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An abstract of
a thesis submitted to the Faculty of Emory College of Arts and Sciences
of Emory University in partial fulfillment
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Abstract

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Porphyromonas gingivalis is the keystone pathogen of periodontitis, a chronic inflammatory disease which causes tooth loss and deterioration of gingiva, alveolar bone, and periodontal ligaments. This bacteria has been linked to a number of peripheral and neurological diseases, including cardiovascular disease, diabetes mellitus, respiratory infection, rheumatoid arthritis, osteoporosis, obesity, pre-term birth, depression, and Alzheimer's disease. *P. gingivalis* interacts with the brain directly via secreted proteases which enter the brain, induce inflammation, and directly affect β-Amyloid and tau proteins. Given how widespread periodontal disease is (greater than 70% of adults over 65 are affected), its range of damaging properties, and growing resistance to many antibiotics, finding new methods of inhibiting *P. gingivalis* growth is paramount. The ethnobotanical approach to drug discovery offers a promising method of discovering new compounds to combat *P. gingivalis* while also addressing the growing problem of antibiotic resistance. In this study, a review of plant medicines used for oral hygiene or symptoms of periodontitis was conducted. From the resulting database of 513 plants, 30 plants were tested against *P. gingivalis*, and 11 of these plants demonstrated high growth inhibition, verifying their traditional medicinal uses. Minimum inhibitory concentration as well as cytotoxicity against human keratinocytes was determined for each of these plant extracts. Promising therapeutic indices for these 11 plants supports further testing including biofilm eradication and bioactivity guided fractionation and potential development as antibiotic pharmaceuticals or additives to oral hygiene products.

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<u>Chapter 1: Introduction – Overview</u>

1. *Porpyromonas gingivalis* – mechanisms of infection and evading host immune response

Periodontitis, a chronic inflammatory disease which causes tooth loss and deterioration of gingiva, alveolar bone, and periodontal ligaments, is caused by several microbes including the keystone pathogen *Porphyromonas gingivalis* [1, 2]. Periodontitis is a slow-developing infection; if gingivitis, which is irritation and inflammation of the gums, is left untreated over long periods of time, pockets can develop between the gums and teeth which provide the breeding ground for *P. gingivalis* to thrive. These deep pockets of bacterial growth eventually lead to the loss of alveolar bone and teeth. Because periodontitis is slow-developing, and can advance if the host is immune compromised, the disease is associated with aging, affecting 47.2% of adults over 30 and 70.1% of adults over 65 [3].

The keystone pathogen of periodontitis, *P. gingivalis,* is a gram negative, rod-shaped, obligate anaerobe which infects periodontal tissues as a secondary infection through interactions with commensal streptococci [2, 4, 5]. *P. gingivalis* has evolved complex mechanisms of evading host antibacterial immune response. The bacteria develops a biofilm, an extracellular matrix composed of various polymeric substances, which protects the pathogen from host immune cells and supports the continued virulence of other infections [4, 6]. *P. gingivalis* also invades host cells without causes apoptosis, replicates within host cells, and travels from cell to cell, thus allowing it to evade immune surveillance [7-9]. The virulent lipopolysaccharide (LPS) of *P. gingivalis* includes a dynamic subunit, Lipid A, with the capacity to change structure, assisting the bacteria in avoiding detection by immune cells [1]. In addition, *P. gingivalis* degrades several components of the complement immune pathway and anti-microbial peptides, including defensins and key pro-inflammatory cytokines [6, 8, 10]. These bacteria also bind to blood cells via complement protein C3, which serves as a transport mechanism and a strategy to avoid detection by phagocytes [1, 11]. Transportation through binding to blood cells has been termed the "haematogenous route."

Because of its ability to evade host immune response and travel from cell to cell as well as via the haematogenous route, *P. gingivalis* can cause and maintain high levels of chronic inflammation in various peripheral organs. Infection with *P. gingivalis* has been linked to cardiovascular disease, diabetes mellitus, respiratory infection, rheumatoid arthritis, osteoporosis, obesity, and pre-term birth [12]. More recently, the link between inflammation and the CNS has been receiving increasing attention, and many studies have begun to investigate the link between *P. gingivalis,* inflammation, and the brain. An increasing volume of clinical and experimental evidence has associated *P. gingivalis* to Alzheimer's disease (AD), dementia, and other forms of cognitive decline. (see Chapter 2 for more detail on the neuro-immune response and the role of *P. gingivalis*)

2. Antibiotic Resistance and the Search for New Antimicrobials

Because of the link between *P. gingivalis* and various diseases, and the pathogen's range of evasive mechanisms for surviving host immune response, identifying new ways of counteracting the bacteria will be important in combating infection and related diseases. Currently, periodontitis is treated with systemic and local antibiotics and occasionally surgery to reach deep-pocket inflammation. In the search for antimicrobial remedies, it is important to consider the rising risk of antibiotic resistance, which is considered by the World Health Organization considers to be a pressing threat to global public health [13]**.** *P. gingivalis* strains show resistance levels as high as 21.56% to metronidazole, 25.49% to amoxicillin, and 23.52% to clindamycin [14]. Significant levels of resistance have also been recorded for penicillin, erythromycin, azithromycin, and tetracycline [15].

While antibiotic resistance is rising, new antimicrobials are increasingly necessary, but in recent years there has in fact been a decrease in the discovery of new antibiotics [16]**.** Natural products, defined as "any FDA-approved, unmodified natural material or compound, semisynthetic derivatives, or synthetic structures which were conceptually derived from a natural product," are a promising source of new antimicrobials [17]. As opposed to single-compound antibiotics, natural products contain up to thousands of chemical compounds, and thus may have many mechanisms of antimicrobial activity. Use of natural products has been found to result in less development of antibiotic resistance [16, 18]**.** Therefore, one of the ways of combating antibiotic resistance is to employ the ethnobotanical approach to drug discovery. A look into the ethnobotanical approach follows:

3. Ethnobotanical Approach to Drug Discovery

The ethnobotanical approach to drug discovery means employing the traditional uses of plants as a tool for discovery of new molecules for pharmaceutical use; ethnobotanical information guides the selection of plant extracts to test in high-throughput screening. There is a vast wealth of recorded ethnobotanical information about the medicinal uses of thousands of plants; traditional medicinal systems all over the world have employed and documented plant-derived drugs for millennia. The earliest documented use of plant medicine, from Mesopotamia circa 2600 BCE, includes records of about 1000 medicinal substances [19]. Some of the most famous examples of other vast ethnobotanical records include the Egyptian Eber's Papyrus, which dates from 1500 BCE and documents more than 700 drugs, multiple Traditional Chinese Medicine documents from 1100 BCE to 659 AD which in total include over 1000 drugs, and, more recently, the Canon of Medicine by Ibn Sina, circa 1037 CE, which compiles over 1000 drugs from Greek and Eastern medicines [19, 20]. The World Health Organization estimates that today, 65% of the global population still relies, for primary healthcare, on traditional medicines principally employing botanically-derived natural products [19].

During the 1990s and early 2000s, high-throughput screening technologies made it possible for compounds created by combinatorial chemistry to be tested rapidly for antimicrobial activity. As a result, there was a decline in testing of natural products, and many pharmaceutical companies even retired their screening libraries of plant extracts [21]. However, combinatorial chemistry as a source of new chemical entities has not been as successful as predicted in the 1990s – in fact, this approach produced only one FDA-approved new chemical entity (sorafendib) from that time until 2014 [21, 22].

Although combinatorial chemistry and the creation of synthetic molecules have become central to the field of pharmaceutical development, the ethnobotanical approach remains one of the most important contributors to new drug discovery. For example, the antimalarial drug quinine was first isolated from the bark of *Cinchona* species, which was used by indigenous populations of the Amazon to treat fevers [19, 23]. Reserpine, used to treat hypertension, comes from the Ayurvedic medicinal plant *Rauvolfia serpentine* [19, 24]. Vinblastine and vincristine, important chemotherapy drugs, were isolated from *Catharanthus roseus,* and Paclitaxel, another key anticancer pharmaceutical, was isolated from the Pacific yew tree, *Taxus brevifolia* [19]*.* Bark of the willow tree was used for millennia in the Mesopotamian region, Egypt, and China as an analgesic, and led to the development of a common household pharmaceutical – aspirin [25].

These examples are only a small selection of the many contributions to pharmacology from the natural world. In fact, 75% of the new small molecules approved for cancer treatment from the 1940s to 2014 were derived from natural products or were synthetic molecules mimicking a natural products [19, 26]. A review of pharmaceuticals from 1981 to 2010 found that 69% of anti-infective drugs were either derived from natural products or based on the structure of natural products [19, 26].

Screening for small molecules with a basis in the ethnobotanical approach is much more likely to yield new active molecules than random screening; the National Cancer Institute found a hit rate of 10.4% for screening random samples, compared to 19.9% when using ethnobotanical information to guide screening [27]. A survey of natural products in current use as pharmaceuticals found that 80% “were used for the same or related ethnomedical purposes” as the plants’ original uses in their respective traditional medicinal systems [19]. The ethnobotanical approach to drug discovery is also brimming with untapped potential for discovery of many new medicinal molecules; for example, some researchers estimate that current knowledge of molecules derived from rainforest plants represent only one eighth of the potential molecules of pharmaceutical significance the rainforests contain [28].

4. Specific Research Question and Project Aims

The aims of this research project were to perform an ethnobotanically-guided screen of plant extracts against the bacteria *Porphyromonas gingivalis*, to determine the extracts’ ability to inhibit growth of the bacteria, and to determine the therapeutic index of each extract by testing for cytotoxicity against human cells. An ethnobotanical review was completed to gather data on plants from traditional medicinal systems across the globe which have been used to treat periodontitis or the symptoms of periodontitis. The plant extracts investigated in this project were selected from the matches between this ethnobotanical search and the Quave Natural Product Library. It was hypothesized that the traditional uses of these plants would be verified by results demonstrating their ability to inhibit *P. gingivalis,* and it was further hypothesized that each plant extract would have low toxicity to human cells, given that these plants have been used to treat oral pain or infection in various medicinal systems.

Chapter 2: Introduction – *Porphyromonas gingivalis* and the Brain

A growing body of evidence links *P. gingivalis* to the development of Alzheimer's disease (AD), dementia, and other forms of cognitive decline. In addition, chronic infection with *P. gingivalis* has been linked to anhedonia [29] and dysphoria [30] in depression, and periodontitis has been found to have links with Schizophrenia [31-33]. Although not linked to *P. gingivalis* specifically, a range of other psychiatric disorders have been found to be associated with levels of pro-inflammatory cytokines [34] and microglial activation [35], both of which are increased when infected with *P. gingivalis.* Alzheimer's disease is currently the most well-studied neuropsychiatric disease associated with *P. gingivalis,* with the largest volume of clinical and experimental evidence [36].

Periodontal infection with the *P. gingivalis* is a risk factor for developing AD [33, 36-41]. There are two main groups of mechanisms by which *P. gingivalis* interacts with brain health – inducing inflammation, and direct interaction via excretion of toxic proteases.

1. The inflammatory connection to AD

The onset of AD has been found to be related to inflammation and immune response [42, 43]. The three major inflammatory pathways of the innate immune response – the classical pathway, the alternative pathway, and the complement pathway – are not only involved in peripheral inflammation, but nearly all components of these pathways have also been detected in the brain [44]. Microglia and astrocytes produce complement proteins and contain many complement receptors [44]. The mRNAs for the proteins of all three inflammatory pathways are significantly upregulated in areas of the brain affected by AD [44]. Inflammation in the AD brain is particularly high at the sites of $A\beta$ plaques and neurofibrillary tangles, where complement fragments and clusters of microglia and astrocytes are all found at significantly heightened levels [44].

Levels of inflammation are associated with severity of AD symptoms. For example, the brains of asymptomatic patients which at autopsy contained enough $A\beta$ and neurofibrillary tangles to qualify for diagnoses with AD contained significantly less inflammatory markers than the brains of symptomatic AD patients [44].

The increased inflammation found near sites of AD-associated cell damage is not only a byproduct of the cellular damage; $A\beta$ specifically activates the complement and alternative pathways, which brings in a flood of inflammatory cells capable of causing neuronal damage if they are not properly regulated [45]. In fact, four key genetic defects associated with AD encode components of the complement cascade: sub-component 1s, complement receptor 1, complement component 9, and clusterin [46]. The resulting dysregulation of the complement cascade leads to sustained complement activation [47], which causes neuronal damage and altered glial function [45]. Since the ability to stop the activation of the inflammatory pathways is dysregulated in the AD brain, any activation point could result in a vicious cycle leading to a state in which the inflammation itself becomes a source of neural damage regardless of the original source of activation [44].

Indeed, there is evidence that an acute inflammatory event such as an infection can increase the inflammatory load in AD and result in an increased rate of cognitive decline [48]. In addition, *Aβ* itself, often thought to be only a pathological by-product, has been found to play a physiological role as an antimicrobial peptide [49]. Therefore, microbial products entering the brain cause an increase in *Aβ,* which in turn activates the dysregulated inflammatory cascade.

2. *P. gingivalis* causes increased inflammatory load and interacts directly with key AD pathologies

P. gingivalis components can directly infiltrate the brain, thus affecting the pathway of increasing *Aβ,* activating an inflammatory response, and beginning a feedback cycle in which the inflammatory response is not shut off. LPS from *P. gingivalis* was found in postmortem AD brains while absent in control samples [50], toxic proteases from *P. gingivalis* were highly elevated in AD brains [51], and antibodies against periodontal bacteria were elevated in AD brains [52]. In addition, cerebrospinal fluid from living AD patients contained *P. gingivalis* DNA [51]. The brains of mice orally infected with *P. gingivalis* contained *P. gingivalis* genomic DNA, which also caused microglia activation, complement activation, and bystander neuronal damage [51, 53]. Interestingly, brain infection and activation of the complement pathway was not found for other oral bacteria [53].

While the mechanism of *P. gingivalis* accessing the brain is not currently known, there are several pieces of evidence which support a hypothesized mechanism. *P. gingivalis* binds to erythrocytes, which allows the bacteria to circulate [11]. Bacterial components can then enter the brain via the circumventricular organs, structures around the third and fourth ventricles not subject to the blood-brain-barrier, where infectious particles and inflammatory mediators access the brain [37]. In addition, *P. gingivalis* can also damage the endothelial cells of the blood-brain-barrier in order to access the brain [51]. Once in the brain, *P. gingivalis* may be able to spread slowly via neuron to neuron transmission, since *P. gingivalis* has been shown to spread from cell-to-cell peripherally [51, 54].

P. gingivalis excretes toxic proteases called gingipains. These proteases have been shown to cleave tau, there is a high correlation between gingipain load and tau load in postmortem brain tissue, and gingipains specifically co-localize with tau tangles [51]. Fragmentation of tau is thought to be a major cause of the formation of tau tangles, so the link between gingipains and tau cleavage suggests that *P. gingivalis* not only causes increased inflammation, but directly interacts with a key AD-specific pathology. Oral infection of *P. gingivalis* also leads to increased production of $A\beta_{1-42}$ in mice [51], which further suggests direct interaction with AD pathology.

Chapter 3: Materials and Methods

1. Literature Review of Oral Health Related Plants

For full tables of ethnobotanical review, see supplementary tables 1-9.

A review of plants used for oral health in various traditional medicinal systems across the globe was conducted. Plants selected for inclusion were those that have been used for oral hygiene, plants used for periodontitis, and plants used for any of the symptoms of periodontitis including toothache, sore mouth, mouth sores/abscesses, loose teeth, and halitosis. Family, genus, and species names were recorded along with information on part of plant used, medicinal system, application (how the plant is processed and used traditionally), and whether the plant has been previously tested against periodontitis microbes. All plants were then cross-checked to determine most recent accepted genus and species names. Next, the database created from this review was cross-checked with the extracts contained in the Quave Natural Product Library (QNPL). Matches were sorted according to priority level. Priority levels were defined as follows:

1 – genus, species, and plant part match

2 – genus and species match, but different plant part

3 – genus and part match

4 – genus, species, and part match but the plant is part of a multi-ingredient medicine

2. Bacterial Strains and Growth Conditions

For protocol design process, see supplementary materials.

Freeze dried *P. gingivalis* strain ATCC 33277 was rehydrated in Brain Heart Infusion (BHI) media supplemented with 5 µg/mL Hemin and 1 µg/mL menadione. Bacteria were grown on supplemented BHI (sBHI) agar plates (5 µg/mL Hemin, 1 µg/mL menadione) and supplemented blood agar plates (5% defibrinated sheep's blood, 5 µg/mL Hemin, 1 µg/mL menadione, 2 g/L yeast extract) [5, 55-60]. Hemin stock was prepared by dissolving 250 mg Hemin in 5 mL of 1M NaOH and 495 mL ddH20 for a final concentration of 0.5 mg/mL. Menadione stock was prepared by dissolving 25 mg menadione in 20 mL 100% EtOH for a final concentration of 1.25 mg/mL. Each stock was filter sterilized, wrapped in tin foil, and stored at 4°C.

Identification of *P. gingivalis* was determined by pigmentation of colonies on supplemented agar (see Fig 3.1) and confirmed by MALDI-TOF.

A **B**

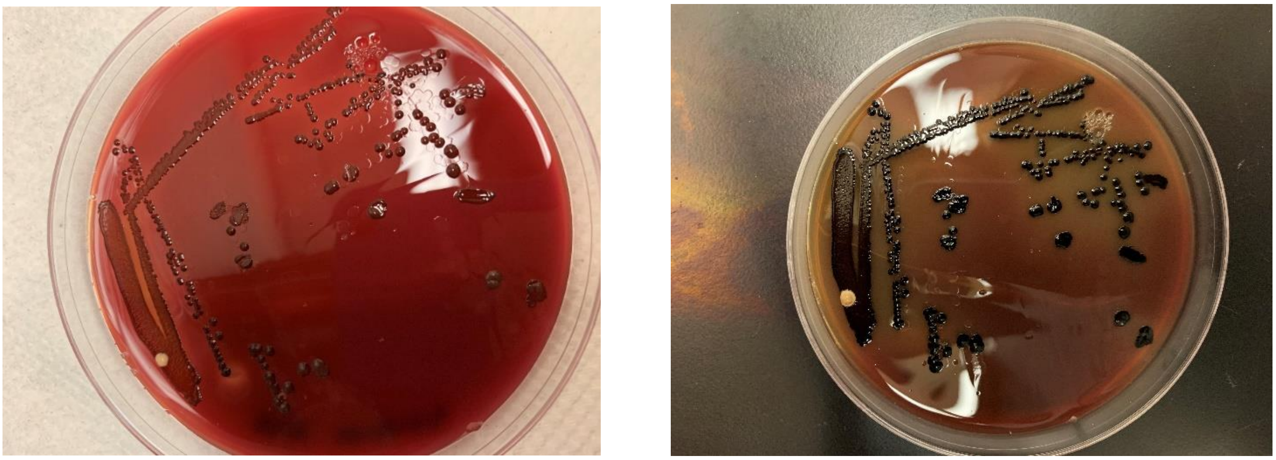

Fig 3.1: ***P. gingivalis colonies*** **on supplemented blood agar**
P. gingivalis develops a highly characteristic black-pigmented colony after incorporating protoheme from blood. **A)** after 10 days of incubation and **B)** after 15 days of incubation in anaerobic chamber at 37°C. White colony seen on the left side of the plate is a growth of yeast, likely from the yeast extract used in the blood agar media.

