/buccal and lingual/palatal surfaces of index teeth, was used to assess oral hygiene. Informed consent was obtained from all subjects.

2.2. Subgingival sampling – DNA extraction

Four subgingival plaque samples, one from the deepest pocket in each quadrant, were obtained from each subject by placing a sterile, size-40 paper point in each pocket for 20 s after removal of supragingival plaque. The four samples were then pooled in a tube containing 500 μl low EDTA TE buffer (Invitrogen, USA). A pooled sample from 4 healthy sites (PD ≤ 3 mm) in each subject was also obtained. The samples (40 in total) were stored at −80 °C until processing.

In preparation for DNA extraction, samples were pelleted by centrifugation at 13,000 rpm for 1 min in a Spectrafuge® 16 M (Labnet international, USA), resuspended in 180 μl lysozyme digestion buffer (25 mM Tris–HCl, pH 8.0, 2.5 mM EDTA, 1% Triton X-100) containing 20 mg/ml lysozyme, and incubated at 37 °C overnight. DNA was then extracted using the Purelink Genomic DNA extraction kit (Invitrogen, USA) following manufactures' instructions; DNA was eluted in a volume of 100 μl and stored at 4 °C for subsequent analysis.

2.3. Quantitative PCR assays

Detection and quantification of total bacteria, fusobacterium spp., prevotella spp., *Parvimonas micra* (previously *Micromonas micra* or *peptostreptococcus micros*), *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* in the DNA samples was performed using the Taqman real-time PCR technology (Holland et al., 1991). Sequences of probes and primers used in the study are shown in Table 1. They were obtained as pre-validated, optimized and ready to use kits from Primerdesign, UK. In addition to the

Table 1
Sequences of primers and probes used in the quantitative PCR assays.

Test species	Sequences 5′–3′	Target gene	Product size	Ref.
Total bacteria	F-primer: AAACTCAAAGGAATTGACGGGG R-primer: TTGCGCTCGTTGCGGGACT Probe: FAM-CTGTCGTCAGCTCGTGTCGTGA-BHQ	16S rRNA	205 bp	This study[a]
Fusobacterium spp.	F-primer: CGCAGAAGGTGAAAGTCCTGTAT R-primer: TGGTCCTCACTGATTCACACAGA Probe: FAM-CTTTGCTCCCAAGTAACATG GAACACGA-BHQ	23S r RNA	101 bp	Suzuki et al. (2004)
Prevotella spp.	F-primer: ACCAGCCAAGTAGCGTGCA R-primer: TGGACCTTCCGTATTACCGC Probe: FAM-AATAAGGACCGGCTAATTCC GTGCCAG-BHQ	16S rRNA	153 bp	Martin et al. (2002)
Parvimonas micra	F-primer: TGAGCAACCTACCTTACACAG R-primer: GCCCTTCTTACACCGATAAATC Probe: FAM-ACCGCATGAGACCACAGAA TCGCA-BHQ	16S rRNA	112 bp	This study[a]
Porphyromonas gingivalis	F-primer: ACGAATCAAAGGTGGCTAAGTT R-primer: TTAGTCGCATTTTCGGCTGAT Probe: FAM-CCTGCTGTTCTCCATTATAAAC CATTACGG-BHQ	fimA	85 bp	This study[a]
Tannerella forsythia	F-primer: GATAGGCTTAACACATGCAAGTC R-primer: GTTGCGGGCAGGTTACATAC Probe: FAM-TTACTCACCCGTGCGCCGGTCG-BHQ	16S rRNA	99 bp	This study[a]
Treponema denticola	F-primer: GGGCGGCTTGAAATAATRATG R-primer: CTCCCTTACCGTTCGACTTG Probe: FAM-CAGCGTTCGTTCTGAGCCA GGATCA-BHQ	16S rRNA	92 bp	This study[a]

[a] Primers and probes were designed as a commercial service by Primerdesign, UK.

Table 2
Clinical characteristics of the study groups.