3. Colony Counting to Standardize CFU/mL

To determine the average number of colony forming units (CFU) per mL, bacterial culture was first incubated in sBHI for 48 hours in anaerobic conditions at 37°C (GasPak container system). A 96-well flat-bottom non-tissue culture treated plate were prepared with 90 µL of sBHI. 10 µL of bacterial culture was added to four wells, and each of these wells was then serial diluted at 10 µL for a total of 8 10-fold dilutions. An 8-channel pipette was then used to transfer 5 µL from each well to an sBHI agar plate. Once droplets were dry, the plate was incubated for 5 in anaerobic conditions at 37°C. The number of colonies were counted in each of the four 10^{-3} droplets. The calculation was as follows:

$$\frac{average\ \#\ colonies\ per\ droplet}{10^{-3}\ * 0.005\ mL} = CFU$$

3. Single Concentration Screens To Select High Activity Extracts

109 extracts from the 21 high priority plants were screened for growth inhibition against *P. gingivalis* at a single concentration (256 µg/mL). All extracts which had higher than 90% inhibition at 256 µg/mL were defined as hits. The hits from this screen, 38 extracts from 17 plants, were then screened at 64 µg/mL and 32 µg/mL. In order to minimize the total time during which *P. ginginvalis* were exposed to open air conditions, the following protocol was designed:

256 µg/mL screen: sBHI media was pre-reduced overnight in an anaerobic chamber, and *P. gingivalis* was then incubated in pre-reduced media for 48 hours in anaerobic conditions at 37°C. Additional sBHI was pre-incubated 24 hours before the screen. On the day of the screen, 94.88 µL of pre-reduced sBHI and 5.12 µL of extract were added to each well of a 96-well non-tissue culture treated flat bottom plate.

All extracts were dissolved in DMSO at 10 mg/mL. Next, 100 µL *P. gingivalis* culture were added to each well. The culture was standardized for a final concentration of 10^6 CFU per well. Untreated control, vehicle (DMSO) control, and antibiotic control (tetracycline 64 µg/mL) were included. Plates were then incubated in anaerobic conditions at 37°C for 72 hours.

64 and 32 µg/mL screens:
The same protocol was repeated with the following changes: 2.56 µL of extracts were added to 197.44 µL sBHI media and completed with 200µL *P. gingivalis* in sBHI media for a final concentration of 10^6 CFU. Wells were then serial diluted once to achieve 32 µg/mL of extract.

Growth inhibition was determined by change in ocular density (OD) from the start of incubation to the final timepoint. Optical density (OD600) was measured using a BioTek Cytation3 plate reader. Growth inhibition was calculated with the following formula:

$$\left(1-\left(\frac{\Delta ODtest}{\Delta ODvehicle}\right)\right)*100 = \%\ growth\ inhibition$$

The 21 hits (>90%growth inhibition) from the 64 µg/mL screen were selected for minimum inhibitory concentration assays (MIC).

4. Minimum Inhibitory Concentration Assays

P. gingivalis was incubated in 5 mL sBHI for 48 hours in an anaerobic GasPak container at 37°C. Working culture was created by standardizing to a concentration of 10^6 CFU using BioTek Cytation3. 96-well flat-bottom non-tissue culture treated plates were used as in the single concentration screens. All extracts which had greater than 90% growth inhibition at 64 µg/mL (table 1) were prepared for serial dilution with a starting concentration of 256 µg/mL. All extracts which had greater than 90% growth inhibition at 32 µg/mL (table 1) were prepared for serial dilution with a starting concentration of 128 µg/mL. For those extracts starting at 256 µg/mL, starting wells were prepared with 189.76 µL of sBHI and 10.24 µL extract, and for those starting at 128 µg/mL, starting wells were prepared with with 194.88 µL of sBHI and 5.12 µL of extract. All extracts were dissolved in DMSO at 10 mg/mL. Untreated control, vehicle (DMSO) control, and antibiotic control (tetracycline 64 µg/mL) were included. 200 µL of working culture of *P. gingivalis* was added to complete starting wells to 400 uL. A total of 8 2-fold serial dilutions were completed for a final concentration of 2 µg/mL (256 µg/mL starting concentration) or 1 µg/mL (128 µg/mL starting concentration). Plates were incubated in anaerobic conditions at 37°C for 72 hours. Optical density was measured using BioTek Cytation3 at the starting point and after 72 hours of incubation. MIC tests were duplicated on a separate day to confirm accuracy. Growth inhibition was calculated using the formula defined above. MIC_{90} was defined as the lowest concentration at which 90% of growth was inhibited compared with vehicle control, and IC_{50} was defined as the lowest concentration at with 50% of growth was inhibited.

5. Cytotoxicity Assays

All extracts which underwent MIC assays were tested for toxicity against human cells. The human immortalized keratinocytes (HaCaT) were grown in Dulbecco's modified Eagle's medium with L-glutamine and 4.5 g/L glucose (Corning, Corning, NY) supplemented with 10% heat-inactivated fetal bovine serum (Seradigm, Randor, PA) and 1X solution of 100 IU Penicillin and 100 µg/mL Streptomycin (Corning, Corning, NY). Cells were incubated in 10 mL of supplemented DMEM in 75 cm^2 flasks (Greiner

Bio-One) at 37°C, 5% CO_2. Upon reaching 80% confluence, cells were passaged by detaching from the flask bottom with 0.25% trypsin, centrifuging at 1500 rpm for 3 minutes, and resuspending in 10 mL of fresh supplemented DMEM. Cells were passaged every 2-3 days.

Toxicity was tested using the LDH cytotoxicity assay. Cell culture was standardized to a concentration of 4×10^4 cells/mL and 200 µL were added to each well of a 96-well tissue culture treated flat bottom microtiter plate (Falcon 35-3075). Plates were incubated for 48 hours at 37°C, 5% CO_2. Media was gently aspirated and 200 µL fresh supplemented DMEM was added to each well. Extracts were added and serial diluted 512-4 µg/mL or 128-1 µg/mL (table 4.3). Cells were then processed after 24 hours of incubation at 37°C, 5% CO_2. Lactate dehydrogenase (LDH) assay was performed following manufacturer's instructions (LDH assay kit, G-Biosciences, St. Louis, MO). Briefly, the assay tests levels of LDH, a cytosolic enzyme which is released upon cell lysis. The assay solution undergoes a coupled enzymatic reaction with LDH to ultimately reduce a tetrazolium salt to a red formazan; the intensity of color is correlated to proportion of cells which underwent lysis due to extract toxicity. Positive control of maximum cytotoxicity was achieved by adding 20 µL of lysis buffer to six wells untreated with extracts, and negative control of level of spontaneous lysis was determined by leaving 6 wells untreated with extract. Optical density was measured using a BioTek Cytation3 plate reader. Cytotoxicity was calculated using the following formula:

$$\frac{(OD\ test\ -\ OD\ spontaneous)}{(OD\ maximum)} * 100 = \%\ cytotoxicity$$

IC_{50} was defined as the lowest concentration at which 50% of the maximum lysis was measured. Therapeutic index was defined as the following:

$$\frac{IC_{50}\ of\ extracts\ against\ human\ keratinocytes}{IC_{50} of\ extracts\ against\ P.\ gingivalis}$$

Chapter 4: Results

1. Literature Review of Oral Health Related Plants

In total, 513 plants were recorded and inputted into a database (for all plant information, see tables S1-9). When cross-checked with the extracts contained in the QNPL, 30 matches were identified. Of the 30 matches, 21 plants were determined to be high priority (priority levels 1 and 2) and were selected for screening. All QNPL extracts from various parts of these plants and various extract solvents were selected, totaling 109 extracts. Since 21 of the 109 extracts were determined to have high activity (see Results part 2) and were selected for further analysis, Table 4.1 contains identification information for each of these 21 extracts. The extracts are ordered alphabetically by family name, then genus name. This order will be followed for reporting of all other data, and plants will be referred to by extract number. See Table S10 for plant IDs of all 109 extracts screened for selection.

Table 4.1: Extract IDs

Extract Number	Family	Species	Part	Extract Solvent
1288	Anacardiaceae	*Pistacia lentiscus* L.	leaves	dH2O
1300	Anacardiaceae	*Pistacia lentiscus* L.	woody parts	95% Ethanol
1892	Anacardiaceae	*Pistacia lentiscus* L.	fruits	95% Ethanol
66	Fabaceae	*Vicia faba* L.	Leaves Flowers Whole Plant Roots Stems	EtOH
350	Fabaceae	*Vicia faba* L.		MeOH
638	Juglandaceae	*Carya tomentosa* (Poir.) Nutt.	Woody Parts	MeOH
640	Juglandaceae	*Carya tomentosa* (Poir.) Nutt.	Fruits	MeOH
126	Juglandaceae	*Juglans regia* L.	Immature Fruits	EtOH
195	Juglandaceae	*Juglans regia* L.	Woody Parts	EtOH

276	Juglandaceae	*Juglans regia* L.	Immature Fruits	MeOH
1511	Juglandaceae	*Juglans r.*	woody stems	95% EtOH
1512	Juglandaceae	*Juglans r.*	woody stems	MeOH
1795	Lauraceae	*Sassafras albidum* (Nutt.) Nees	stems	80% Ethanol
1161	Meliaceae	*Azadirachta indica* A.Juss.	Leaves Woody Stem	95% Ethanol
747	Myricaceae	*Myrica cerifera* L.	Leaves Flowers	MeOH
294	Oleaceae	*Olea europaea* L.	Leaves	MeOH
124	Rubiaceae	*Vitis vinifera var.aglianico* L.	Leaves	EtOH
336	Rubiaceae	*Vitis vinifera var.aglianico* L.	Leaves	MeOH
337	Rubiaceae	*Vitis vinifera var.aglianico* L.	Stems	MeOH
1111	Rutaceae	*Citrus sinensis* (L.) Osbeck	Woody Parts	MeOH
1290	Rutaceae	*Zanthoxylum armatum* DC.	fruits, seeds	95% Ethanol

Table 4.1: Extract IDs
Each extract number is paired with plant family, genus and species, part of plant, and extract solvent.

2. Single Concentration Screens: Selection of Extracts for Analysis

Screening all 109 extracts from 21 plants which were priority levels 1 and 2 at 256 µg/mL yielded 38 extracts from 17 plants which were chosen for further testing at 64 µg/mL and 32 µg/mL (fig4.1, table 4.2). Extracts were chosen if they demonstrated greater than 90% growth inhibition, standard deviations which included 90% growth inhibition, or high standard deviations (>15). Because MIC_{90} is the value of interest, and the exact percent of inhibition at 256 µg/mL was not a value of interest, extracts with high standard deviations were included in further tests rather than repeating the 256 µg/mL test. It was expected that testing at these lower concentrations would narrow down the list of extracts with very high anti- *P. gingivalis* activity, which would be promising targets for MIC testing.

Screening at these 64 µg/mL and 32 µg/mL yielded 21 extracts from 11 plants that were considered hits (>90% growth inhibition at 64 µg/mL). Ten of these extracts, from 7 plants, also had greater than 90% growth inhibition at 32 µg/mL (fig4.1, table 4.2). These 21 hits were then selected for MIC.

Single concentration screens data is reported in table 4.2 for the 21 extracts selected for MIC testing. For single concentration screen data from all 109 extracts screened, see table S10.

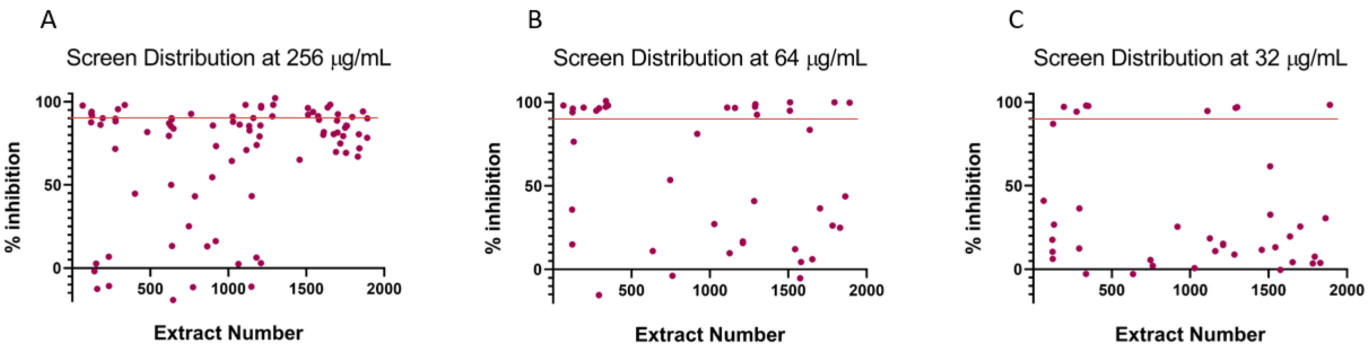

Fig 4.1: Distribution of % Inhibition for 109 Extracts at 256 µg/mL, 64 µg/mL and 32 µg/mL
The extracts are arranged by extract number (x-axis) and % of inhibition (y-axis), demonstrating the range of extract activity. Red lines represent 90% growth inhibition**.** (A) Distribution of inhibitory activity at 256 µg/mL. The 38 extracts which had greater than 90% inhibition, standard deviations crossing 90% inhibition, or high standard deviations, were chosen for the next screens. (B) Distribution of inhibitory activity at 64 µg/mL. All extracts with greater than or equal to 90% inhibition (red line) were chosen for further study (21 extracts). (C) Distribution of inhibitory activity at 32 µg/mL; 7 extracts had greater than 90% inhibition.

Table 4.2: Single Concentration Screens at 256 μg/mL, 64 μg/mL, and 32 μg/mL

Extract Number	Species	Average % Inhibition at 265 μg/mL	Average % Inhibition at 64 μg/mL	Average % Inhibition at 32 μg/mL
1288	*Pistacia lentiscus*	98.2 ± 0.6	97.36 ± 1.51	(-)105.21 ± 6.69
1300	*Pistacia lentiscus*	102.2 ± 6.07	92.55 ± 3.50	97.05 ± 2.02
1892	*Pistacia lentiscus*	90.09 ± 1.59	99.72 ± 0.96	98.41 ± 0.88
66	*Vicia faba*	97.75 ± 5.31	98.12 ± 0.55	41.00 ± 10.53
350	*Vicia faba*	108.11 ± 2.62	98.05 ± 0.24	97.76 ± 0.13
638	*Carya tomentosa*	273.47 ± 17.82	131.04 ± 6.17	107.09 ± 1.97
640	*Carya tomentosa*	359.06 ± 65.04	195.76 ± 9.93	154.45 ± 11.86
126	*Juglans regia*	91.79 ± 3.04	96.17 ± 1.26	86.98 ± 2.93
195	*Juglans regia*	90.19 ± 1.51	96.87 ± 0.36	97.25 ± 0.13
276	*Juglans regia*	89.89 ± 0.97	94.92 ± 1.97	94.36 ± 0.57
1511	*Juglans regia*	96.3 ± 3.31	94.99 ± 1.04	61.60 ± 9.02
1512	*Juglans regia*	92.19 ± 0.6	99.93 ± 0.32	32.61 ± 7.22
1795	*Sassafras albidum*	90.89 ± 1.25	99.93 ± 0.24	7.52 ± 0.98
1161	*Azadirachta indica*	90.29 ± 1.39	96.66 ± 1.37	10.85 ± 16.34
747	*Myrica cerifera*	25.13 ± 61.23*	53.5 ± 44.18*	5.53 ± 2.47

294	*Olea europaea*	106.61 ± 6.36	96.38 ± 1.05	36.37 ± 10.77
124	*Vitis vinifera var.aglianico*	93.99 ± 2.97	94.05 ± 0.15	6.22 ± 1.48
336	*Vitis vinifera var.aglianico*	98.1 ± 6.13	97.22 ± 0.48	(-)2.75 ± 5.57
337	*Vitis vinifera var.aglianico*	136.04 ± 14.59	100.77 ± 0.43	97.98 ± 0.63
1111	*Citrus sinensis*	98.2 ± 0.79	96.78 ± 0.32	94.79 ± 0.57
1290	*Zanthoxylum armatum*	117.12 ± 1.97	98.82 ± 0.84	96.6 ± 0.33

Table 4.2: Single Concentration Screens at 256 µg/mL, 64 µg/mL, and 32 µg/mL
Percent of growth inhibition is reported as averages with standard deviation (n=3). At 256 µg/mL, extracts with >90% growth inhibition, standard deviations allowing for >90% growth inhibition, or high standard deviations, were chosen for further study. Extracts with >90% growth inhibition at 64 µg/mL or 32 µg/mL were chosen for MIC. *Extract 747 was included in MIC; further tests were necessary to determine accurate anti-*P. gingivalis* activity due to high standard deviation in the single-concentration screens.

3. MIC

The 21 selected high-activity extracts were tested for growth inhibition against *P. gingivalis,* and MIC_{90} was determined for each extract (fig 4.2, table 4.3). MIC_{90} values ranged from 128 µg/mL (747) to 8 µg/mL (1892).

4. Cytotoxicity

All 21 high-activity extracts were tested for cytotoxic effects on human keratinocytes (fig 4.2, table 4.3). Extracts were tested at starting concentrations of 512 µg/mL (512-4 µg/mL) with the exception of samples 350 and 337, which were tested at starting concentrations of 128 µg/mL (128-1 µg/mL) due to low supply of extract in the QNPL. When IC_{50} was undetectable at the highest concentration testable, IC_{50} was reported as >512 µg/mL. None of the plant extracts tested had high cytotoxicity; IC_{50} ranged from 256 µg/mL to greater than 512 µg/mL. Sixteen samples had IC_{50} values higher than the possibility of testing, two had IC_{50} values of 512 µg/mL, one had an IC_{50} value of 256 µg/mL, and both samples tested at 128 µg/mL had undetectable toxicity. When IC_{50} was undetectable, therapeutic index was calculated using the highest concentration of extract tested, and reported as greater than the resulting value (table 4.3). Therapeutic indices ranged from 4 (1795) to greater than 256 (1892).