	Khat non-chewers ($n=10$)	Khat chewers ($n=10$)	P
Gender (M/F %)	60/40	90/10	0.03*
Age, median (interquartile range)	37.5 (30.0–45.0)	40.0 (32.0–45.0)	NS
Plaque index, median (interquartile range)	1.55 (1.18–2.08)	1.48 (1.31–1.53)	NS
Pocket depth at sampled sites, median (interquartile range)	6.38 (5.38–7.88)	5.50 (5.00–6.75)	NS

* Statistically significant; chi square. NS, not significant; Mann–Whitney test.

primer/probe mix, each kit included a plasmid-based quantification standard, which was used in a 10-fold serial dilution to construct standard curves for absolute quantification of the test species, and to confirm efficiency, linearity and sensitivity of the assays.

Specificity of primers was initially confirmed by blasting them against eubacterial sequence databases at the National Center for Biotechnology Information (NCBI; http://www.ncbi.nlm.nih.gov/tools/primer-blast/index.cgi?LINK_LOC=BlastHome). To further confirm specificity, and rather than testing the primer sets against a limited number of bacterial strains, each set was tested against a pooled subgingival DNA sample representing 5 periodontitis patients using a SYBR Green real-time PCR assay followed by disassociation curve analysis. A single disassociation peak that is identical to the positive standard peak was used as a proof of specificity of a primer set.

Quantitative PCR assays were performed in 20 μl reactions, consisting of 10 μl mastermix with ROX (Primerdesign, UK), 1 μl primers/probe mix, 5 μl template DNA (or positive standard), and 4 μl PCR-grade water, on an ABI 7000 real-time PCR system (Applied Biosystems, USA) using the following cycling program: an initial enzyme activation step at 95 °C for 10 min, and 40 cycles of a denaturation step at 95 °C for 15 s, and an annealing/extension step at 60 °C for 1 min. Data was acquired through the FAM channel.

Threshold cycle values obtained were converted into absolute counts in copies per reaction using the standard curves; values were then converted into copies per sample by multiplying by a factor of 20 (since 5 μl of the eluted DNA was included in the reaction). Relative counts (as % total bacteria) were simply calculated by dividing the absolute counts of the test species by that of total bacteria in the same sample and multiplying by 100.

2.4. Statistical analysis

Data available for each subject were gender, age, mean plaque index, mean pocket depth, log-transformed absolute counts and relative counts of the study species. Examining data with the Kolmogorov–Smirnov statistic revealed non-normal distribution. Consequently data was summarized for each study group in terms of medians and interquartile ranges. The Mann–Whitney test was used to compare clinical variables between the khat chewers and non-chewers. Significance of differences between the two groups in absolute and relative counts of the test species at the healthy, and separately the periodontitis sites were sought using ordinal regression with clinical variables as covariates. The logit and negative log–log link functions were used for absolute and relative counts, respectively. A significance level of 0.05 was considered. All tests were performed using SPSS 17.

3. Results

3.1. Study subjects

The clinical characteristics of the two study groups are shown in Table 2. The khat chewers tended to be older, but to have lower median PI scores and PD at sampled sites; however, the differences were not statistically significant. There were significantly more males among the chewers (90%) compared to the non-chewers (60%).

3.2. The real-time PCR assays

The quantitative PCR assays for all tested organisms showed excellent linearity ($R^2 > 0.99$) over a dynamic range of 5–10^6 copies per reaction (data not shown), achieving a theoretical sensitivity of 100 copies per sample (assuming a DNA extraction of 100%). In the SYBR Green – pooled DNA assay, all primer sets produced single disassociation peaks corresponding to standard peaks, indicating high specificity (data not shown). No PCR inhibition was encountered as assessed by the total bacterial assays. The median log total bacterial DNA recovered was 8.69 (range 7.73–9.61). With the exception of *Porphyromonas gingivalis*, all tested species were detected in 100% of the samples; *Porphyromonas gingivalis* was not seen in only one sample (2.5%).

3.3. Khat chewing and absolute microbial counts

The median log-transformed absolute counts of the test species in subgingival plaque samples from the healthy and periodontitis sites in both study groups is shown in Table 3. More total bacteria were recovered from the healthy and periodontitis sites of the khat chewers; however, the difference was only significant for the periodontitis sites (OR = 20; $P = 0.006$). The khat chewers also harbored significantly higher absolute counts of *Treponema denticola* in samples from both the healthy and periodontitis sites with odds ratio of 3.13 and 13, respectively ($P = 0.05$ and 0.001, respectively). On the other hand, samples from the healthy sites of the khat chewers contained significantly lower absolute counts of *Porphyromonas gingivalis* with OR of 0.07 ($P = 0.01$); similar difference was noted for the periodontitis sites but it was statistically insignificant. No significant differences were detected for the remaining species.

3.4. Khat chewing and relative microbial counts

Comparison between the two study groups in terms of the relative counts (% total bacteria) of the test species revealed considerably more differences (Table 4). As with the absolute counts, *Treponema denticola* was detected at higher relative counts in the khat chewers, but the differences were only significant for samples from healthy sites (OR = 2.97; $P = 0.03$). In contrast, *Porphyromonas gingivalis* was present in significantly lower relative counts in subgingival plaque samples from the healthy as well as the periodontitis sites of the khat chewers with OR of 0.25 and 0.15, respectively ($P = 0.02$ and 0.005, respectively). In addition, the periodontitis sites from the khat chewers harbored lower relative counts of fusobacteria, prevotellae, and *Parvimonas micra* (OR = 0.20, 0.11 and 0.18, respectively; $P = 0.005$, 0.002, 0.003, respectively); similar differences were noted at the healthy sites, but only fusobacteria and prevotellae maintained a statistical significance (OR = 0.33 and 0.32; $P = 0.03$ and 0.025, respectively).

Taking all pathogens together, the subgingival plaque samples from the khat chewers were found to harbor lower relative counts

Table 3
Median log absolute counts (interquartile range) of the test species in subgingival plaque samples by chewing and sampled site status.

Species	Healthy sites			Periodontitis sites		
	Khat non-chewers $n = 10$	Khat chewers $n = 10$	OR[a] (95% CI)	Khat non-chewers $n = 10$	Khat chewers $n = 10$	OR[a] (95% CI)
Total bacteria	8.60 (7.99–8.78)	8.68 (8.53–8.97)	2.78 (0.43–18.2)	8.55 (8.37–8.84)	9.01 (8.75–9.18)	20.0** (2.46–201)
Fusobacterium spp.	6.73 (6.24–6.87)	6.21 (5.62–6.85)	1.15 (0.18–7.11)	6.37 (5.96–7.17)	6.55 (5.98–7.09)	0.88 (0.14–5.59)
Prevotella spp.	6.45 (5.56–6.77)	6.02 (5.78–6.55)	0.42 (0.07–2.69)	6.39 (5.89–6.94)	6.42 (6.08–6.79)	1.96 (0.30–12.9)
Parvimonas micra	4.89 (4.00–5.40)	4.17 (3.58–4.79)	1.75 (0.28–11.15)	5.37 (4.78–5.70)	5.42 (4.94–5.61)	0.59 (0.09–3.86)
Porphyromonas gingivalis	4.21 (3.25–5.66)	3.03 (2.26–5.84)	0.07** (0.01–0.58)	5.58 (4.07–5.95)	5.45 (2.60–6.53)	0.34 (0.07–2.29)
Tannerella forsythia	5.99 (4.84–6.31)	6.19 (5.63–6.55)	4.35 (0.13–5.70)	6.15 (5.73–6.76)	6.83 (6.51–7.05)	6.67 (0.91–49.0)
Treponema denticola	5.03 (3.42–5.80)	6.02 (4.61–6.72)	3.13* (1.14–8.60)	6.01 (5.30–6.10)	6.71 (6.28–6.94)	13.0*** (3.46–49.5)

Ordinal regression adjusting for the effect of clinical variables.
[a] *Odds* in the khat chewers relative to the non-chewers.
* $P \leq 0.05$.
** $P \leq 0.01$.
*** $P \leq 0.001$.

Table 4
Median relative counts (interquartile range) as % total bacteria of the test species in subgingival plaque samples by chewing and sampled site status.