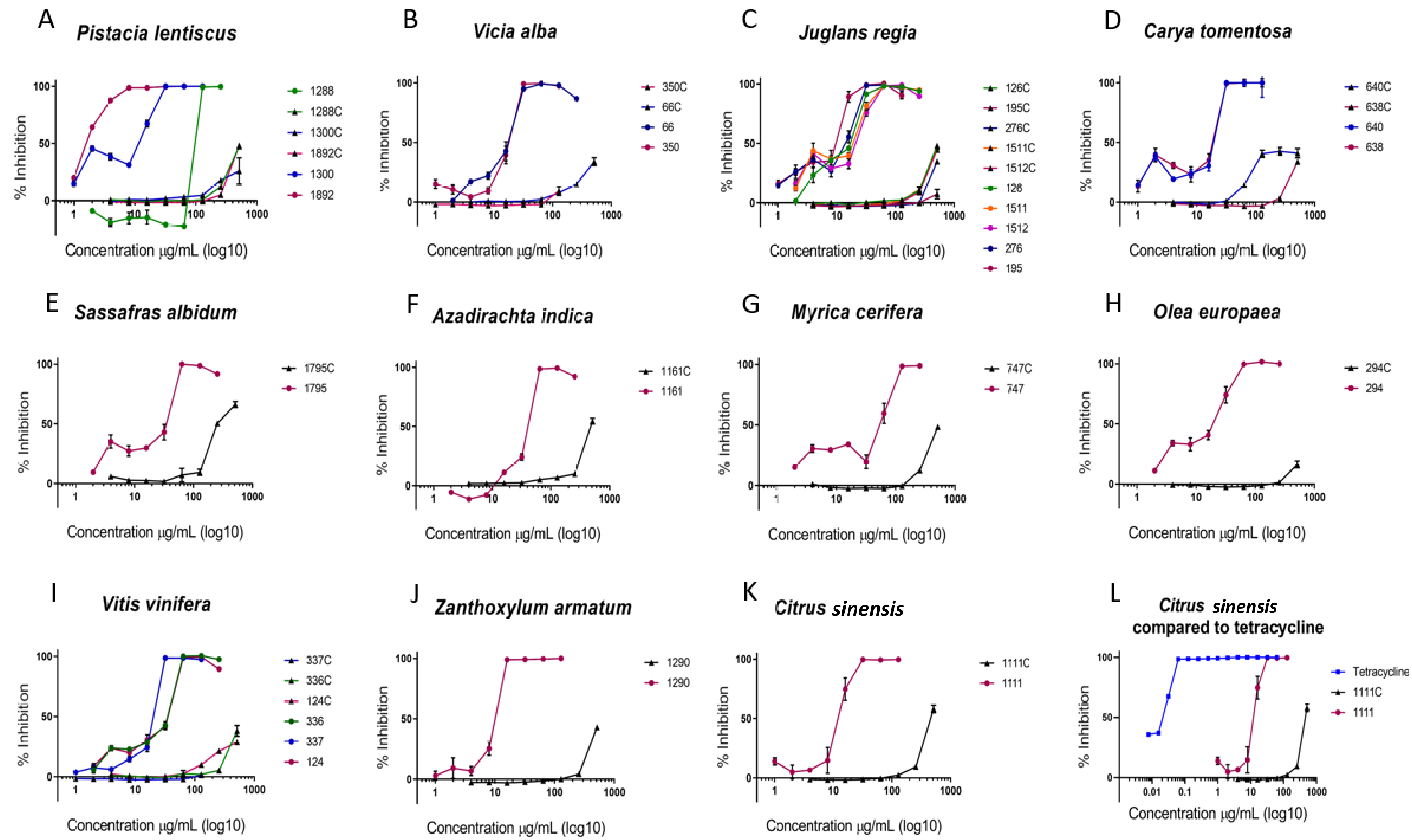

Fig. 4.2: MIC and Cytotoxicity Dose Response Curves

(A-K) 21 extracts from 11 plants are shown, representing different plant parts and extract solvents. Error bars represent SEM (n=3). Extract number with suffix C denotes cytotoxicity against HaCat cells. When error bars are not present, they are too small to be visible beyond the data point. Values are cutoff at 100% inhibition for visibility. (L) Tetracycline dose response curve is shown in relation to *Citrus sinensis* as an example of the scale of dose-response for a single-compound antibiotic compared to crude plant extracts.

Table 4.3: MIC$_{90}$ and Cytotoxicity Data from Selected Extracts

Extract Number	Species	MIC$_{90}$ *P. gingivalis* (µg/mL)	IC$_{50}$ *P. gingivalis* (µg/mL)	IC$_{50}$ HaCaT (µg/mL)	% toxicity at IC$_{50}$, or at highest concentration tested	Therapeutic Index
1288	*Pistacia lentiscus*	64	64	>512	47.51 ± 4.02	>8
1300	*Pistacia lentiscus*	32	16	>512	25.93 ± 20.02*	>32
1892	*Pistacia lentiscus*	8	2	>512	48.23 ± 2.62	>256
66	*Vicia faba*	32	32	>512	33.95 ± 5.84	>16
350	*Vicia faba*	32	32	>128**	9.28 ± 6.38	>4
638	*Carya tomentosa*	32	32	>512	33.76 ± 2.44	>16
640	*Carya tomentosa*	32	16	>512	41.04 ± 6.80	>16
126	*Juglans regia*	64	32	>512	48.06 ± 2.59	>16
195	*Juglans regia*	32	16	>512	7.37 ± 6.93	>32
276	*Juglans regia*	32	16	>512	34.72 ± 4.27	>32
1511	*Juglans regia*	64	32	>512	46.91 ± 2.16	>16
1512	*Juglans regia*	64	32	>512	44.53 ± 2.36	>16
1795	*Sassafras albidum*	64	64	256	50.44 ± 3.69	4
1161	*Azadirachta Indica*	64	64	512	54.49 ± 4.41	8

747	*Myrica cerifera*	128	64	>512	48.43 ± 2.14	>8
294	*Olea europaea*	64	32	>512	16.43 ± 4.93	>16
124	*Vitis vinifera var.aglianico*	64	64	>512	28.77 ± 3.65	>8
336	*Vitis vinifera var.aglianico*	32	64	>512	38.08 ± 7.74	>8
337	*Vitis vinifera var.aglianico*	32	32	>128**	1.3 ± 2.03	>4
1111	*Citrus sinensis*	32	16	512	58.08 ± 5.47	32
1290	*Zanthoxylum armatum*	16	16	>512	43.02 ± 3.77	>32

* high standard deviation due to one well with less volume
** highest concentration tested due to low supply of extract

Table 4.3: MIC_{90} and Cytotoxicity Data from Selected Extracts
Extract number is shown with MIC_{90} and IC_{50} against *P. gingivalis,* IC_{50} against human keratinocytes, percent of toxicity at the IC_{50} or the highest concentration tested if no IC_{50} was detectable, and therapeutic index. Error is standard deviation (n=3). MIC_{90}'s and IC_{50}'s are integer proportions with no relevant statistical error.

Chapter 5: Discussion

From a larger review of 513 plants that have been used for oral health across various traditional medicines, 109 extracts from 30 plants were tested, and 21 extracts from 11 plants were found to have high activity against *P. gingivalis.* A brief overview of each of these 11 plants follows:

1. *Pistacia lentiscus*:

From the Anacardiaceae family, this plant is a relative of the common pistachio, *Pistacia vera*. The resin from *P. lentiscus,* known as mastic, has been used medicinally in traditional medicinal systems widely in the middle east area [61] as well as other areas including Japan, where it is recorded in Kampo, the traditional medicinal system [62]. The mastic is recorded as being used for tooth disease, toothache, or gum inflammation [61, 62]. Crude extract from the fruits of *P. lentiscus* (sample 1892) had the lowest MIC_{90} in this study (MIC_{90} 8 µg/mL). While the fruits are not the part of the plant typically used for oral health, the fruits have been shown to have hepatoprotective and antidiabetic properties [63].

While the resin has been tested against *P. gingivalis* [64] [65] [66], there were no results in the literature for testing extracts of the fruits, which was the extract with the highest MIC_{90} in this study (1892). The chemistries of the resin [64], the fruits [63, 67], and the aerial parts [62] have been explored, which is a good starting point for bioactivity guided fractionation. Oleanolic acid (MIC_{90} 9.8) and 24Z-isomasticadienolic acid (MIC_{90} 2.4), from the resin had comparable MIC values to the crude extract of the fruits [64], so it would be fruitful to test whether the activity in the fruits may be due to these compounds by bioactivity guided fractionation, or if the activity is due to a different compounds present only or in much higher quantities in the fruits, which may be the case as other studies have not reported either compound in the fruits [63, 67].

2. *Vicia faba*

Vicia faba is the common fava bean. The ground dried beans have been used for sore mouth in North American traditional medicines [28]. The plant has also been used for prostate disorders and cancer in the Palestinian area [68], as a depilatory and diuretic in Bozüyük (Bilecik–Turkey) [69], for colitis and to get thorns out of skin in Italy [70], and for kidney problems in Turkey, [71], and for hemorrhoids, inflammation, and fever in 10^{th} century Cairo [72]. Crude whole-plant extract from *V. faba* had MIC_{90} as low as 32 µg/mL, highly promising for further study and pharmaceutical development, and this plant has not been tested against *P. gingivalis* before.

3. Carya Tomentosa

Carya tomentosa (more recently known as *Carya alba),* native to the southeastern United States, is a relative of the walnut often called mocker nut. The Cherokee used the inner bark of this plant to heal a sore mouth [73], and the plant is known as “wanéí” [74].

C. tomentosa (MIC_{90} 32 µg/mL) has not been previously tested against *P. gingivalis*; in fact nothing could be found in the literature about its chemical composition, although other *Carya* species like pecan have been tested for antitumor effects [75], antioxidant capacities [76], and mitigating effects of high fat diet [77].

4. *Juglans regia*

Juglans regia (best MIC_{90} 32 µg/mL) is commonly known as the Persian Walnut. In Pakistan and India, the stems and bark have been used for teeth cleaning as chewing sticks [28, 78]. Although the plant has not been previously tested against *P. gingivalis*, the compound juglone, known to be in *Juglans regia* [79], has activity against *P. gingivalis* [80] and has been shown to reduce periodontal biofilms of other bacteria [81]. These results in the literature make *J. regia* a promising target of further research, and much work has been done on its chemistry [82], which could help guide bioactivity guided fractionation.

5. *Sassafras albidum*

Commonly known as sassafras, *Sassafras albidum* (MIC_{90} 64 µg/mL) has been used as a chewing stick for teeth cleansing in the North America, particularly in the Appalachia and Ozarks regions [28, 78]. In addition to oral hygiene, the plant has been used for blood pressure, arthritis, insomnia, and to "clean the kidneys" by native inhabitants of the Appalachian region [83], and for diabetes symptoms by the Iroquois and Ojibwa [84]. The plant has not been tested against *P. gingivalis* but has activity against *Streptococcus* species [85-87].

6. *Azadirachta indica*

Azadirachta indica (MIC_{90} 64 µg/mL), is commonly called neem. It has been used as a chewing stick in Pakistan [88] and the Arabian Peninsula [89] [90]. In Karnataka, India, the powder of the inner bark is held in the mouth for half an hour for toothache [91]. It has also been used for a wide variety of other traditional uses; *A. indica* is recorded in Ayurveda for use in skin diseases, inflammation, and fevers, which have been verified in modern studies [92]. It is also known to be efficacious as an insect repellant [92].

A. indica has been found to have efficacy in vitro against periodontitis; a chip with the oil was placed in the pockets in gums, and *P.gingivalis* growth was reduced over time [93]. One study was found which previously tested for MIC *A. indica* against *P. gingivalis,* but the results (MIC 500 µg/mL) were unreliable due to lack of vehicle control as use of gentamycin, which is known to have no activity against *P. gingivalis*, as the antibiotic control [94].

The chemistry of *A. indica* has been previously explored, showing abundance of limonoids and phenolics [92].

7. Myrica cerifera

Myrica cerifera (MIC_{90} 128 µg/mL), also known as *Morella cerifera*, is commonly called the bayberry tree. Its root bark is made into a decoction for oral hygiene in the Southern U.S. [28]. The bark has also been used as a powder and decoction to prevent dental decay in the Florida area [90].

In addition to its uses for oral health, it has been used widely by Native Americans for other uses: Louisiana Choctaws used the boiled leaves and stems as a fever remedy, the Houma boiled the leaves as an antihelmintic, the Koasati used the plant for stomachache, the Micmac used the roots for headache and topically for inflammation [95] and the Nahua termed it Ahuaxochitl and used it for heart ailments [96]. It was also shown by modern science to have antithrombin activity [95].

No previous studies were found testing *M. cerifera* against *P. gingivalis.*

8*. Olea europaea*

Olea europaea (MIC_{90} 64 µg/mL) is the common olive. It has been used as a chewing stick in the middle east area [28], and the chewing stick from this plant is called "zaitoon" in Pakistan [88]. In addition to its uses in oral hygiene, in southern Africa, the leaves are used to make an eye lotion, to treat colds and throat problems, and as a styptic [97].

O. europaea extracts from both the leaves and oil have been tested against *P. gingivalis* [64]. The whole leaf crude extract was not reported, but individual compounds were tested, with the best compound being maslinic acid (MIC 4.9 µg/mL) [64]. This study provides as basis of comparison for the bioactivity guided fractionation of the crude leaf extract tested.

9*. Vitis vinifera*

The grape vine, *Vitis vinifera* (best MIC_{90} 32 µg/mL), has been used in oral hygiene as a toothpaste made from the ashes of burnt branches [28]. In addition, many parts of the plant have been recorded in Islamic scriptures as a treatment for a wide variety of ailments [98], and it has been used in Turkey as a tea for respiratory and intestinal ailments [99].

While the seeds and fruits have been tested against *P. gingivalis* [100], the stems and leaves, which were tested in this study, have not. One study showed extracts from seeds had inhibitory and antibiofilm activity against *P. gingivalis* only at very high concentrations (2000 µg/mL), so the values of 32 and 64 µg/mL from the leaves and stems in this study might indicate that the leaves/stems are a better source for pharmaceutical search [101]. The chemistry of the stalks has been evaluated [102, 103], which provides good background for beginning bioactivity guided fractionation.

10. *Citrus sinensis*

The orange tree, *Citrus sinensis* (MIC_{90} 32 µg/mL) has been used as a chewing stick in west Africa [78, 104], and in Malaya as a decoction made from the leaves for sore mouth [28].

No previous studies were found testing *Citrus sinensis* leaves or stems against *P. gingivalis*. One study found poor results testing a water extract of the fruit peel [105], and another found efficacy of a 4% ethanolic extract of the peel against gingival inflammation [106]. The chemistry of stems has been explored, and extracts have been tested against other oral pathogens [107].

11. *Zanthoxylum armatum*

Finally, *Zanthoxylum armatum* (MIC_{90} 16 µg/mL) is used as a chewing stick in India [28, 108]. Its common name is the winged prickly ash. Native Americans crush the bark and apply to gums for relief, and it is known as the toothache tree [109]. The fruits and bark have also been used in India for cancer and digestive ailments [108].

The chemistry of the plant has been explored, and it has been found to have strong inhibitory properties against many bacteria, but has not been tested against *P. gingivalis* [109].

These 11 plants represent a promising collection of natural products which could be further explored for use as pharmaceuticals. Each of these plants was used in various traditional medicinal systems as every-day oral hygiene care or as treatments for symptoms related to periodontitis; therefore, the traditional uses of these plants were verified by this study.

Future directions based on this research include biofilm testing and bioactivity guided fractionation. When the extracts are fractioned into component compounds, we expect to see even lower values for MIC and higher therapeutic indices. One likely product of these results could be a development of a mouthwash which includes the tested extracts and/or fractions from these extracts. Because they were found to have high growth inhibitory properties, and *P. gingivalis* is a slow-developing bacteria which can take years to start flourishing in the oral cavity, long-term growth inhibition as part of a daily oral hygiene routine could be highly effective. Periodontal infections affect 47.2% of adults over 30 and 70.1% of adults over 65, so developing long-term preventatives could have far-reaching impact, especially given that *P. gingivalis* has been linked to so many diseases, including cardiovascular disease, diabetes mellitus, respiratory infection, rheumatoid arthritis, osteoporosis, obesity, pre-term birth, and Alzheimer's disease [3].

Table 5.1: Comparison of Tested Extracts and Traditional Uses

QNPL Extract ID				**Traditional Use**			
Extract Number	**Family**	**Species**	**Part**	**Part Used**	**Medicinal System/ Area**	**Application**	**Citation**
1288	Anacardiaceae	*Pistacia lentiscus*	leaves	resin	Turkey	mastic	[28]
1300	Anacardiaceae	*Pistacia lentiscus*	woody parts	resin	Kampo: "Yo-Nyuko olibanum	chewing gums with oil extracts	[62]
1892	Anacardiaceae	*Pistacia lentiscus*	fruits				
66	Fabaceae	*Vicia faba*	Leaves Flowers Whole Plant Roots Stems	bean	North America	ground dried beans for sore mouth	[28]
350	Fabaceae	*Vicia faba*					
638	Juglandaceae	*Carya tomentos*	Woody Parts	inner bark	Cherokee	doctor chews inner bark and blows into mouth for sore mouth	[73]
640	Juglandaceae	*Carya tomentos*	Fruits				

126	Juglandaceae	*Juglans regia*	Immature Fruits	stem, bark	Pakistan, India	Chewing Sticks	[78], [28]
195	Juglandaceae	*Juglans regia*	Woody Parts				
276	Juglandaceae	*Juglans regia*	Immature Fruits				
1511	Juglandaceae	*Juglans regia*	woody stems				
1512	Juglandaceae	*Juglans regia*	woody stems				
1795	Lauraceae	*Sassafras albidum*	stems	twig	United States; particularly Appalachia and Ozarks	Chewing Sticks	[78], [28]
1161	Meliaceae	*Azadirachta indica*	Leaves Woody Stem	bark	Karnataka, India	powdered inner bark held in mouth for half and hour for toothache	[91]
				twig	Indian Subcontinent (called "datun")	Cleaning Sticks	[78], [93]
747	Myricaceae	*Myrica cerifera*	Leaves Flowers	root bark	Southern U.S.	decoction	[28]
294	Oleaceae	*Olea europaea*	Leaves	twig	Middle East	Chewing Sticks	[28]
124	Rubiaceae	*Vitis vinifera var.aglianico*	Leaves	ashes of burnt branches	England	Toothpaste	[28]
336	Rubiaceae	*Vitis vinifera var.aglianico*	Leaves				
337	Rubiaceae	*Vitis vinifera var.aglianico*	Stems				

1111	Rutaceae	*Citrus sinensis*	Woody Parts	twig, branch	W. Africa	Chewing Sticks	[78]
1290	Rutaceae	*Zanthoxylum armatum*	fruits, seeds	wood, bark	India	Chewing Sticks	[28]

Table 5.1: Comparison of Tested Extracts and Traditional Uses
(Left Column) Each sample tested is reported with family name, genus and species name, and part of plant. (Right Column) The part of each plant which is used in traditional medicine, the medicinal system or area of the world from which the medicinal use is documented, and the method of use for each plant. The part of each plant tested against *P. gingivalis* (left column) can be compared to the part of plant traditionally used for periodontitis symptoms (right column).