Species	Healthy sites			Periodontitis sites		
	Khat non-chewers $n = 10$	Khat chewers $n = 10$	OR[a] (95% CI)	Khat non-chewers $n = 10$	Khat chewers $n = 10$	OR[a] (95% CI)
Fusobacterium spp.	1.142 (0.480–2.120)	0.404 (0.150–0.880)	0.33* (0.13–0.93)	0.809 (0.302–2.560)	0.470 (0.180–0.950)	0.20** (0.06–0.61)
Prevotella spp.	0.760 (0.302–0.980)	0.285 (0.120–0.513)	0.32* (0.12–0.87)	0.724 (0.268–1.755)	0.355 (0.188–0.411)	0.11** (0.03–0.43)
Parvimonas micra	0.017 (0.007–0.041)	0.003 (0.001–0.011)	0.50 (0.17–1.49)	0.040 (0.021–0.100)	0.023 (0.010–0.046)	0.18** (0.05–0.56)
Porphyromonas gingivalis	0.011 (7E-4–0.227)	2E-4 (2E-5–0.105)	0.25** (0.07–0.77)	0.121 (0.004–0.227)	0.053 (3E-5–0.289)	0.15** (0.04–0.57)
Tannerella forsythia	0.275 (0.047–0.622)	0.302 (0.127–0.481)	1.97 (0.77–5.06)	0.353 (0.135–1.467)	0.569 (0.254–2.032)	0.74 (0.27–1.99)
Treponema denticola	0.054 (0.002–0.211)	0.185 (0.015–0.513)	2.98* (1.10–8.01)	0.209 (0.056–0.579)	0.514 (0.217–0.729)	2.40 (0.90–6.24)

Ordinal regression adjusting for the effect of clinical variables.
[a] *Odds* in the khat chewers relative to the non-chewers.
* $P \leq 0.05$.
** $P \leq 0.01$.

of total periodontal pathogens; however, differences were only significant at sites with periodontal destruction (Fig. 1).

4. Discussion

Previous findings suggest that khat may have a favorable effect on periodontal microbiota, but the evidence remains inconclusive; therefore, further investigation is warranted. The effect of khat chewing on subgingival microbiota in vivo has been previously assessed in young adults with healthy periodontium using the DNA–DNA checkerboard hybridization assay (Al-hebshi and Skaug, 2005a). The purpose of the current study was to carry that further, by investigating subgingival microbial shifts produced by the habit in samples from subjects with chronic periodontitis using the highly sensitive real-time PCR technology.

A total of 40 subgingival samples from 10 khat chewers and 10 khat non-chewers were analyzed. Tough criteria were used for selection of the study subjects that, in fact, it took 6 months to recruit the sample. The khat chewers were predominantly males, supporting the fact that khat chewing is mainly a male habit. The khat chewers had less median PI and PD scores; however, the differences were not significant. Nevertheless, these findings are in line with other reports supporting the notion that khat chewing may have a mechanical cleansing effect on dental biofilm (Jorgensen and Kaimenyi, 1990; Al-hebshi and Al-ak'hali, 2010).