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Supplementary Materials:

Protocol Design Process: Culturing *Porphyromonas Gingivalis*
Several methods were tested to optimize *P. gingivalis* growth. The protocol design process is detailed in the following sample protocols and final protocol:

Method 1: *P. gingivalis* initial growth from freeze-dried

Preparation of Broth

Materials Required

TSB
BHI
Hemin
Menadione
K2HPO4
DI water

A) Preparation of Hemin Stock solution

1. Prepare solution with 0.5g hemin and 1.74g K2HPO4 in 100mL DI water. Boil for 5 minutes while stirring on hot plate.

*work in dark room since hemin is light sensitive

*wrap glass bottle in aluminum foil

B) Preparation of TSB supplemented broth (TSBhm)

1. Add 100µL hemin stock, 100 µg menadione, and 3g TSB to 100mL DI water.

*work in dark room since hemin and menadione are light sensitive

*pre-wrap glass bottle with aluminum foil

*weigh menadione in the dark

*to get 100 µg menadione, create stock solution by adding 10mg to 10mL of DI water, and add 100µL of this solution to the media

2. Autoclave 121ºC for 15 mins

C) Preparation of BHI supplemented broth (BHIhm)

1. Add 100µL hemin stock, 100 µg menadione, and 3.7g BHI to 100mL DI water.

2. Autoclave 121ºC for 15 mins

Preparation of Agar

Materials Required

TSB
BHI agar
Hemin Stock
Menadione
Agar
Yeast Extract
L-cysteine hydrochloride

A) Prepare TSB agar

1. Add 100µL hemin stock, 100 µg menadione, 1.5g agar, and 3g TSB to 100mL DI water.

    *work in dark room since hemin and menadione are light sensitive

    *pre-wrap glass bottle with aluminum foil

    *weigh menadione in the dark

2. Autoclave 121ºC for 15 mins

3. Pour agar into 5 plates, 20 mL each.

4. Store plates in opaque box.

B) Prepare BHI agar

1. Add 100µL hemin stock, 100 µg menadione, 5.2g BHI agar to 100mL DI water.

2. Repeat 2-4 from part A.

B) Prepare ATCC recommended agar

1. Add 100µL hemin stock, 100 µg menadione, 1.5g agar, 3g TSB, 0.5g yeast extract, and 0.05g l-cysteine hydrochloride to 100mL DI water.

2. Repeat 2-4 from part A.

Reviving Freeze-Dried *P. gingivalis*

Materials Required

TSB
BHI agar

Hemin Stock
Menadione
Agar
Yeast Extract
L-cysteine hydrochloride

1. Rehydrate bacteria pellet in 1 mL of TSBhm

    *work in dark room since hemin and menadione are light sensitive

2. Streak plates from the 1 mL solution:

    2 TSBhm plates

    2 BHIhm plates

    2 ATCC recommended plates

3. Incubate 1 of each plate in 37ºC and 1 of each plate in anaerobic chamber.

4. Prepare 3 tubes of 6mL TSBhm and 3 tubes of 6mL BHIhm.

5. Add 150 µL of bacteria solution to each tube.

6. Incubate 1 from each broth type in 37ºC, 1 in anaerobic chamber, and 1 in C02 incubator.

7. Check for growth after 24 hours.

Method 1 Results:

After incubating for 48 hours, there was no growth. The likely explanation was that the hemin and menadione, necessary for *P. gingivalis* survival, were degraded in the autoclave. Therefore, 5µg/mL hemin and 1µg/mL menadione were added to the two 6mL tubes in the anaerobic chamber containing the bacteria. At 72 hours post addition of extra supplements, growth was visible in the tubes. Freezer stocks were made from these tubes.

Test for Method 1: sBHI v.s. sTSB growth on 96-well plates, Ampicillin vs. Tetracycline

sBHI was chosen as the best media for *P. gingivalis* growth, and tetracycline was selected as the positive control for future experiments. Further protocol design was developed with sBHI only.

Method 2: *P. gingivalis* media and agar

Preparation of Hemin Stock Solution

Materials Required

Hemin powder
1M NaOH
ddH2O

1. Dissolve 250mg of hemin powder in 5mL of 1M NaOH

2. Add the dissolved hemin in NaOH to 495mL ddH2O.

3. Sterilize with a bottle-cap filter.

4. Wrap the bottle of stock solution in tin foil **(hemin is light sensitive)** and store at 4°C.

Note: the final concentration of hemin will be 0.5mg/mL

Preparation of Menadione Stock Solution

Materials Required

Menadione powder
100% EtOH

1. Dissolve 50mg of menadione powder in 25mL of 100% EtOH.

2. Sterilize with a bottle-cap filter.

3. Wrap the bottle of stock solution in tin foil **(menadione is light sensitive)** and store at 4°C.

Note: the final concentration of hemin will be 2mg/mL

Preparation of sBHI Broth

Materials Required

BHI
Hemin Stock
Menadione Stock

1. Make BHI as per standard BHI protocol.

2. After autoclaving, add appropriate amounts of menadione stock and hemin stock. Final concentration of menadione should be **1µg/mL** and final concentration of hemin should be **5µg/mL.**

See table for reference:

Volume Broth	500mL	400mL	300mL	200mL	100mL	50mL	25mL	10mL	5mL
Volume Hemin Stock	5mL	4mL	3mL	2mL	1mL	500uL	250uL	100uL	50uL
Volume Menadione Stock	250uL	200uL	150uL	100uL	50uL	25uL	12.5uL	5uL	2.5uL

Note: Add hemin and menadione directly to culture media instead of making a large stock. For example, when starting MIC and culturing 5mL of bacteria in a 14mL tube, add 50µL of hemin stock and 2.5µL of menadione stock directly to the 14mL tube.

Preparation of sBHI Agar

Materials Required

BHI agar powder
Hemin Stock
Menadione Stock
Yeast Extract
L-cysteine
ddH2O

1. Prepare BHI agar mix as per standard protocol.
2. Add 1g/L yeast extract
3. Autoclave at 121°C for 15 minutes.
4. Keep the agar mix stirring while it is cooling.
5. When the agar mix is cool enough to comfortably touch the glass, add 0.5g/L-cysteine, 500µL/L menadione stock, and 10mL/L hemin stock.
6. Pour 20mL of sBHI agar mix into each petri dish.
7. Store the dishes in an opaque cardboard box at 4°C.

Preparation of sBlood Agar

Materials Required

TSA powder
Yeast Extract
Hemin Stock
Menadione Stock
5% defibrinated Sheep's Blood

1. Prepare TSA agar mix as per standard protocol.
2. Add 2g/L yeast extract.
3. Autoclave at 121°C for 15 minutes.

4. Keep the agar mix stirring while it is cooling.
5. When the agar mix is cool enough to comfortably touch the glass, add 50mL/L defibrinated sheep's blood (***for final proportion of 5% sheep's blood)***, 500µL/L menadione stock, and 10mL/L hemin stock.
6. Pour 20mL of sBlood agar mix into each petri dish.
7. Store the dishes in an opaque cardboard box at 4°C.

Tables S1-9: Oral Health Plants Review

Table S1: Chewing Sticks

Family	Species (reported name)	Species (accepted name)	Part Used	Medicinal System	Application	Tested on P. gingivalis?	Citation
Amaranthaceae	*Achyranthes aspera* L.		branch	Arabia, Panama	Chewing Sticks	?	[28]
Amaranthaceae	*Achyranthes aspera* L.		stem	India	Chewing Sticks		[91]
Amaranthaceae	*Aerva tomentosa* Forssk.	*Aerva javanica* var. *bovei* Webb	root	Red sea area	Chewing Sticks	?	[28]
Anacardiaceae	*Rhus natalensis* Bernh. ex C.Krauss	*Searsia natalensis* (Bernh. ex C.Krauss) F.A. Barkley		Kenya	Chewing Sticks	Y	[110] [110], [78]
Anacardiaceae	*Sorindeia warneckei* Engl.	*Sorindeia grandifolia* Engl.		Nigerian	Chewing Sticks	Y, ATCC33277	[111]
Apocynaceae	*Funtumia elastica* (Preuss) Stapf			Ghana	Chewing Sticks	?	[28]
Apocynaceae	*Landolphia owariensis* P.Beauv.			Tropical Africa	Chewing Sticks	?	[28]
Arecaceae	*Cocos nucifera* L.		peduncle	Middle East	Chewing Sticks	?	[28]
Asclepiadaceae	*Gongronema latifolium* Benth.		stem	Sierra Leone	Chewing Sticks	?	[28]
Asteraceae	*Vernonia amygdalina* Delile		roasted root, stem	W. Africa	Chewing Sticks	?	[28]
Asteraceae	*Vernonia amygdalina* Delile		Root and stem	Nigerian	Chewing Sticks	Y, ATCC33277	[111]
Asteraceae	*Vernonia colorata* (Willd.) Drake	*Gymnanthemum coloratum* (Willd.) H.Rob & B.Kahn	roasted root, stem	W. Africa	Chewing Sticks	?	[28]

Betulaceae	*Alnus glutinosa* (L.) Gaertn.		inner bark	England	Chewing Sticks	N, active against gram+ bacteria and myrobacterium	[28]
Betulaceae	*Betula lenta* L.			United States; particularly Appalachia and ozarks	Chewing Sticks	N, active against gram+ bacteria and myrobacterium	[78], [28]
Betulaceae	*Betula lutea* (Britton) Rehder	*Betula alleghaniensis* Britton		United States; particularly Appalachia and ozarks	Chewing Sticks	N, active against gram+ bacteria and myrobacterium	[78], [28]
Bignoniaceae	*Newbouldia laevis* (P.Beauv.) Seem.		twig	Nigerian	Chewing Sticks	?	[28]
Bignoniaceae	*Stereospermum kunthianum* Cham.		stem	W. Africa	Chewing Sticks	?	[28]
Boraginaceae	*Cordia gharaf* Ehrenb. ex Asch.	*Cordia sinensis* Lam.	twig	Middle East	Chewing Sticks	?	[28]
Boraginaceae	*Ehretia thonningiana* R.Br. ex Fresen.	*Ehretia cymose* Thonn.	stem	Tropical Africa	Chewing Sticks	?	[28]
Capparidaceae	*Maerua crassifolia* Forssk.		twig	W. Africa	Chewing Sticks	?	[28]
Clusiaceae	*Allanblackia floribunda* Oliv.		Root; Twig	W. Africa	Chewing Sticks	?	[28]
Clusiaceae	*Allanblackia parviflora* A.Chev.		Root; Twig	W. Africa	Chewing Sticks	?	[28]
Clusiaceae	*Garcinia afzelii* Engl.		Twig; root; twig; root, stem	W. Africa	Chewing Sticks	?	[28]
Clusiaceae	*Garcinia kola* Heckel		Twig; root; twig; root, stem	W. Africa	Chewing Sticks	?	[28]
Clusiaceae	*Garcinia mangostana* L.		Twig; root; twig; root, stem	W. Africa	Chewing Sticks	?	[28]
Clusiaceae	*Garcinia mannii* Oliv.		Twig; root; twig; root, stem	W. Africa	Chewing Sticks	?	[28]
Clusiaceae	*Pentadesma butyracea* Sabine		Root	W. Africa	Chewing Sticks	?	[28]

Combretaceae	*Anogeissus leiocarpus* (DC.) Guill. & Perr.	*Anogeissus leiocarpa* (DC.) Guill. & Perr.		Nigerian	Chewing Sticks	Y, ATCC33277	[111], [28]
Combretaceae	*Guiera senegalensis* J.F.Gmel		root, stem	W. Africa	Chewing Sticks	?	[28]
Combretaceae	*Terminalia glaucescens* Planch. ex Benth.		root	W. Africa	Chewing Sticks	?	[28]
Combretaceae	*Terminalia glaucescens*			Nigerian	Chewing Sticks	Y, ATCC33277	[111]
Connaraceae	*Agelaea obliqua* (P.Beauv.) Baker	*Agelaea pentagyna* (Lam.) Baill.	Fruit	W. Africa	Chewing Sticks	?	[28]
Connaraceae	*Agelaea trifolia* (Lam.) Baill.	*Agelaea pentagyna* (Lam.) Baill.	stem	Nigerian	Chewing Sticks	?	[28]
Connaraceae	*Castanola paradoxa* Schellenb.	*Agelaea paradoxa* Gilg	Stem	W. Africa	Chewing Sticks	?	[28]
Connaraceae	*Manotes expansa* Sol. ex Planch.		stem	Liberia	Chewing Sticks	?	[28]
Cornaceae	*Cornus florida* L.		Twig	United States	Chewing Sticks	?	[28]
Dichapetalaceae	*Dichapetalum guineense* (DC.) Keay	*Dichapetalum madagascariense* Poir.	Stem	W. Africa	Chewing Sticks	?	[28]
Ebenaceae	*Diospyros barteri* Hiern		stem	Ghana	Chewing Sticks	?	[28]
Ebenaceae	*Diospyros elliotii* (Hiern) F.White		twig, stem	W. Africa	Chewing Sticks	?	[28]
Ebenaceae	*Diospyros heudelotii* Hiern		twig	Seirra Leone	Chewing Sticks	?	[28]
Ebenaceae	*Diospyros loureiroana* G.Don			S. Africa	Chewing Sticks	?	[28]
Ebenaceae	*Diospyros lycioides* Desf.		root	Namibia, Zambia	Chewing Sticks	N	[78], [28]

Ebenaceae	*Diospyros tricolor* (Schumach. & Thonn.) Hiern		twig, stem	W. Africa	Chewing Sticks	?	[28]
Ebenaceae	*Euclea divinorum* Hiern			Kenya	Chewing Sticks	Y	[110], [78], [28]
Ebenaceae	*Euclea fructuosa* Hiern	*Euclea natalensis* subsp. *obovata* F.White		Kenya	Chewing Sticks	Y	[110], [78], [28]
Ebenaceae	*Euclea multiflora* Hiern	*Euclea natalensis* subsp. *obovata* F.White		Kenya	Chewing Sticks	Y	[110], [78], [28]
Ericaceae	*Gaultheria procumbens* L.		root	United States; particularly Appalachia and ozarks	Chewing Sticks	?	[78], [28]
Euphorbiaceae	*Acalypha fruticosa* Forssk.		twig	E. Africa	Chewing Sticks	?	[28]
Euphorbiaceae	*Alchornea cordifolia* (Schumach. & Thonn.) Mull.Arg		Root, stem	W. Africa	Chewing Sticks	?	[28]
Euphorbiaceae	*Antidesma venosum* E.Mey. ex Tul.		twig	Nigerian	Chewing Sticks	?	[28]
Euphorbiaceae	*Cleidion gabonicum* Baill.		twig	Ghana	Chewing Sticks	?	[28]
Euphorbiaceae	*Drypetes floribunda* (Mull.Arg) Hutch.		Stem	W. Africa	Chewing Sticks	?	[28]
Euphorbiaceae	*Jatropha curcas* L.		stem	India	Chewing Sticks	?	[28]
Euphorbiaceae	*Mallotus oppositifolius* (Geiseler) Mull.Arg			W. Africa	Chewing Sticks	?	[28]
Euphorbiaceae	*Phyllanthus engleri* Pax		fruit	Tanzania	Chewing Sticks	?	[28]

Euphorbiaceae	*Phyllanthus muellerianus* (Kuntze) Exell		twig, bark strips	Nigerian	Chewing Sticks	?	[28]
Euphorbiaceae	*Phyllanthus reticulatus* Poir.		twig	S. Africa	Chewing Sticks	?	[28]
Euphorbiaceae	*Tetrorchidium didymostemon* (Baill.) Pax & K.Hoffm.		stem	S. Africa	Chewing Sticks	?	[28]
Fabaceae	*Abrus schimperi* Baker		stem	E. Africa	Chewing Sticks	?	[28]
Fabaceae	*Acacia arabica* (Lam.) Willd.	*Acacia nilotica* (L.) Delile	twig	Tropical Africa	Chewing Sticks	?	[28]
Fabaceae	*Acacia kamerunensis* Gand.		pounded root	Ghana	Chewing Sticks	?	[28]
Fabaceae	*Acacia modesta* Wall.		twig	Pakistan	Chewing Sticks	?	[28]
Fabaceae	*Acacia nilotica* (L.) Delile			Tropical Africa	Chewing Sticks	?	[28]
Fabaceae	*Acacia pennata* (L.) Willd.			Tropical Africa	Chewing Sticks	?	[28]
Fabaceae	*Acacia pentagona* (Shum. & Thonn.) Hook.f.		twig	Tropical Africa	Chewing Sticks	?	[28]
Fabaceae	*Baphia nitida* Lodd.		stem	W. Africa	Chewing Sticks	?	[28]
Fabaceae	*Burkea africana* Hook.		twig	Nigerian	Chewing Sticks	?	[28]
Fabaceae	*Cassia auriculata* L.	*Senna auriculata* (L.) Roxb.	twig	India	Chewing Sticks	?	[28]
Fabaceae	*Cassia sieberiana* DC.		root	Seirra Leone	Chewing Sticks	?	[28]
Fabaceae	*Daniellia oliveri* (Rolfe) Hutch. & Dalziel		twig	Ghana	Chewing Sticks	?	[28]
Fabaceae	*Delonix elata* (L.) Gamble		twig	E. Africa	Chewing Sticks	?	[28]

Fabaceae	*Dialium guineense* Willd.		twig	W. Africa	Chewing Sticks	?	[28]
Fabaceae	*Distemonanthus benthamianus* Baill.			Nigerian	Chewing Sticks	Y, ATCC33277	[111]
Fabaceae	*Eriosema griseum* Baker		stem	W. Africa	Chewing Sticks	?	[28]
Fabaceae	*Griffonia simplicifolia* (DC.) Baill.		stem	W. Africa	Chewing Sticks	?	[28]
Fabaceae	*Humboldtia laurifolia* M. Vahl		twig	Ceylon	Chewing Sticks	?	[28]
Fabaceae	*Hymenostegia afzelii* (Oliv.) Harms		twig	W. Africa	Chewing Sticks	?	[28]
Fabaceae	*Lonchocarpus spp.*			W. Africa	Chewing Sticks	?	[28]
Fabaceae	*Mezoneuron spp.*	*Caesalpinia spp.*	twig	Liberia	Chewing Sticks	?	[28]
Fabaceae	*Millettia thonningii* (Schum. & Thonn.) Baker		stem, peeled bark	Nigerian	Chewing Sticks	?	[28]
Fabaceae	*Piliostigma reticulatum* (DC.) Hochst.	*Bauhinia reticulata* (DC.)	root, twig	Tropical Africa	Chewing Sticks	?	[28]
Fabaceae	*Piliostigma thonningii* (Schum.) Milne-Redh.	*Bauhinia thonningii* Schum.	root, twig	Tropical Africa	Chewing Sticks	?	[28]
Fabaceae	*Pongamia pinnata* (L.) Pierre		twig	Ceylon	Chewing Sticks	?	[28]
Fabaceae	*Pterocarpus marsupium* (Roxb.)		twig	India	Chewing Sticks	?	[28]
Fabaceae	*Tamarindus indica* (L.)		twig	W. Africa	Chewing Sticks	?	[28]
Flacourtiaceae	*Casearia barteri* Mast.		Stem	W. Africa	Chewing Sticks	?	[28]
Hamamelidaceae	*Liquidambar styraciflua* L.			United States; particularly Appalachia and ozarks; Eastern U.S., Mexico	Chewing Sticks	?	[78], [28]

Juglandaceae	*Carya alba* (L.) Nutt. ex Elliot		twig	United States; particularly Appalachia and ozarks	Chewing Sticks	?	[28]
Juglandaceae	*Carya laciniosa* (F.Michx.) G.Don		twig	United States; particularly Appalachia and ozarks	Chewing Sticks	?	[28]
Juglandaceae	*Carya pallida* (Ashe) Engelm. & Graebn.		twig	United States; particularly Appalachia and ozarks	Chewing Sticks	?	[28]
Juglandaceae	*Juglans regia* L.		stem, bark	Pakistan	Chewing Sticks	N	[78], [28]
Lamiaceae	*Ocimum gratissimum* L.		twig	Ghana	Chewing Sticks	?	[28]
Lamiaceae	*Vitex simplicifolia* Oliv.	*Vitex madiensis* Oliv.	stem	Nigerian	Chewing Sticks	?	[28]
Lamiaceae	*Vitex simplicifolia* Oliv.	*Vitex madiensis* Oliv.	stem	Nigerian	Chewing Sticks	?	[28]
Lauraceae	*Lindera benzoin* (L.) Blume			United States; particularly Appalachia and ozarks	Chewing Sticks	?	[78], [28]
Lauraceae	*Sassafras albidum* (Nutt.) Nees		twig	United States; particularly Appalachia and ozarks	Chewing Sticks	?	[78], [28]
Lecthidaceae	*Napoleonaea vogelii* Hook. & Planch.		twig, bark strips	W. Africa	Chewing Sticks	?	[28]
Loganiaceae	*Strychnos afzelii* Gilg		stem	W. Africa	Chewing Sticks	?	[28]
Meliaceae	*Azadirachta indica* A.Juss.		twig	Indian Subcontinent; called “datun”	Cleaning Sticks	N	[78], [93]

Meliaceae	*Carapa procera* DC.		twig	W. Africa	Chewing Sticks	?	[28]
Meliaceae	*Khaya senegalensis* (Desv.) A.Juss.		peeled stem	Nigerian	Chewing Sticks	?	[28]
Menispermaceae	*Penianthus zenkeri* (Engl). Diels		Twig, root	W. Africa	Chewing Sticks	?	[28]
Menispermaceae	*Sphenocentrum jollyanum* Pierre		Root	W. Africa	Chewing Sticks	?	[28]
Menispermaceae	*Tiliacora dielsiana* Hutch. & Dalziel		stem	W. Africa	Chewing Sticks	?	[28]
Musaceae	*Musa sapientum* L.	*Musa paradisiaca* L.	pounded peduncle	Ghana	Chewing Sticks	?	[28]
Nyssacea	*Nyssa sylvatica* Marshall		twig	United States	Chewing Sticks	?	[28]
Olacaceae	*Coula edulis* Baill.			W. Africa	Chewing Sticks	?	[28]
Olacaceae	*Olax gambecola* Baill.		Twig	W. Africa	Chewing Sticks	?	[28]
Olacaceae	*Olax subscorpioides* Oliv.		Twig	W. Africa	Chewing Sticks	?	[28]
Oleaceae	*Ligustrum medium* Franch. & Sav.	*Ligustrum ovalifolium* Hassk.	stem	Japan	Chewing Sticks	?	[28]
Oleaceae	*Olea europaea* L.		twig	Middle East	Chewing Sticks	?	[28]
Pandanaceae	*Pandanus spp.*		pedicel	Oceania	Chewing Sticks	?	[28]
Passifloraceae	*Androsiphonia adenostegia* Stapf.		twig	Liberia	Chewing Sticks	?	[28]
Passifloraceae	*Smeathmannia pubescens* Sol. ex R.Br.		twig	Liberia	Chewing Sticks	?	[28]
Poaceae	*Bothriochloa saccharoides* (Sw.) Rydb.			United States	Chewing Sticks	?	[28]
Poaceae	*Saccharum officinarum* L.		stem	United States	Chewing Sticks	?	[28]

Poaceae	*Zizania aquatica* L.		stem	United States	Chewing Sticks	?	[28]
Polygalaceae	*Carpolobia alba* G.Don		twig; stem	W. Africa	Chewing Sticks	?	[28]
Polygalaceae	*Carpolobia lutea* G.Don		twig; stem	W. Africa	Chewing Sticks	?	[28]
Rhamnaceae	*Gouania lupuloides* (L.) Urb.		stem	United States; Florida	Chewing Sticks	?	[28]
Rhamnaceae	*Gouania polygama* Lam.		twig	Honduras	Chewing Sticks	?	[28]
Rhamnaceae	*Lasiodiscus mildbraedii* Engl.		stem	W. Africa	Chewing Sticks	?	[28]
Rosaceae	*Parinari curatellifolia* Planch. ex Benth.		twig, bark strips	W. Africa	Chewing Sticks	?	[28]
Rosaceae	*Potentilla rubra* Haller f.	*Comarum palustre* L.	root	Mexico	Chewing Sticks	?	[28]
Rubiaceae	*Adina microcephala* (Delile) Hiern	*Breonadia salicina* (Vahl) Hepper & J.R.I.Wood	twig	Tropical Africa	Chewing Sticks	?	[28]
Rubiaceae	*Aulacocalyx jasminiflora* Hook.f.		twig	W. Africa	Chewing Sticks	?	[28]
Rubiaceae	*Aulacocalyx jasminiflora* Hook.f.			Tropical Africa	Chewing Sticks	?	[28]
Rubiaceae	*Coffea ebracteolata* (Hiern) Brenan		stem	W. Africa	Chewing Sticks	?	[28]
Rubiaceae	*Craterispermum caudatum* Hutch.		Stem	W. Africa	Chewing Sticks	?	[28]
Rubiaceae	*Craterispermum laurinum* (Poir.) Benth.		Stem	W. Africa	Chewing Sticks	?	[28]
Rubiaceae	*Massularia acuminata* (G.Don) Bullock ex Hoyle		stem	Nigerian	Chewing Sticks	Y, ATCC33277	[111], [28]
Rubiaceae	*Mussaenda afzelii* G.Don		calyx lobe	W. Africa	Chewing Sticks	?	[28]

Rubiaceae	*Mussaenda erythrophylla* Schumach. & Thonn.		root	Tropical Africa	Chewing Sticks	?	[28]
Rubiaceae	*Nauclea latifolia* Sm.	*Neonauclea excelsa* (Sm.) E.A.Bruce	root	Nigerian, Ghana	Chewing Sticks	Y, ATCC33277	[111], [28]
Rubiaceae	*Oxyanthus speciosus* DC.		twig	Tropical Africa	Chewing Sticks	?	[28]
Rubiaceae	*Oxyanthus tenuis* Stapf	*Oxyanthus subpunctatus* (Hiern) Keay	twig	Tropical Africa	Chewing Sticks	?	[28]
Rubiaceae	*Psychotria subobliqua* Hiern			W. Africa	Chewing Sticks	?	[28]
Rubiaceae	*Psychotria vogeliana* Benth.			Tropical Africa	Chewing Sticks	?	[28]
Rubiaceae	*Randia maculate* DC.	*Rothmannia longiflora* Salisb.	stem	Ghana	Chewing Sticks	?	[28]
Rubiaceae	*Rothmannia longiflora* Salisb.			Tropical Africa	Chewing Sticks	?	[28]
Rubiaceae	*Sarcocephalus esculentus* Afzel. ex Sabine	*Sarcocephalus latifolius* (Sm.) E.A.Bruce	root	W. Africa	Chewing Sticks	?	[28]
Rubiaceae	*Uncaria talbotii* Wernham		stem	Ghana	Chewing Sticks	?	[28]
Rutaceae	*Aegle marmelos* (L.) Correa		twig	India	Chewing Sticks	?	[28]
Rutaceae	*Cassia sleberlanba* Oliv.			W. Africa	Chewing Sticks	N	[78]
Rutaceae	*Citrus aurantiifolia* (Christm.) Swingle			W. Africa	Chewing Sticks	N	[78]
Rutaceae	*Citrus sinensis* (L.) Osbeck		peeled twig	Ghana	Chewing Sticks	?	[28]
Rutaceae	*Clausena anisate* (Willd). Hook.f. ex Benth.		stem	W. Africa	Chewing Sticks	?	[28]

Rutaceae	*Glycosmis pentaphylla* (Retz.) DC.		twig	India	Chewing Sticks	?	[28]
Rutaceae	*Teclea verdoorniana* Exell & Mendonca	*Vepris verdoorniana* (Exell & Mendonca) Mziray	twig	W. Africa	Chewing Sticks	?	[28]
Rutaceae	*Zanthoxylum alatum* Roxb.	*Zanthoxylum armatum* DC.	wood, bark	India	Chewing Sticks	?	[28]
Rutaceae	*Zanthoxylum chalybeum* Engl.		twig	S. Africa	Chewing Sticks	?	[28]
Rutaceae	*Zanthoxylum deremense* (Engl.) Kokwaro		twig	S. Africa	Chewing Sticks	?	[28]
Rutaceae	*Zanthoxylum viride* (A.Chev.) P.G. Waterman			Ghana	Chewing Sticks	?	[28]
Rutaceae	*Zanthoxylum zanthoxyloides* (Lam.) Zepern. & Timler		stem	W. Africa	Chewing Sticks	?	[28]
Salicaceae	*Populus spp.*		twig	United States; particularly Appalachia and ozarks	Chewing Sticks	?	[78], [28]
Salicaceae	*Salix lucida* Muhl.		twig	United States; particularly Appalachia and ozarks	Chewing Sticks	?	[28]
Salvadoraceae	*Salvadora persica* L.		Roots, twigs, stems	Greek, Roman, India, Middle East, Africa	Chewing Sticks	Y, ATCC33277	[78], [112], [113]
Sapindaceae	*Allophylus africanus* P.Beauv.		root, twig	W. Africa	Chewing Sticks	?	[28]
Sapindaceae	*Lecaniodiscus cupanoides* Planch. ex Benth.		stem	W. Africa	Chewing Sticks	?	[28]

Sapindaceae	*Paullinia pinnata* L.		root	Tropical Africa	Chewing Sticks	?	[28]
Sapotaceae	*Butyrospermum paradoxum* (C.F. Gaertn.) Hepper	*Vitellaria paradoxa* C.F.Gaertn.		Nigerian	Chewing Sticks	Y, ATCC33277	[111]
Sapotaceae	*Synsepalum dulcificum* (Schumach. & Thonn.) Daniell		twig	W. Africa	Chewing Sticks	?	[28]
Smilacaceae	*Smilax prolifera* Roxb.	*Smilax perfoliata* Lour.	stem	Himalayas	Chewing Sticks	?	[28]
Sterculiaceae	*Cola laurifolia* Mast.		twig	W. Africa	Chewing Sticks	?	[28]
Sterculiaceae	*Nesogordonia papaverifera* (A. Chev.) Capuron ex N. Halle		twig	W. Africa	Chewing Sticks	?	[28]
Tiliaceae	*Glyphaea brevis* (Spreng.) Monach.		twig	W. Africa	Chewing Sticks	?	[28]
Tiliaceae	*Grewia mollis* Juss.		bark, leaf	W. Africa	Chewing Sticks	?	[28]
Velloziaceae	*Vellozia equisetoides* (Baker) Baker	*Xerophyta equisetoides* Baker	stem	Tanzania	Chewing Sticks	?	[28]
Verbenaceae	*Lantana trifolia* L.		twig	E. Africa	Chewing Sticks	?	[28]
Violaceae	*Rinorea subintegrifolia* (P.Beauv.) Kuntze		Stem	W. Africa	Chewing Sticks	?	[28]

Table S2: Native American Plants

Family	Species (reported name)	Species (accepted name)	Part Used	Medicinal System	Application	Tested on P. gingivalis?	Citation
Acoracae	*Acorus calamus* L.		root	Shinnecock	for halitosis	periodontitis symptoms	[28]
Anacardiaceae	*Rhus copallinum* L.		berries	Delaware	mouthwash for halitosis	periodontitis symptoms	[28]
Anacardiaceae	*Rhus glabra* L.		bark, leaves	Jemez, Sanpoil	chewed	used for periodontitis	[28]
Anacardiaceae	*Rhus hirta* (L.) Sudw.	*Rhus typhina* L.	gall-infected leaves	Ojibwa	infusion	periodontitis symptoms	[28]
Anacardiaceae	*Rhus trilobata* Nutt.		bark, leaves	Jemez, Sanpoil	chewed	used for periodontitis	[28]

Anacardiaceae	*Rhus virens* Lindh. ex A. Gray		bark, leaves	Jemez, Sanpoil	chewed	used for periodontitis	[28]
Apiaceae	*Angelica tomentosa* S.Watson		root	Pomo, Kashaya	chewed for halitosis	periodontitis symptoms	[28]
Aspelniaceae	*Asplenium pseudofalcatum* Hillebr.		leaf, but, fruit	Hawaiian	leaf ashes, nut juice, fruit milk mixed	periodontitis symptoms	[28]
Asteraceae	*Achillea lanulosa* Nutt.	*Achillea millefolium* L.	leaf, stem	Crow, "paswat"	tea held in mouth for sore gums	?	[114] [115]
Asteraceae	*Artemisia campestris* L.		leaves	Blackfoot	chewed for halitosis	periodontitis symptoms	[28]
Asteraceae	*Artemisia tilesii* Ledeb.		aboveground parts	Tanana	for mouth sores	periodontitis symptoms	[28]
Asteraceae	*Balsamorhiza sagittate* (Nutt.) Nutt.		root	Cheyenne, Blackfoot	chewed for mouth sores	?	[114] [28]
Asteraceae	*Chrysopsis graminifolia* (Michx.) Elliott	*Pityopsis graminifolia* (Michx.) Elliott	entire plant	Choctaw	ashes rubed on gums to treat mouth sores	?	[114]
Asteraceae	*Cirsium remotifolium* (Hook.) DC.		root	Kwakiutl	infusion	periodontitis symptoms	[28]
Asteraceae	*Echinacea angustifolia* DC.		leaf, root	Cheyenne	tea or chewed for sore mouth and gums	?	[114]
Asteraceae	*Echinacea pallida* (Nutt.) Nutt.		root, leaves	Cheyenne	powdered	used for periodontitis	[28]
Asteraceae	*Gnaphalium obtusifolium* L.	*Pseudognaphalium obtusifolium* (L.) Hilliard & B.L.Burtt.		Cherokee	chewed for sore mouth	periodontitis symptoms	[28]
Asteraceae	*Gutierrezia sarothrae*		root	Shosone	sucked for mouth sores	?	[114]

	(Pursh) Britton & Rusby						
Asteraceae	*Haplopappus spinulosus* (Pursh) DC.	*Xanthisma spinulosum* (Pursh) D.R.Morgan & R.L.Hartm.	leaf, root	Navajo	rubbed on gums	?	[114]
Asteraceae	*Pityopsis graminifolia* (Michx.) Nutt.		whole plant	Choctaw	ashes	periodontitis symptoms	[28]
Asteraceae	*Sanvitalia aberti* A. Gray		whole plant	Navajo	mouthwash or chewed for sore mouth	periodontitis symptoms	[114] [28]
Asteraceae	*Schkuhria multiflora* Hook. & Arn.		whole plant	Navajo	chewed for mouth sores	?	[114]
Asteraceae	*Solidago californica* Nutt.		?	Miwok	Tea held in mouth for toothache	?	[114]
Asteraceae	*Solidago odora* Aiton		root	Cherokee	chewed	periodontitis symptoms	[28]
Asteraceae	*Stenotus lanuginosus* (A.Gray) Greene		plant	Navajo		used for periodontitis	[28]
Asteraceae	*Vernonia noveboracensis* (L.) Michx.		root	Cherokee	Tea for soft gums	?	[114]
Asteraceae	*Xanthium strumarium* L.		burs	Paiute	rubbed on gums	used for periodontitis	[28]
Berberidaceae	*Berberis vulgaris* L.		root, bark	Micmac, penobscot	pounded	used for periodontitis	[28]
Berberidaceae			plant	Hopi		used for periodontitis	[28]

	Mahonia fremontii (Torr.) Fedde					
Berberidaceae	*Mahonia spp.*	bark	Cowlitz	mouthwash for sore mouth	periodontitis symptoms	[28]
Betulaceae	*Alnus incana* (L.) Moench	bark	Malecite	for mouth ulcers	periodontitis symptoms	[28]
Caprifoliaceae	*Lonicera involucrata* (Richarson) Banks ex Spreng.	leaves	Quinault	chewed for sore mouth	periodontitis symptoms	[28]
Crassulaceae	*Sedum integrifolium* (Raf.) A.Nelson	roots	Eskimo	chewed	periodontitis symptoms	[28]
Crassulaceae	*Sedum spathulifolium* Hook.	plant	Thompson		used for periodontitis	[28]
Cupressaceae	*Juniperus occidentalis* Hook.	leaf	Shoshoni	poultice	used for periodontitis	[28]
Ericaceae	*Andromeda polifolia* L.		Southwest Native Americans; "Hobefzobal"	used for periodontitis	?	[115]
Ericaceae	*Arctostaphylos uva-ursi* (L.) Spreng.	plant	Blackfoot	infusion	used for periodontitis	[28]
Ericaceae	*Menziesia ferruginea* Sm.	nectar	Hesquiat	eated for halitosis	periodontitis symptoms	[28]
Ericaceae	*Oxydendrum arboreum* (L.) DC	bark	Cherokee	chewed for mouth ulcers	periodontitis symptoms	[28]