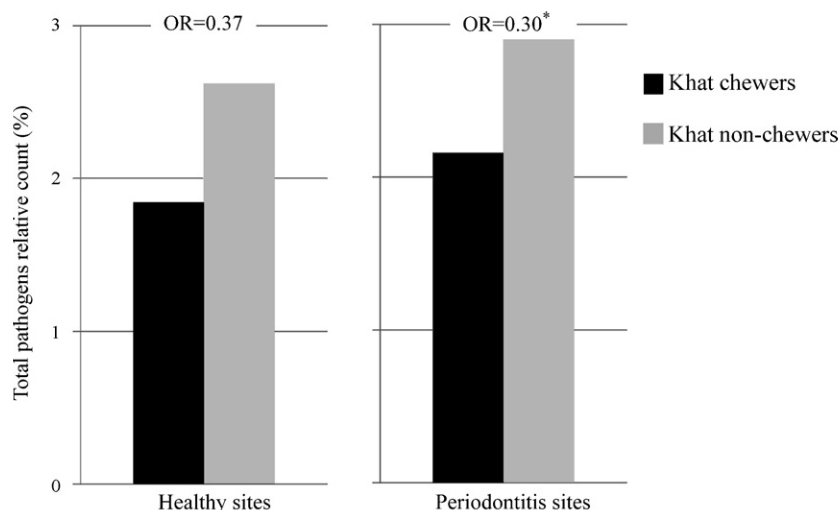

Fig. 1. Clustered bars of median relative counts of total pathogens as % total bacteria in subgingival plaque samples from healthy and periodontitis sites from the khat chewers ($n = 10$) and the khat non-chewers ($n = 10$). *$P \leq 0.05$; ordinal regression adjusting for the effect of clinical variables.

A panel of known major periodontal pathogens was assessed in this study. *Porphyromonas gingivalis*, *Tannerella forsythia* and *Treponema denticola*, or the so called the red complex are strongly associated with chronic periodontitis. The rest belong to the orange complex, which is also associated, but to a lesser extent, with the disease (Socransky et al., 1998). These species are present in high proportions subgingivally at sites with periodontal destruction, but they are still found at lower numbers at healthy sites (Haffajee et al., 1999). The advantage of real-time PCR is that it allows sensitive and accurate quantification of species in a sample, not only in absolute terms but relative to total bacterial. Because of variation in sampling procedures, relative quantification represents a more reliable measure of a species in a mixed bacterial sample (Lyons et al., 2000). The current view of chronic periodontitis as consequence of ecological shifts of the bacterial community in dental biofilm further underscores the importance of relative quantification (Marsh, 2003). For the purpose of this study more weight was given for differences in relative counts rather than absolute counts.

Comparisons were made using an ordinal regression model adjusting for the possible confounding effects of age, gender, PI, and PD. In spite of having lower PI and PD scores the khat chewers were found to harbor more total bacteria subgingivally particularly at the periodontitis sites, suggesting that khat encourages growth of all or a subset of bacteria in subgingival biofilm. However, the khat chewers had significantly lower relative counts of four of the tested periodontal pathogens, including *Porphyromonas gingivalis*, at both healthy and diseased sites (Table 4) which is consistent with previously reported antibacterial effect of crude khat extract against the same species (Al-hebshi et al., 2006). By suppressing these pathogens, khat chewing seems to interfere with, or at least slow down, periodontal destruction especially in established pockets. *Tannerella forsythia* was not found to be influenced by khat chewing in this study, which is in disagreement with previous observations (Al-hebshi and Skaug, 2005a; Al-hebshi et al., 2006), but this could be a result of differences in study samples and detection method used. As an outlier, *Treponema denticola* was present at higher relative counts in the khat chewers but only at the healthy sites. This should be assessed carefully since *Treponema denticola* is a well-established periodontal pathogen. However, this bacterium has been repeatedly shown to work in synergism with other pathogens, particularly *Porphyromonas gingivalis* (Sela, 2001). Therefore, the observed increase of *Treponema denticola* in isolation may not represent a risk for the healthy sites of the khat chewers. In fact, *Treponema denticola* has been previously detected in isolation at higher frequency at healthy sites than in periodontitis sites (Mineoka et al., 2008).

Overall, khat chewing decreased total pathogen burden subgingivally in spite of increasing total subgingival bacterial count, suggesting that khat encourages growth of some other species. It is probably difficult to speculate which specific species contributed to the increase of total bacteria. However, crude khat extract was previously found to increase the proportion of *Streptococcus oralis* in the Zurich biofilm by 39% (Al-hebshi, 2005). In line with that, we also noted that khat extract enhances the growth of oral streptococci in liquid broth (unpublished data). Additionally, khat was previously shown to be associated with higher prevalence and levels of *Veillonella parvula* in subgingival biofilm (Al-hebshi and Skaug, 2005a). Since streptococci and veillonella spp. are generally considered as health-compatible species (Socransky et al., 1998), it could be speculated that the higher total bacterial counts could be attributed, at least in part, to an increase in health-compatible species. In this respect, it would have been a major addition to this study if some health-compatible species were also assessed.