Fabaceae	*Desmodium nudiflorum* (L.) DC.		root	Cherokee	chewed	used for periodontitis	[28]
Geraniaceae	*Geranium maculatum* L.		root, leaf	Meswaki, Ojibwa	infusion	used for periodontitis	[28]
Geraniaceae	*Geranium oreganum* Howell		root, leaf	Meswaki, Ojibwa	infusion	used for periodontitis	[28]
Grossulariaceae	*Ribes lobbii* A. Gray		roots	Kwakiutl	poultice	periodontitis symptoms	[28]
Haemodoraceae	*Lachnanthes caroliniana* (Lam.) Dandy		root	Cherokee	decoction for sore mouth	periodontitis symptoms	[28]
Hydrophyllaceae	*Hydrophyllum virginianum* L.		root	Iroquois	chewed; decoction	periodontitis symptoms	[28]
Juglandaceae	*Carya laciniosa* (F.Michx.) G.Don		bark	Cherokee	chewed	periodontitis symptoms	[28]
Juglandaceae	*Carya pallida* (Ashe) Engelm. & Graebn.		bark	Cherokee	chewed	periodontitis symptoms	[28]
Juglandaceae	*Carya tomentosa* (Lam.) Nutt.	*Carya alba* (L.) Nutt. ex Elliot	inner bark	Cherokee	doctor chews inner bark and blows into mouth for sore mouth	?	[73]
Juglandaceae	*Juglans cinerea* L.		buds	Iroquois	infusion	periodontitis symptoms	[28]
Lamiaceae	*Mentha canadensis* L.		Flowers	Cree	ground, rubbed on gums	used for periodontitis	[28]

Lauraceae	*Persea planifolia*	seed	Mahuna	powder	used for periodontitis	[28]
Malvaceae	*Sphaeralcea fendleri* A. Gray	whole plant	Navajo, Kayenta	Infusion	periodontitis symptoms	[28]
Moraceae	*Artocarpus altilis* (Parkinson ex F.A.Zorn) Fosberg	Latex	Hawaiian	for mouth sores	periodontitis symptoms	[28]
Myrtaceae	*Syzygium malaccense* (L.) Merr. & L.M.Perry	bark, whole plant	Hawaiian	juice of whole plant	periodontitis symptoms	[28]
Nyctaginaceae	*Abronia fragrans* Nutt. ex Hook.		Navajo Ramah	Infusion	used for periodontitis	[28]
Nymphaeaceae	*Nymphaea odorata* Aiton	Root	Chippewa	pulverized root for mouth sores	periodontitis symptoms	[28]
Olaceae	*Ximenia americana* L.	root	Seminole	mouthwash	used for periodontitis	[28]
Oleaceae	*Syringa vulgaris* L.	bark, leaves	Iroquois	chewed by children	periodontitis symptoms	[28]
Oxalidaceae	*Oxalis corniculata* L.	leaf	Cherokee	chewed for sore mouth	periodontitis symptoms	[28]
Oxalidaceae	*Oxalis stricta* L.	whole plant	Iroquois	infusion for halitosis	periodontitis symptoms	[28]
Pedaliaceae	*Prosopis velutina* (Wooton) Britton & Rose	Gum	Pima	Decoction	used for periodontitis	[28]

Pinaceae	*Abies grandis* (Douglas ex D.Don) Lindl.		root	Kwakiutl	root held in mouth	used for periodontitis	[28]
Pinaceae	*Abies lasiocarpa* (Hook.) Nutt.		needles	Flathead	pounded and mixed with lard	used for periodontitis	[28]
Pinaceae	*Picea glauca* (Moench) Voss		young tips	Tanana	decoction with blackberry stems	periodontitis symptoms	[28]
Pinaceae	*Picea sitchensis* (Bong.) Carriere		pitch	Haisla, Hanaksiala	chewed for halitosis	periodontitis symptoms	[28]
Pinaceae	*Pseudotsuga menziessi*	*Pseudotsuga menziesii* (Mirb.) Franco	bud tips	Swinomish	Chewed	periodontitis symptoms	[28]
Polemoniaceae	*Phlox gracilis* (Douglas ex Hook.) Greene	*Microsteris gracilis* (Douglas ex Hook.) Greene	whole plant	Navajo, Ramah	Poultice	periodontitis symptoms	[28]
Polygalaceae	*Polygala senega* L.		root	Cree	for sore mouth	periodontitis symptoms	[28]
Polygonaceae	*Eriogonum spp.*		flowers, leaves	Southwest Native Americans; "Pasvaat"	infusion, mouthwash; for periodontisis held in mouth a few minutes daily		[115]
Polygonaceae	*Polygonum amphibium* L.	*Persicaria amphibia* (L.) Delarbre	roots	Cree	poultice for mouth blisters	periodontitis symptoms	[28]

Polygonaceae	*Rumex crispus* L.	leaf	Navajo, Ramah	infusion	periodontitis symptoms	[28]
Polygonaceae	*Rumex hymenosepalus* Torr.	root	Pima	root held in mouth	used for periodontitis	[28]
Polypodiaceae	*Pleopeltis polypodioides* (L.) E.G. Andrews & Windham	Frond	Houma	mouthwash	used for periodontitis	[28]
Polypodiaceae	*Polypodium glycyrrhiza* D.C. Eaton	Rhizomes	Bella Coola, Hesquiat	eaten for halitosis	periodontitis symptoms	[28]
Polypodiaceae	*Polypodium hesperium* Maxon	rhizomes	Thompson		used for periodontitis	[28]
Primulaceae	*Dodecatheon pulchellum* (Raf.) Merr.	leaf	Blackfoot	infusion for children	periodontitis symptoms	[28]
Ranunculaceae	*Coptis trifolia* (L.) Salisb.	root	Potawatomi	infusion	used for periodontitis	[28]
Ranunculaceae	*Xanthorhiza simplicissima* Mashall	stem	Cherokee	chewed	periodontitis symptoms	[28]
Rhamnaceae	*Ceanothus americanus* L.	bark	Iroquois	decoction for sore mouth	periodontitis symptoms	[28]

Rhamnaceae	*Ceanothus fendleri* A.Gray	leaf	Keres	chewed	periodontitis symptoms	[28]
Rosaceae	*Fragaria vesca* L.	root	Iroquois	decoction	periodontitis symptoms	[28]
Rosaceae	*Geum triflorum* Pursh	root	Blackfoot	infusion	used for periodontitis	[28]
Rosaceae	*Malus coronaria* (L.) Mill.	bark	Cherokee	infusion for sore mouth	periodontitis symptoms	[28]
Rosaceae	*Prunus americana* Marshal	root bark	Meskwaki	Astringent	periodontitis symptoms	[28]
Rosaceae	*Prunus emarginata* (Douglas ex Hook.) Walp.	root	Kwakiutl	poultice for mouth sores for children	periodontitis symptoms	[28]
Rosaceae	*Rosa spp.*	roots	Crow	Infusion	used for periodontitis	[28]
Rosaceae	*Rubus spp.*	stem	Tanana	decoction with *Picea glauca*	periodontitis symptoms	[28]
Salicaceae	*Populus tremuloides* Minchx.	Leaves	Haisla, Hanaksiala		used for periodontitis	[28]
Salicaceae	*Salix arbusculoides* Andersson	leaves	Eskimo, Kuskokwagmuit	chewed	periodontitis symptoms	[28]
Salicaceae	*Salix fuscescens* Andersson	leaves	Eskimo	chewed		[28]

						periodontitis symptoms	
Salicaceae	*Salix humilis* Marshall		roots	Catawba	mouthwash	periodontitis symptoms	[28]
Salicaceae	*Salix nigra* Marshall			Iroquois		used for periodontitis	[28]
Salicaceae	*Salix planifolia* Pursh		whole plant	Eskimo, Nunivak	chewed	periodontitis symptoms	[28]
Salicaceae	*Salix rotundifolia* Trautv.		whole plant	Eskimo, Nunivak	chewed	periodontitis symptoms	[28]
Salicaceae	*Salix sericea* Marshall			Iroquois		used for periodontitis	[28]
Salicaceae	*Salix tristis* Aiton	*Salix humilis* var. *tristis* (Aiton) Griggs	root	Catawba	mouthwash for sore gums	?	[73]
Santalaceae	*Comandra umbellata* (L.) Nutt.		whole plant	Navajo, Kayenta	mouthwash	periodontitis symptoms	[28]
Saxifragaceae	*Heuchera cylindrica* Douglas		root	Thompson	chewed	used for periodontitis	[28]

Table S3: Plants Used for Periodontitis and Periodontitis Symptoms

Family	Species (reported name)	Species (accepted name)	Part Used	Medicinal System	Application	Tested on P. gingivalis?	Citation	Notes
Acanthaceae	*Barleria prionitis* L.					used for periodontitis	[28]	
Aizoaceae	*Carpobrotus acinaciformis* (L.) L.Bolus		fruit, leaf	S. Africa	boiled; for sore mouth	periodontitis symptoms	[28]	
Aliaceae	*Allium sativum* L.		bulb	Europe, India	chewed for toothache	used for periodontitis	[28] [91]	
Anacardiaceae	*Mangifera indica* L.		leaf	India	chewed	Y	[116]	
Anacardiaceae	*Pistacia lentiscus* L.			Turkey	mastic	used for periodontitis	[28]	
Anacardiaceae	*Rhus glabra* L.		root, bark, leaves	Jemez, Sanpoil	bark and leaves chewed; root decoction	used for periodontitis	[28]	native north american
Anacardiaceae	*Rhus trilobata* Nutt.		bark, leaves	Jemez, Sanpoil	chewed	used for periodontitis	[28]	native north american
Anacardiaceae	*Rhus virens* Lindh. ex A. Gray		bark, leaves	Jemez, Sanpoil	chewed	used for periodontitis	[28]	native north american
Apiaceae	*Heracleum maximum* W. Bartram		root	Eastern U.S.	powder rubbed on gums	used for periodontitis	[28]	
Asteraceae	*Echinacea pallida* (Nutt.) Nutt.		root, leaves	Cheyenne	powdered	used for periodontitis	[28]	native north american
Asteraceae	*Solidago virgaurea* L.		plant	England	decoction	used for periodontitis	[28]	
Asteraceae	*Spilanthes mauritiana*		Flowers	England, S. Africa	chewed	used for periodontitis	[28]	

	(A.Rich. ex Pers.) DC.							
Asteraceae	*Stenotus lanuginosus* (A.Gray) Greene		plant	Navajo		used for periodontitis	[28]	native north american
Asteraceae	*Xanthium strumarium* L.		burs	Paiute	rubbed on gums	used for periodontitis	[28]	native north american
Berberidaceae	*Berberis vulgaris* L.		root, bark	Micmac, penobscot	pounded	used for periodontitis	[28]	native north american
Berberidaceae	*Mahonia fremontii* (Torr.) Fedde		plant	Hopi		used for periodontitis	[28]	native north american
Bignonaceae	*Tecomaria capensis* (Thunb.) Spach	*Tecoma capensis* (Thunb.) Lindl.	bark	S. Africa	powder rubbed on gums	used for periodontitis	[28]	
Burseraceae	*Commiphora myrrha* (Nees) Engl.		resin	Near East	resin	used for periodontitis	[28]	
Cornaceae	*Cornus florida* L.		bark	Eastern North America	decoction for sore mouth	periodontitis symptoms	[28]	native north american
Crassulaceae	*Sedum spathulifolium* Hook.		plant	Thompson		used for periodontitis	[28]	native north american
Cupressaceae	*Juniperus occidentalis* Hook.		leaf	Shoshoni	poultice	used for periodontitis	[28]	native north american
Ebenaceae	*Diospyros virginiana* L.		bark	Eastern North America	boiled bark decoction for sore mouth of babies	periodontitis symptoms	[28]	native north american
Eleocarpaceae	*Elaeocarpus floribundus* Blume					used for periodontitis	[28]	

Ericaceae	*Arctostaphylos uva-ursi* (L.) Spreng.		plant	Blackfoot	infusion	used for periodontitis	[28]	native north american
Euphorbiaceae	*Acalypha phleoides* Cav.		plant	Mexico	mouthwash	used for periodontitis	[28]	
Euphorbiaceae	*Flueggea virosa* (Roxb. ex Willd.) Royle		boiled roots	Philiphines	mouthwash	used for periodontitis	[28]	
Euphorbiaceae	*Gelonium multiflorum* A.Juss.	*Suregada multiflora* (A.Juss.) Baill.	Bark	Himalayas	tonic	used for periodontitis	[28]	
Euphorbiaceae	*Hymenocardia acida* Tul.		roots	S. Africa	ashed roots for mouth infection	periodontitis symptoms	[28]	
Euphorbiaceae	*Jatropha dioica* Sesse			Mexico	tea	used for periodontitis	[28]	
Euphorbiaceae	*Sapium ellipticum* (Hochst.) Pax	*Shirakiopsis elliptica* (Hochst.) Esser	bark	Central Africa	decoction as mouthwash (for ulceration of gums and loose teeth caused by scurvy)	periodontitis symptoms	[28]	
Fabaceae	*Acacia pennata* (L.) Willd.		Leaves + sugar and cumin	Himalayas	chewed	used for periodontitis	[28]	
Fabaceae	*Acacia spp.*			India		used for periodontitis	[28]	contain tannins
Fabaceae	*Albizia lebbeck* (L.) Benth.		root bark	Himalayas	powdered	used for periodontitis	[28]	
Fabaceae	*Desmodium nudiflorum* (L.) DC.		root	Cherokee	chewed	used for periodontitis	[28]	native north american
Fabaceae	*Glycyrrhiza glabra* L.		roots	Himalayas		used for periodontitis	[28]	
Fabaceae	*Mimosa palmeri* Rose		Bark	Mexico	chewed	used for periodontitis	[28]	

Fabaceae	*Vicia faba* L.		bean	North America	ground dried beans for sore mouth	periodontitis symptoms	[28]	native north american
Fagaceae	*Quercus alba* L.		Bark	North America	decoction for sore mouth	periodontitis symptoms	[28]	native north american
Fagaceae	*Quercus spp.*			India		used for periodontitis	[28]	contain tannins
Geraniaceae	*Geranium maculatum* L.		root, leaf	Meswaki, Ojibwa	infusion	used for periodontitis	[28]	native north american
Geraniaceae	*Geranium oreganum* Howell		root, leaf	Meswaki, Ojibwa	infusion	used for periodontitis	[28]	native north american
Juglandaceae	*Juglans regia* L.			India		used for periodontitis	[28]	contain tannins
Krameriaceae	*Krameria triandra* Ruiz & Pav.	*Krameria lappacea* (Dombey) Burdet & B.B. Simpson		India		used for periodontitis	[28]	contain tannins
Lamiaceae	*Mentha canadensis* L.		Flowers	Cree	ground, rubbed on gums	used for periodontitis	[28]	native north american
Lamiaceae	*Thymus vulgaris* L.					used for periodontitis	[28]	phenolic thymol
Lauraceae	*Persea planifolia*		seed	Mahuna	powder	used for periodontitis	[28]	native north american
Meliaceae	*Aglaia spp.*		bark	Philiphines	chewed	used for periodontitis	[28]	
Myricaceae	*Myrica cerifera* L.	*Morella cerifera* (L.) Small.	root bark	Southern U.S.	decoction	used for periodontitis	[28]	
Myrtaceae	*Syzygium aromaticum* (L.) Merr. & L.M. Perry					Y	[28] [117]	eugenol

Nyctaginaceae	*Abronia fragrans* Nutt. ex Hook.			Navajo Ramah	infusion	used for periodontitis	[28]	native north american
Olaceae	*Ximenia americana* L.		root	Seminole	mouthwash	used for periodontitis	[28]	native north american
Orchidaceae	*Dendrobium nobile* Lindl.			China	Polyherbal	used for periodontitis	[28]	
Pedaliaceae	*Prosopis velutina* Wooton		gum	Pima	decoction	used for periodontitis	[28]	native north american
Pinaceae	*Abies grandis* (Douglas ex D.Don) Lindl.		root	Kwakiutl	root held in mouth	used for periodontitis	[28]	native north american
Pinaceae	*Abies lasiocarpa* (Hook.) Nutt.		needles	Flathead	pounded and mixed with lard	used for periodontitis	[28]	native north american
Pinaceae	*Pseudotsuga taxifolia* (Lindl.) Britton	*Pseudotsuga menziesii* (Mirb.) Franco	bud tips	U.S.	chewed for mouth sores	periodontitis symptoms	[28]	native north american
Polygonaceae	*Eriogonum atrorubens* Engelm.		root	Mexico	chewed	used for periodontitis	[28]	
Polygonaceae	*Rumex hymenosepalus* Torr.		root	Pima	root held in mouth	used for periodontitis	[28]	native north american
Polypodiaceae	*Drynaria fortunei* (Kunze ex Mett.) J.Sm.	*Drynaria roosii* Nakaike		China	Polyherbal	used for periodontitis	[28]	

Polypodiaceae	*Pleopeltis polypodioides* (L.) E.G. Andrews & Windham		frond	Houma	mouthwash	used for periodontitis	[28]	native north american
Polypodiaceae	*Polypodium hesperium* Clute		rhizomes	Thompson		used for periodontitis	[28]	native north american
Pteridaceae	*Pellaea calomelanos* (Sw.) Link		rhizomes	S. Africa	decoction for mouth cankers	periodontitis symptoms	[28]	
Ranunculaceae	*Aconitum napellus* L.					used for periodontitis	[28]	alkaloids
Ranunculaceae	*Coptis groenlandica* (Oeder) Fernald	*Coptis trifolia* subsp. *Groenlandica* (Oeder) Hulten	roots	Eastern North America	chewed for mouth sores	periodontitis symptoms	[28]	native north american
Ranunculaceae	*Coptis trifolia* (L.) Salisb.		root	Potawatomi	infusion	used for periodontitis	[28]	native north american
Ranunculaceae	*Hydrastis canadensis* L.		roots	Eastern North America	chewed for mouth sores	used for periodontitis	[28]	alkaloids
Rhamnaceae	*Gouania lupuloides* (L.) Urb.		stem	West Indies		used for periodontitis	[28]	
Rosaceae	*Fragaria vesca* L.		fruit, root, leaves	England	lotions and gargles for ulcers and sore mouth	used for periodontitis	[28]	
Rosaceae	*Geum triflorum* Pursh		root	Blackfoot	infusion	used for periodontitis	[28]	native north american
Rosaceae	*Potentilla fulgens* T.T.Yu & C.L.Li	*Potentilla lineata* Trevir.	root	Himalayas	tonic	used for periodontitis	[28]	