The microbial shifts highlighted above, i.e. the decrease of total pathogens relative to total bacteria, are suggestive of a prebiotic effect. A prebiotic is defined as non-digestible oligosaccharides that affect the proliferation of resident *intestinal* commensal bacteria that may then exert probiotic effects (Roberfroid, 2007). In fact, it has been previously reported that carbohydrates constitute 30% of crude aqueous khat extract (Al-hebshi et al., 2005); therefore, the presence of a prebiotic oligosaccharide cannot be ruled out. Khat is, therefore, the very first reported natural substance with *potential* prebiotic effect in connection with oral bacteria, which opens up a new prospective for oral health research (see Supplementary material).

5. Conclusions

Further evidence is provided here that khat chewing probably modifies the composition of subgingival microbial community in compatibility with periodontal health, in a way similar to the effects of prebiotics on intestinal microflora. However, a larger scale study addressing the dose-dependent effects of the plant on a larger panel of periodontal species is required before a decisive conclusion can be made.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jep.2010.08.051.

References

Adeoya-Osiguwa, S.A., Fraser, L.R., 2005. Cathine and norephedrine, both phenylpropanolamines, accelerate capacitation and then inhibit spontaneous acrosome loss. Human Reproduction 20, 198–207.

Al-Habori, M., 2005. The potential adverse effects of habitual use of Catha edulis (khat). Expert Opinion on Drug Safety 4, 1145–1154.

Al-Habori, M., Al-Mamary, M., 2004. Long-term feeding effects of Catha edulis leaves on blood constituents in animals. Phytomedicine 11, 639–644.

Al-hebshi, N., 2005. Khat and oral microbiota – a study with relevance to periodontitis and dental caries (PhD thesis). University of Bergen, Bergen.

Al-hebshi, N., Al-haroni, M., Skaug, N., 2006. In vitro antimicrobial and resistance-modifying activities of aqueous crude khat extracts against oral microorganisms. Archives of Oral Biology 51, 183–188.

Al-hebshi, N.N., Al-ak'hali, M.S., 2010. Experimental gingivitis in male khat (Catha edulis) chewers. Journal of the International Academy of Periodontology 12, 56–62.

Al-hebshi, N.N., Nielsen, O., Skaug, N., 2005. In vitro effects of crude khat extracts on the growth, colonization, and glucosyltransferases of *Streptococcus mutans*. Acta Odontologica Scandinavica 63, 136–142.

Al-hebshi, N.N., Skaug, N., 2005a. Effect of khat chewing on 14 selected periodontal bacteria in sub- and supragingival plaque of a young male population. Oral Microbiology and Immunology 20, 141–146.

Al-hebshi, N.N., Skaug, N., 2005b. Khat (Catha edulis) – an updated review. Addiction Biology 10, 299–307.

Dimba, E., Gjertsen, B.T., Francis, G.W., Johannessen, A.C., Vintermyr, O.K., 2003. Catha edulis (Khat) induces cell death by apoptosis in leukemia cell lines. Annals of the New York Academy of Sciences 1010, 384–388.

Elhag, H., Mossa, J.S., El-Olemy, M.M., 1999. Antimicrobial and cytotoxic activity of the extracts of khat callus cultures. Center for New Crops & Plant Products: West Lafayette. Available from: http://www.hort.purdue.edu/newcrop/proceedings1999/v4-463.html (accessed 30.05.10).

Fitzgerald, J., 2009. Khat: a literature review. Centre for Culture, Ethnicity and Health, Melbourne. Available from: http://www.ceh.org.au/downloads/Khat_report_FINAL.pdf (accessed 30.05.10).

Graziani, M., Milella, M.S., Nencini, P., 2008. Khat chewing from the pharmacological point of view: an update. Substance Use and Misuse 43, 762–783.

Haffajee, A.D., Socransky, S.S., Feres, M., Ximenez-Fyvie, L.A., 1999. Plaque microbiology in health and disease. In: Newman, H.N., Wilson, M. (Eds.), Dental Plaque Revisited: Oral Biofilms in Health and Disease. BioLine, Cardiff, UK, pp. 255–282.