Rosaceae	*Rosa spp.*		roots	Crow	infusion	used for periodontitis	[28]	native north american
Rubiaceae	*Psychotria spp.*		sap	Solomon Islands	decoction for sore mouth	periodontitis symptoms	[28]	
Rubiaceae	*Uncaria gambir* (Hunter) Roxb.			India		used for periodontitis	[28]	contain tannins
Rutaceae	*Citrus aurantifolia*	*Citrus aurantiifolia* (Christm.) Swingle	leaf	Malaya	decoction for sore mouth	periodontitis symptoms	[28]	
Rutaceae	*Zanthoxylum alatum* Roxb.	*Zanthoxylum armatum* DC.	stick	Himalayas	chewed	used for periodontitis	[28]	alkaloids
Rutaceae	*Zanthoxylum capense* (Thunb.) Harv.		root	S. Africa	decoction for mouth ulcers	periodontitis symptoms	[28]	
Salicaceae	*Populus tremuloides* Michx.		leaves	Haisla, Hanaksiala		used for periodontitis	[28]	native north american
Salicaceae	*Salix nigra* Marshall			Iroquois		used for periodontitis	[28]	native north american
Salicaceae	*Salix sericea* Marhsall			Iroquois		used for periodontitis	[28]	native north american
Sapotaceae	*Mimusops elengi* L.			India		used for periodontitis	[28]	contain tannins
Saxifragaceae	*Heuchera cylindrica* Douglas		root	Thompson	chewed	used for periodontitis	[28]	native north american
Solanaceae	*Lycium barbarum* L.			China	Polyherbal	used for periodontitis	[28]	
Solanaceae	*Lycium chinense* Mill.			China	Polyherbal	used for periodontitis	[28]	
Solanaceae	*Solanum merkeri* Dammer					used for periodontitis	[28]	

Solanaceae	*Solanum verbascifolium* L.	unresolved	leaf	Solomon Islands	decoction for sore mouth	periodontitis symptoms	[28]
Symplocaceae	*Symplocos racemose* Roxb.		bark	India	decoction gargled	used for periodontitis	[28]

Table S4: Plants Used for Tooth Coloring

Family	Species	Species (accepted name)	Part Used	Medicinal System	Application	Tested on P. gingivalis?	Citation	Notes
Annonaceae	*Hexalobus senegalensis* A. DC.	*Hexalobus monopetalus* (A.Rich.) Engl. & Diels	fruits	Nigeria, Morroco; Bornu, Adamawa, Wanuamwezi	reddening			
Arecaceae	*Cocos nucifera* L.		fruit rind	Japan, "Ohaguro"	blackening; tannic extract mixed with iron			tannins
Bigoniaceae	*Arrabidaea chica* (Bonpl.) Verl.	*Fridericia chica* (Bonpl.) L.G. Lohmann			blackening	active against Streptococcus sanguis and Actinomyces naeslundii	[28]	polyphenolic substances
Cucurbitaceae	*Luffa petola* Ser.	*Luffa cylindrica* (L.) M.Roem.	gourd juice	Japan, "Ohaguro"	blackening; tannic extract mixed with iron			tannins
Euphorbiaceae	*Agrostistachys borneensis* Becc.		stems	Dyaks of Singhi, Malaysia	blackening			tannins
Euphorbiaceae	*Antidesma spp.*		bark	Philiphines	blackening; ashes from burned bark			tannins
Euphorbiaceae	*Homonoia riparia* Lour.		juice	Java	blackening			tannins
Fagaceae	*Quercus cyclophora* Endl.	*Lithocarpus cyclophorus* (Endl.) A.Camus	acorns	Japan, "Ohaguro"	blackening; tannic extract mixed with iron			tannins

Icacinaceae	*Calatola costaricensis* Standl.			blackening	active against Streptococcus sanguis and Actinomyces naeslundii	[28]	polyphenolic substances
Melastomataceae	*Melastoma malabathricum* L.	woody parts	Singapore	blackening; wood tar			tannins
Moraceae	*Ficus religiosa* L.	resin	Gujarat	reddening			
Nyctaginaceae	*Neea floribunda* Poepp. & Endl.			blackening	active against Streptococcus sanguis and Actinomyces naeslundii	[28]	polyphenolic substances
Nyctaginaceae	*Neea parviflora* Poepp. & Endl.		Caqueta, Columbia/Peru; Putumayo, Columbia/Pero	blackening			
Rubiaceae	*Manettia divaricate* Wernham		Peru	blackening	active against Streptococcus sanguis and Actinomyces naeslundii	[28]	polyphenolic substances
Rubiaceae	*Paederia foetida* L.	bark	Philiphines	blackening; ashes from burned bark			tannins
Rubiaceae	*Schradera marginalis* Standl.		Citara, Columbia	blackening, chewed			
Sterculiaceae	*Cola acuminata* (P.Beauv.) Schott & Endl.	nut	Hausa	tooth coloring; chewed			

Table S5: Chewing Sponges

Family	Species	Part Used	Medicinal System	Application	Citation
Combretaceae	*Terminalia glaucescens* Planch. ex Benth.	inner fiber of branches	Ghana	Chewing Sponge	[28]
Combretaceae	*Terminalia ivorensis* A.Chev.	inner fiber of branches	Ghana	Chewing Sponge	[28]
Fabacea	*Acacia pennata* (L.) Willd.	inner fiber of branches	Ghana	Chewing Sponge	[28]
Malvaceae	*Hibiscus rosa-sinensis* L.	inner fiber of branches	Ghana	Chewing Sponge	[28]
Malvaceae	*Hibiscus rostellatus* Guill. & Perr.	inner fiber of branches	Ghana	Chewing Sponge	[28]
Stemonuraceae	*Lasianthera Africana* P.Beauv.	inner fiber of branches	Ghana	Chewing Sponge	[28]

Table S6: Plants used for Toothpastes

Family	Species (reported name)	Species (accepted name)	Part Used	Medicinal System	Application	Tested on P. gingivalis?	Citation
Acoraceae	*Acorus calamus* L.		powdered root	Europe/North America	Toothpaste	?	[28]
Anacardiaceae	*Lannea grandis* Engl.	*Lannea coromandelica* (Houtt.) Merr.	powdered bark	India	Toothpaste	?	[28]
Burseraceae	*Commiphora myrrha* (Nees) Engl.		powdered gum resin	Near East	Toothpaste	?	[28]
Combretaceae	*Myrobalanifera spp.*		powdered fruit	India	Toothpaste	?	[28]
Ericaceae	*Gaultheria procumbens* L.		cortex	North America	Toothpaste	?	[28]
Fabaceae	*Caesalpinia pulcherrima* (L.) Sw.			Nicaragua	Toothpaste	?	[28]
Fabaceae	*Peltophorum pterocarpum* (DC.) K.Heyne		powdered bark	Southeast Asia	Toothpaste	?	[28]
Krameriaceae	*Krameria triandra* Ruiz & Pav.	*Krameria lappacea* (Dombey) Burdet & B.B. Simpson	powdered root	Bolivia, Peru	Toothpaste	?	[28]
Lessoniaceae	*Macrocystis pyrifera* L. C.Ag.		?		Toothpaste	?	[28]
Polygonaceae	*Rumex crispus* L.		powdered root	North America	Toothpaste	?	[28]
Rhamnaceae	*Gouania lupuloides* (L.) Urb.		powdered stem	Central America	Toothpaste	?	[28]
Rubiaceae	*Cinchona officinalis* L.		powdered bark	Europe/North America	Toothpaste	?	[28]
Rubiaceae	*Vitis vinifera* L.		ashes of burnt branches	England	Toothpaste	?	[28]

Table S7: Cleaning Gums and Quids

Family	Species (reported name)	Species (accepted name)	Part Used	Medicinal System	Application	Citation
Anacardiaceae	*Toxicodendron diversilobum* (Torr. & A.Gray) Greene		plant	Karok	Gum	[28]
Apiaceae	*Osmorhiza occidentalis* (Nutt.) Torr.		roots	Blackfoot	Gum	[28]
Apocynaceae	*Apocynum cannabinum* L.		latex	Kiowa	Gum	[28]
Asceliadaceae	*Asclepias spp.*		latex, seed silk	Kawaiisu, Karok, Mendocino, Acoma, Zuni	Gum	[28]
Asceliadaceae	*Sarcostemma cynanchoides* Decne.		sap boiled	Pima	Gum	[28]
Asteraceae	*Agoseris aurantiaca* (Hook.) Greene		root	Karok	Gum	[28]
Asteraceae	*Chloracantha spinosa* (Benth.) G.L.Nesom		stems	Navajo	Gum	[28]
Asteraceae	*Chrysothamnus spp.*		roots	Paiute, Gosiute	Gum	[28]
Asteraceae	*Dugaldia hoopesii* (A.Gray) Rydb.	*Hymenoxys hoopesii* (A.Gray) Bierner	roots	Navajo	Gum	[28]
Asteraceae	*Encelia farinosa* A.Gray ex Torr.		gum	Papago, Pima	Gum	[28]
Asteraceae	*Hymenopappus filifolius* Hook.		root	Zuni	Gum	[28]

Asteraceae	*Hymenoxys richardsonii* (Hook.) Cockerell		root	Navajo, Isleta, Keres Western	Gum	[28]
Asteraceae	*Lactuca tatarica* (L.) C.A.Mey		root	Apache White Mountain, Navajo, Zuni	Gum	[28]
Asteraceae	*Lygodesmia spp.*		root	Lakota, Navajo Ramah, Washo	Gum	[28]
Asteraceae	*Senecio spp.*		root, latex	Navajo Ramah, Aleut	Gum	[28]
Asteraceae	*Silphium laciniatum* L.		stems	Dakota, Omaha, Pawnee, Ponca	Gum	[28]
Asteraceae	*Stephanomeria pauciflora* (Torr.) A.Nelson		root, fuzz	Kawaiisu, Navajo, Paiute	Gum	[28]
Asteraceae	*Stephanomeria spinosa* (Nutt.) Tomb	*Pleiacanthus spinosus* (Nutt.) Rydb.	root, fuzz	Kawaiisu, Navajo, Paiute	Gum	[28]
Asteraceae	*Tragopogon porrifolius* L.		latex	Thompson	Gum	[28]
Chenopodiaceae	*Chenopodium californicum* S.Watson		latex	Cahuilla	Gum	[28]
Cupressaceae	*Thuja plicata* Donn. ex D.Don		resin	Kwakiutl Southern, Montana Indian	Gum	[28]
Euphorbiaceae	*Croton xalapensis* Hook.f.		gum	Mexico	Gum	[28]
Euphorbiaceae	*Euphorbia marginata* Pursh			Kiowa	Gum	[28]
Fabaceae	*Myroxylon balsamum* (L.) Harms		gum	South America	Gum	[28]

Geraniaceae	*Erodium cicutarium* (L.) L'Her.	roots	Digueno	Gum	[28]
Hamamelidaceae	*Liquidambar styraciflua* L.	gum	Cherokee	Gum	[28]
Lamiaceae	*Mentha spp.*	fresh leaves	Kiowa	Gum	[28]
Moraceae	*Ficus spp.*		Seminole	Gum	[28]
Pinaceae	*Abies amabilis* (Douglas ex Loudon) J.Forbes	pitch, inner bark gum	Nitinaht, Shuswap	Gum	[28]
Pinaceae	*Abies grandis* (Douglas ex D.Don) Lindl.	pitch, inner bark gum	Nitinaht, Shuswap	Gum	[28]
Pinaceae	*Larix occidentalis* Nutt.	solidified resin	Flathead, Okanagan-Colville, Thompson	Gum	[28]
Pinaceae	*Picea glauca* (Moench) Voss	resin	Algonquin Quebec, Cree Woodlands, Eskimo Alaska, Tanana Upper	Gum	[28]
Pinaceae	*Picea mariana* (Mill.) Britton, Sterns & Poggenb.	resin	Cree Woodlands	Gum	[28]
Pinaceae	*Picea sitchensis* (Bong.) Carriere	resin	Haisla, Hanaksiala, Hesquiat, Kwakiutl Souther, Makah, Quinault	Gum	[28]
Pinaceae	*Pinus banksiana* Lamb.	resin	Blackfoot, Hesquiat	Gum	[28]

Pinaceae	*Pinus edulis* Engelm.	resin	Navajo Ramah	Gum	[28]
Pinaceae	*Pinus monophylla* Torr. & Frem.	resin	Paiute Northern	Gum	[28]
Pinaceae	*Pinus monticola* Douglas ex D.Don	resin	Salish coast	Gum	[28]
Pinaceae	*Pinus ponderosa* Douglas ex C.Lawson	resin	Cheyenne, Okanaga-Colville, Paiute	Gum	[28]
Pinaceae	*Pseudotsuga menziesii* (Mirb.) Franco	resin	Apache White Mountain, Gosiute	Gum	[28]
Pinaceae	*Tsuga heterophylla* (Raf.) Sarg.	resin	Hesquiat	Gum	[28]
Polygonaceae	*Rumex hymenosepalus* Torr.	roots	Pima	Gum	[28]
Salicaceae	*Dalea candida* Willd.	plant	Santa Clara	Gum	[28]
Salicaceae	*Dalea lasiathera* A.Gray	root	Zuni	Gum	[28]
Salicaceae	*Dalea purpurea* Vent.	root	Lakota, Ponca	Gum	[28]
Salicaceae	*Populus angustifolia* E.James	buds	Navajo	Gum	[28]
Salicaceae	*Populus deltoides* Marshall	buds	Apache White Mountain, Chiricahua, Isleta, Mescalero, Zuni	Gum	[28]
Salicaceae	*Populus fremontii* S.Watson	catkins	Havasupai	Gum	[28]

Sapotaceae	*Sideroxylon lanuginosum* Michx.	outer bark mucilage	Kiowa	Gum	[28]
Ulmaceae	*Ulmus rubra* Muhl.	inner fresh bark	Kiowa	Gum	[28]

Table S8: Plants from Kampo

Family	Species (reported name)	Species (accepted name)	Part Used	Medicine Name; Medicinal System	Application	Tested on P. gingivalis?	Citation
				“hainosan;” TCM and Kampo	oral administration, boiled in 20-fold weight of water for 30 minutes and filtered, lyophilized for powder extracts & suspended in water	Y	[56, 118]
Rutaceae	*Citrus aurantium* L.		Dried immature fruit		2.25g		[56, 118]
Paeoniaceae	*Paeonia lactiflora* Pall.		Dried root		2.25g		[56, 118]
Campanulaceae	*Platycodon grandifloras* (Jacq.) A.DC.		Dried root		1.125g		[56, 118]
				“hangeshashinto;” Kampo	Oral administration, boiled in 20-fold weight of water for 30 minutes and filtered, lyophilized for powder extracts & suspended in water	Y	[56, 118]
Araceae	*Pinellia ternata* (Thunb.) Makino		Dried tuber		2.5g		[56, 118]

Lamiaceae	*Scutellaria baicalensis* Georgi		Dried root		1.25g		[56, 118]
Fabaceae	*Glycyrrhiza uralensis* Fisch.		Dried root and stolon		1.25g		[56, 118]
							[56, 118]
Rhamnaceae	*Ziziphus jujuba* Mill.		Dried fruit		1.25g		[56, 118]
Araliaceae	*Panax ginseng* C.A.Mey		Dried root		1.25g		[56, 118]
Ranunculaceae	*Coptis japonica* (Thunb.) Makino		Dried rhizome		0.5g		[56, 118]
Zingiberaceae	*Zingiber officinale* Roscoe		Dried rhizome after being steamed		1.25g		[56, 118]
				"Juzentaihoto (JTT);" Kampo		Y	[62]
Araliaceae	*Panax ginseng*						[62]
Fabaceae	*Astragali Radix*	*Astragalus spp.*					[62]
Apiaceae	*Angelicae Radix*	*Angelica sinensis* (Oliv.) Diels					[62]
Scrophulariaceae	*Rehmanniae Radix*	*Rehmannia spp.*					[62]
Asteraceae	*Atractylodis lanceae*	*Atractylodes lancea* (Thunb.) DC.	Rhizome				[62]
Lauraceae	*Cinnamomi cortex*	*Cinnamomum verum* J.Presl					[62]
Polyporaceae	*Poria cocos* F.A.Wolf	*Wolfiporia extensa* (Peck) Ginns					[62]
Paeoniaceae	*Paeoniae Radix*	*Paeonia spp.*					[62]

Apiaceae	*Ligustici Rhizome*	*Ligusticum striatum* DC.					[62]
Fabaceae	*Glycyrrhizae Radix*	*Glycyrrhiza glabra* L.					[62]
Fabaceae	*Spatholobus suberectus* Dunn		Dried stems	"Jixueteng;" Kampo		Y	[62]
Anacardiaceae	*Pistacia lentiscus* L.		resin	Mastic; “Yo-Nyuko olibanum;” Kampo	chewing gums with oil extracts	Y	[62]

Table S9: Plants from Karnataka India

Family	Species (reported name)	Species (accepted name)	Part Used	Medicinal System	Application	Tested on P. gingivalis?	Citation
Acanthaceae	*Blepharis repens* (Vahl) Roth	*Blepharis integrifolia* (L.f.) E.Mey & Drege ex Schinz	young parts	Karnataka, India	Chewed and held in mouth for toothache		[91]
Amaranthaceae	*Achyranthes aspera* L.		whole plant	Karnataka, India	Dried, burnt; ashes + salt massage gums		[91]
Anacardiaceae	*Mangifera indica* L.		bark	Karnataka, India	powdered bark held in mouth for toothache		[91]
Araceae	*Acorus calamus* L.		rhizome	Karnataka, India	paste applied to teeth and gums		[91]
Asclepiadaceae	*Calotropis gigantea* (L.) Dyrand.		latex; whole plant	Karnataka, India	cotton + latex placed on tooth for toothache; whole dried plant ash massage gums	used for periodontitis	[91]
Asclepiadaceae	*Pergularia daemia* (Forssk.) Chiov.		latex	Karnataka, India	cotton + latex placed on tooth for toothache		[91]
Basellaceae	*Basella alba* L.		leaves	Karnataka, India	Chewed and held in mouth for toothache		[91]
Capparaceae	*Capparis sepiaria* L.		leaves	Karnataka, India	baked powder massaged on teeth and gums		[91]
Cariaceae	*Carica papaya* L.		latex	Karnataka, India	latex of unripened fruit applied with cotton for toothache		[91]

Convolvulaceae	*Merremia chryseides* (Ker Gawl.) Hallier f.	*Merremia hederacea* (Burm. F.) Hallier f.	whole plant	Karnataka, India	decoction gargled for toothache		[91]
Euphorbiaceae	*Jatropha curcas* L.		latex	Karnataka, India	applied for toothache		[91]
Euphorbiaceae	*Jatropha gossypiifolia* L.		latex	Karnataka, India	applied with cotton for toothache		[91]
Euphorbiaceae	*Ricinus communis* L.		seed oil	Karnataka, India	applied for toothache		[91]
Fabaceae	*Caesalpinia coriaria* (Jacq.) Willd.		fruits	Karnataka, India	powder massaged on teeth and gums for toothache		[91]
Fabaceae	*Cassia hirsuta* L.	*Senna hirsuta* (L.) H.S.Irwin & Barneby	seeds	Karnataka, India	powder massaged on teeth and gums for protection		[91]
Fabaceae	*Cassia tora* L.	*Senna tora* (L.) Roxb.	seeds	Karnataka, India	powder massaged on teeth and gums for protection	used for periodontitis	[91]
Lamiaceae	*Leucas aspera* (Willd.) Link		whole plant	Karnataka, India	powder massaged on teeth and gums for protection		[91]
Lamiaceae	*Ocimum sanctum* L.	*Ocimum tenuiflorum* L.	leaves	Karnataka, India	crushed with salt; applied for toothache		[91]
Malvaceae	*Gossypium herbaceum* L.		seeds	Karnataka, India	paste applied to teeth and gums		[91]
Meliaceae	*Azadirachta indica* A.Juss.		bark	Karnataka, India	powdered inner bark held in mouth for half and hour for toothache		[91]
Mimosaceae	*Acacia nilotica* (L.) Delile		bark	Karnataka, India	decoction gargled for periodontitis; chewed and held in mouth for tootache	used for periodontitis	[91]