Halbach, H., 1972. Medical aspects of the chewing of khat leaves. Bulletin of the World Health Organization 47, 21–29.

Hassan, N.A., Gunaid, A.A., Murray-Lyon, I.M., 2007. Khat (Catha edulis): health aspects of khat chewing. Eastern Mediterranean Health Journal 13, 706–718.

Holland, P.M., Abramson, R.D., Watson, R., Gelfand, D.H., 1991. Detection of specific polymerase chain reaction product by utilizing the 5′–3′ exonuclease activity of Thermus aquaticus DNA polymerase. Proceedings of the National Academy of Sciences of the United States of America 88, 7276–7280.

Jorgensen, E., Kaimenyi, J.T., 1990. The status of periodontal health and oral hygiene of Miraa (catha edulis) chewers. East African Medical Journal 67, 585–590.

Kalix, P., 1996. Catha edulis, a plant that has amphetamine effects. Pharmacy World and Science 18, 69–73.

Kalix, P., Braenden, O., 1985. Pharmacological aspects of the chewing of khat leaves. Pharmacological Reviews 37, 149–164.

Kennedy, J.G., 1987a. Qat and health. In: The Flower of Paradise: The Institutionalized Use of the Drug Qat in North Yemen. D. Reidel Pub. Co., Dordrecht, pp. 212–232.

Kennedy, J.G., 1987b. A short history of qat and its use. In: The Flower of Paradise: The Institutionalized Use of The Drug Qat in North Yemen. D. Reidel Pub. Co., Dordrecht, pp. 60–78.

Lemessa, D., 2001. Khat (Catha edulis): Botany, Distribution, Cultivation, Usage and Economics in Ethiopia. UN-Emergencies Unit for Ethiopia, Addis Ababa. Available from: www.telecom.net.et/~undp-eue/reports/khat_0501.doc.

Lyons, S.R., Griffen, A.L., Leys, E.J., 2000. Quantitative real-time PCR for *Porphyromonas gingivalis* and total bacteria. Journal of Clinical Microbiology 38, 2362–2365.

Marsh, P.D., 2003. Are dental diseases examples of ecological catastrophes? Microbiology 149, 279–294.

Martin, F.E., Nadkarni, M.A., Jacques, N.A., Hunter, N., 2002. Quantitative microbiological study of human carious dentine by culture and real-time PCR: association of anaerobes with histopathological changes in chronic pulpitis. Journal of Clinical Microbiology 40, 1698–1704.

Mineoka, T., Awano, S., Rikimaru, T., Kurata, H., Yoshida, A., Ansai, T., Takehara, T., 2008. Site-specific development of periodontal disease is associated with increased levels of *Porphyromonas gingivalis*, *Treponema denticola*, and *Tannerella forsythia* in subgingival plaque. Journal of Periodontology 79, 670–676.

Roberfroid, M., 2007. Prebiotics: the concept revisited. Journal of Nutrition 137, 830S–837S.

Sela, M.N., 2001. Role of *Treponema denticola* in periodontal diseases. Critical Reviews Oral Biology and Medicine 12, 399–413.

Silness, J., Loee, H., 1964. Periodontal disease in pregnancy. Ii. Correlation between oral hygiene and periodontal condtion. Acta Odontologica Scandinavica 22, 121–135.

Socransky, S.S., Haffajee, A.D., Cugini, M.A., Smith, C., Kent Jr., R.L., 1998. Microbial complexes in subgingival plaque. Journal of Clinical Periodontology 25, 134–144.

Suzuki, N., Yoshida, A., Saito, T., Kawada, M., Nakano, Y., 2004. Quantitative microbiological study of subgingival plaque by real-time PCR shows correlation between levels of *Tannerella forsythensis* and *Fusobacterium* spp. Journal of Clinical Microbiology 42, 2255–2257.

World Health Organization, 1997. Oral Health Surveys: Basic Methods. World Health Organization, Geneva.