Mimosaceae	*Prosopis juliflora* (Sw.) DC.	leaves	Karnataka, India	ground with tobacco and lime for toothache		[91]
Moraceae	*Streblus asper* Lour.	bark	Karnataka, India	ground with copper sulphate and held in mouth for toothache		[91]
Myrtaceae	*Psidium guajava* L.	young leaves	Karnataka, India	chewed with sold for toothache		[91]
Olacaceae	*Ximenia americana* L.	root	Karnataka, India	paste applied to teeth and gums for toothache		[91]
Oxalidaceae	*Oxalis corniculata* L.	leaves	Karnataka, India	Chewed and held in mouth for toothache		[91]
Papaveraceae	*Argemone mexicana* L.	root	Karnataka, India	crushed and applied for toothache		[91]
Salvadoraceae	*Azima tetracantha* Lam.	leaves	Karnataka, India	crushed and applied for toothache		[91]
Solanaceae	*Solanum surattense* Burm. f.	seeds, fruits	Karnataka, India	fumes from burning seeds held in mouth for toothache; powdered fruit massaged on gums for periodontitis	used for periodontitis	[91]
Solanaceae	*Solanum violaceum* Ortega	fruits	Karnataka, India	powder applied for toothache		[91]
Verbenaceae	*Vitex negundo* L.	leaves	Karnataka, India	crushed with salt; applied for toothache		[91]
Zygophyllaceae	*Tribulus terrestris* L.	fruits	Karnataka, India	baked, powdered; massaged on gums for toothace		[91]

Table S10: Screen Results at 256 µg/mL, 64 µg/mL, and 32 µg/mL

Extract Number	Family	Species	Part	Extract Solvent	Average % Inhibition at 265 µg/mL	Average % Inhibition at 64 µg/mL	Average % Inhibition at 32 µg/mL
1283	Anacardiaceae	*Pistacia lentiscus*	leaves	95% Ethanol	91.29 ± 3.14	40.92 ± 3.42	8.82 ± 7.05
1288	Anacardiaceae	*Pistacia lentiscus*	leaves	dH2O	98.2 ± 0.6	97.36 ± 1.51	(-)105.21 ± 6.69
1300	Anacardiaceae	*Pistacia lentiscus*	woody parts	95% Ethanol	102.2 ± 6.07	92.55 ± 3.50	97.05 ± 2.02
1457	Anacardiaceae	*Pistacia lentiscus*	woody parts	dH2O	65.17 ± 27.41	(-)21.22 ± 14.02	11.64 ± 10.54
1891	Anacardiaceae	*Pistacia lonisus*	leaves	95% Ethanol	78.38 ± 10.39		
1892	Anacardiaceae	*Pistacia lonisus*	fruits	95% Ethanol	90.09 ± 1.59	99.72 ± 0.96	98.41 ± 0.88
142	Asteraceae	*Achillea millefolium L.*	Inflorescence	EtOH	-1.95 ± 3.19	_	_
153	Asteraceae	*Achillea millefolium L.*	Leaves Stems	EtOH	2.7 ± 12.32	_	_
159	Asteraceae	*Achillea millefolium L.*	Flowers Leaves Stems	EtOH	-12.51 ± 9.46	_	_
234	Asteraceae	*Achillea millefolium L.*	Leaves Stems Flowers	MeOH	6.81 ± 8.74	_	_
235	Asteraceae	*Achillea millefolium L.*	Inflorescence	MeOH	-10.81 ± 6.79	_	_
646	Asteraceae	*Achillea millefolium L.*	Stems Leaves	MeOH	-19.22 ± 14.46	_	_
656	Asteraceae	*Achillea millefolium L.*	Leaves Stems	MeOH	-33.03 ± 18.72	_	_
1654	Ebenaceae	*Diospyros virginiana L.*	leaves	80% Ethanol	98.3 ± 24.75	6.12 ± 3.88	4.27 ± 7.14
1676	Ebenaceae	*Diospyros virginiana L.*	leaves	80% Ethanol	80.58 ± 8.41	_	_
1737	Ebenaceae	*Diospyros virginiana L.*	stems	80% Ethanol	79.38 ± 4.25	_	_

1755	Ebenaceae	*Diospyros virginiana L.*	immature fruits	80% Ethanol	69.37 ± 6.29	_	_
1841	Ebenaceae	*Diospyros virginiana L.*	woody stems	80% Ethanol	72.07 ± 2.35	_	_
66	Fabaceae	*Vicia faba L.*	Leaves Flowers Whole Plant Roots Stems	EtOH	97.75 ± 5.31	98.12 ± 0.55	41.00 ± 10.53
350	Fabaceae	*Vicia faba L.*		MeOH	108.11 ± 2.62	98.05 ± 0.24	97.76 ± 0.13
1150	Fabaceae	*Tamarindus indica L.*	Leaves	95% Ethanol	43.24 ± 40.79	_	_
619	Fagaceae	*Quercus alba L.*	Bark	MeOH	79.48 ± 2.72	_	_
620	Fagaceae	*Quercus alba L.*	Galls	MeOH	87.09 ± 1.31	_	_
632	Fagaceae	*Quercus alba L.*	Leaves	MeOH	85.49 ± 2	_	_
647	Fagaceae	*Quercus alba L.*	Bark	dH2O	-41.54 ± 9	_	_
648	Fagaceae	*Quercus alba L.*	Galls	dH2O	83.93 ± 0.64	_	_
1201	Fagaceae	*Quercus alba L.*	woody parts	MeOH	79.18 ± 1.42	_	_
1209	Fagaceae	*Quercus alba L.*	woody parts	dH2O	3 ± 6.64	_	_
634	Hamamelidaceae	*Liquidambar styraciflua L.*	Woody Parts	MeOH	50.05 ± 3.19	_	_
636	Hamamelidaceae	*Liquidambar styraciflua L.*	Leaves	MeOH	90.19 ± 4.38	11.00 ± 22.72	(-)2.82 ± 11.27
637	Hamamelidaceae	*Liquidambar styraciflua L.*	Fruits Seeds	MeOH	89.39 ± 8.68	_	_
1637	Hamamelidaceae	*Liquidambar styraciflua L.*	roots	80% Ethanol	96.6 ± 3.24	83.51 ± 2.47	19.67 ± 1.54
1691	Hamamelidaceae	*Liquidambar styraciflua L.*	leaves	80% Ethanol	69.87 ± 18.57	_	_

126	Juglandaceae	*Juglans regia L.*	Immature Fruits	EtOH	91.79 ± 3.04	96.17 ± 1.26	86.98 ± 2.93
180	Juglandaceae	*Juglans regia L.*	Leaves	EtOH	86.19 ± 3.46	_	_
195	Juglandaceae	*Juglans regia L.*	Woody Parts	EtOH	90.19 ± 1.51	96.87 ± 0.36	97.25 ± 0.13
275	Juglandaceae	*Juglans regia L.*	Woody Parts	MeOH	71.67 ± 6.04	_	_
276	Juglandaceae	*Juglans regia L.*	Immature Fruits	MeOH	89.89 ± 0.97	94.92 ± 1.97	94.36 ± 0.57
277	Juglandaceae	*Juglans regia L.*	Leaves	MeOH	88.29 ± 3.34	_	_
638	Juglandaceae	*Carya tomentosa (Poir.) Nutt.*	Woody Parts	MeOH	273.47 ± 17.82	131.04 ± 6.17	107.09 ± 1.97
639	Juglandaceae	*Carya tomentosa (Poir.) Nutt.*	Leaves	MeOH	13.31 ± 60.26	_	_
640	Juglandaceae	*Carya tomentosa (Poir.) Nutt.*	Fruits	MeOH	359.06 ± 65.04	195.76 ± 9.93	154.45 ± 11.86
1511	Juglandaceae	*Juglans r.*	woody stems	95% EtOH	96.3 ± 3.31	94.99 ± 1.04	61.60 ± 9.02
1512	Juglandaceae	*Juglans r.*	woody stems	MeOH	92.19 ± 0.6	99.93 ± 0.32	32.61 ± 7.22
1582	Juglandaceae	*Carya tomentosa (Poir.) Nutt.*	leaves	80% Ethanol	89.19 ± 5.24	4.38 ± 15.66	(-)3.76 ± 6.15
1701	Juglandaceae	*Carya tomentosa (Poir.) Nutt.*	bark	80% Ethanol	81.48 ± 0.92	_	_
1703	Juglandaceae	*Carya tomentosa (Poir.) Nutt.*	woody stems	80% Ethanol	92.59 ± 8.63	36.46 ± 7.85	25.60 ± 8.18
1544	Lauraceae	*Sassafras albidum (Nutt.) Nees*	leaves	80% Ethanol	93.89 ± 0.17	12.11 ± 32.59	13.16 ± 5.33
1697	Lauraceae	*Sassafras albidum (Nutt.) Nees*	roots	80% Ethanol	88.69 ± 2	_	_
1795	Lauraceae	*Sassafras albidum (Nutt.) Nees*	stems	80% Ethanol	90.89 ± 1.25	99.93 ± 0.24	7.52 ± 0.98

1161	Meliaceae	*Azadirachta indica A.Juss.*	Leaves Woody Stem	95% Ethanol	90.29 ± 1.39	96.66 ± 1.37	10.85 ± 16.34
1148	Moraceae	*Artocarpus altilis (Parkinson ex F.A.Zorn) Fosberg*	Leaves	95% Ethanol	-11.21 ± 87.12	_	_
747	Myricaceae	*Myrica cerifera L.*	Leaves Flowers	MeOH	25.13 ± 61.23	53.5 ± 44.18	5.53 ± 2.47
762	Myricaceae	*Myrica cerifera L.*	Woody Parts Woody Stem	MeOH	92.79 ± 2.46	(-)3.83 ± 15.87	2.17 ± 2.26
865	Myricaceae	*Myrica cerifera L.*	Woody Stem	dH2O	13.11 ± 6.24	_	_
902	Myricaceae	*Myrica cerifera L.*	Branches Stems	MeOH	85.79 ± 4	_	_
1116	Myricaceae	*Myrica cerifera L.*	Bark	MeOH	70.97 ± 4.05	_	_
1126	Myricaceae	*Myrica cerifera L.*	Bark	dH2O	-26.53 ± 32.29	9.74 ± 14.17	18.51 ± 7.08
1180	Myricaceae	*Myrica cerifera L.*	stems, branches	dH2O	6.31 ± 10.23	_	_
132	Oleaceae	*Olea europaea L.*	Leaves	EtOH	109.51 ± 3.42	76.41 ± 2.17	26.68 ± 2.85
293	Oleaceae	*Olea europaea L.*	Woody Parts	MeOH	95.6 ± 3.08	(-)15.38 ± 1.59	12.44 ± 4.23
294	Oleaceae	*Olea europaea L.*	Leaves	MeOH	106.61 ± 6.36	96.38 ± 1.05	36.37 ± 10.77
122	Polygonaceae	*Rumex crispus L.*	Fruits Leaves Stems Aerial Parts	EtOH	93.19 ± 1.14	35.77 ± 5.79	17.64 ± 4.93
378	Polygonaceae	*Rumex crispus L.*		MeOH	-44.54 ± 36.52	_	_
402	Polygonaceae	*Rumex crispus L.*		MeOH	44.74 ± 25.75	_	_
784	Polypodiaceae	*Pleopeltis polypodioides (L.) E.G. Andrews & Windham*	Whole Plant	MeOH	-23.57 ± 14.23	_	_
786	Polypodiaceae	*Pleopeltis polypodioides (L.) E.G. Andrews & Windham*	Whole Plant	dH2O	43.14 ± 5.29	_	_

120	Rubiaceae	*Vitis vinifera var.aglianico*	Stems	EtOH	87.69 ± 6.82	_	_
123	Rubiaceae	*Vitis vinifera var.aglianico*	Fruits	EtOH	92.69 ± 2.27	14.96 ± 10.63	10.48 ± 14.91
124	Rubiaceae	*Vitis vinifera var.aglianico*	Leaves	EtOH	93.99 ± 2.97	94.05 ± 0.15	6.22 ± 1.48
335	Rubiaceae	*Vitis vinifera var.aglianico*	Fruits	MeOH	-49.95 ± 29.86	_	_
336	Rubiaceae	*Vitis vinifera var.aglianico*	Leaves	MeOH	98.1 ± 6.13	97.22 ± 0.48	(-)2.75 ± 5.57
337	Rubiaceae	*Vitis vinifera var.aglianico*	Stems	MeOH	136.04 ± 14.59	100.77 ± 0.43	97.98 ± 0.63
481	Rubiaceae	*Vitis rotundifolia Michx.*	Leaves Stems	MeOH	81.83 ± 1.49	_	_
1613	Rubiaceae	*Vitis rotundifolia Michx.*	leaves	80% Ethanol	81.78 ± 10	_	_
1760	Rubiaceae	*Vitis rotundifolia Michx.*	roots	80% Ethanol	85.59 ± 6	_	_
1831	Rubiaceae	*Vitis rotundifolia Michx.*	woody stems	80% Ethanol	67.07 ± 64.46	24.91 ± 3.79	3.76 ± 1.27
1837	Rubiaceae	*Vitis rotundifolia Michx.*	immature fruits	80% Ethanol	80.48 ± 0.79	_	_
498	Rutaceae	*Citrus × sinensis (L.) Osbeck*	Fruit Rind	MeOH	-50.35 ± 8.79	_	_
1111	Rutaceae	*Citrus sinensis (L.) Osbeck*	Woody Parts	MeOH	98.2 ± 0.79	96.78 ± 0.32	94.79 ± 0.57
1236	Rutaceae	*Citrus sinensis (L.) Osbeck*	woody parts	MeoH	-50.9 ± 7.43	_	_

1290	Rutaceae	*Zanthoxylum armatum*	fruits, seeds	95% Ethanol	117.12 ± 1.97	98.82 ± 0.84	96.6 ± 0.33
774	Salicaceae	*Salix nigra Marshall*	Leaves	MeOH	-11.51 ± 8.87	_	_
897	Salicaceae	*Salix nigra Marshall*	Leaves	dH2O	54.65 ± 14.25	_	_
922	Salicaceae	*Salix nigra Marshall*	Flowers Fruits Leaves	MeOH	73.37 ± 6.59	_	_
1023	Salicaceae	*Salix nigra Marshall*	Branches	MeOH	64.46 ± 6.69	_	_
1028	Salicaceae	*Salix nigra Marshall*	Woody Stem	MeOH	91.09 ± 2.5	27.14 ± 18.01	0.72 ± 5.51
1030	Salicaceae	*Salix nigra Marshall*	Woody Stem	dH2O	87.99 ± 4.92	_	_
1031	Salicaceae	*Salix nigra Marshall*	Branches	dH2O	-32.43 ± 7.72	_	_
1065	Salicaceae	*Salix nigra Marshall*	Leaves Fruits Flowers	dH2O	2.4 ± 9.67	_	_
1071	Salicaceae	*Salix nigra Marshall*	Woody Stem	MeOH	-46.35 ± 2.72	_	_
1073	Salicaceae	*Salix nigra Marshall*	Leaves	MeOH	86.29 ± 2.33	_	_
1134	Salicaceae	*Salix nigra Marshall*	Bark	MeOH	85.49 ± 2.27	_	_
1136	Salicaceae	*Salix nigra Marshall*	Bark	MeOH	82.78 ± 4.03	_	_
1185	Salicaceae	*Salix nigra Marshall*	Bark	MeOH	-24.02 ± 17.53	_	_
1206	Salicaceae	*Salix nigra Mars hall*	bark	dH2O	85.79 ± 8.11	_	_
1210	Salicaceae	*Salix nigra Mars hall*	woody stems	dH2O	96.55 ± 0.21	15.80 ± 2.95	15.26 ± 11.56
1211	Salicaceae	*Salix nigra Mars hall*	bark	dH2O	97.5 ± 0.76	16.77 ± 5.39	14.53 ± 3.96
1576	Salicaceae	*Salix nigra Marshall*	leaves	80% Ethanol	91.39 ± 3.8	(-)5.22 ± 5.12	(-)0.36 ± 3.29
1749	Salicaceae	*Salix nigra Marshall*	bark	80% Ethanol	84.38 ± 4.26	_	_
1783	Salicaceae	*Salix nigra Marshall*	roots	80% Ethanol	105.01 ± 9.82	26.23 ± 0.73	3.47 ± 15.98
919	Sapotaceae	*Sideroxylon celastrinum (Kunth) T.D. Penn.*	Stems	MeOH	16.22 ± 72.77	81.14 ± 3.56	25.52 ± 6.95

1182	Sapotaceae	*Sideroxylon celastrinum (Kunth) T.D. Penn.*	leaves, stems	MeOH	73.97 ± 9.99	_	_
1190	Sapotaceae	*Sideroxylon celastrinum (Kunth) T.D. Penn.*	leaves, stems	dH2O	-33.93 ± 7.28	_	_
1225	Sapotaceae	*Sideroxylon celastrinum (Kunth) T.D. Penn.*	stems	dH2O	-35.74 ± 7.77	_	_
1609	Sapotaceae	*Sideroxylon lanuginosum Michx.*	leaves	80% Ethanol	80.18 ± 6.5	_	_
1718	Sapotaceae	*Sideroxylon lanuginosum Michx.*	leaves	80% Ethanol	74.97 ± 3.32	_	_
1756	Sapotaceae	*Sideroxylon lanuginosum Michx.*	woody stems	80% Ethanol	85.69 ± 1.14	_	_
1864	Sapotaceae	*Sideroxylon lanuginosum Michx.*	bark	80% Ethanol	94.19 ± 1.42	43.70 ± 5.25	30.59 ± 4.34

Table S10: Results from single concentration screens at 256 µg/mL, 64 µg/mL, and 32 µg/mL
Percent of growth inhibition is reported as averages with standard deviation (n=3). At 256 µg/mL, extracts with >90% growth inhibition, standard deviations allowing for >90% growth inhibition, or high standard deviations, were chosen for further study. Extracts with >90% growth inhibition at 64 µg/mL or 32 µg/mL were chosen for MIC. Extract 747 was included in MIC; further tests were necessary to rule it out as a high activity extract due to high standard deviation in the single-concentration screen.