# STUDY OF PLANTS USED AGAINST INFECTIONS BY CALIFORNIA NATIVE AMERICAN TRIBES

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ABSTRACT

Study of Plants Used Against Infections by California

Native American Tribes

Maria Jose Rojas Soto

The objectives of this research were to evaluate the antibacterial activity and to determine the chemical composition of a list of medicinal plants used by Native Americans in California. *Artemisia californica, Mimulus aurantiacus, Equisetum telmateia, Equisetum hyemale,* and *Marah fabacea* were selected from a list of plants reported as having been used for ailments related to infections by tribes located in California. The extracts obtained through steam distillation from *E. telmateia, E. hyemale* and *M. fabacea* were assayed for *in vitro* antibacterial activity against 16 Gram-negative and 6 Gram-positive bacteria using disk diffusion assays and measuring the diameters of inhibition zones. *E. telmateia* showed the most promising antibacterial activity. The extracts from *A. californica, M. aurantiacus* and *E. telmateia* were analyzed for chemical composition, finding eucalyptol, thujone, eugenol, caryophyllene, germacrene D, and propanal as some of the secondary metabolites identified using GC-MS. Our results suggest that *E. telmateia* can be a potential source for novel antimicrobials against pathogenic bacteria.

Keywords: medicinal plants, essential oil, chemical composition, UTIs, antibacterial activity, GC-MS, disk diffusion assay, ethnobotany, Chumash.

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# Chapter 1

## INTRODUCTION

The current “trend” of medicinal plants is one of the many ways’ humans have found to relieve pain and cure illness, its origin goes up to 60,000 years ago, during the Paleolithic age (Sewell & Rafieian-Kopaei, 2014). It involves the medicinal use of herbs to treat and prevent diseases, enhancing general health and wellbeing (Xiong & Guan, 2017) based almost exclusively on experience gained from trial and error (Sewell & Rafieian-Kopaei, 2014). It has been estimated that more than a half of available medicines today contain ingredients derived from plants (Ma J. et al., 2005; Murphy 1999; Sewell & Rafieian-Kopaei, 2014), but only 6% of all plants on Earth have been studied for medicinal activity (Fabricant & Farnsworth, 2001).

In California, the heritage of human experience goes back thousands of years before the arrival of the Spanish explorers in 1542. Native American peoples in the Far West once lived free in large family groups or clans manipulating nature resources for food, basketry, tools, clothing, medicine, and more. The deserts and mountain ranges of the Pacific Coast isolated these early settlers from the cultures that developed in neighboring Mexico and the western United States. Thus, divided and isolated, the original Californians were a diverse population, separated by language into as many as 135 distinct dialects. Tribes included the Karok, Maidu, Cahuilleno, Mojave, Yokuts, Pomo, Paiute, Modoc, Chumash, and on and on (Library of Congress collection). The Chumash Indians, who occupied the mainland and offshore islands in the vicinity of Santa Barbara, California, were coastal people with maritime lifestyle and skillful harvesters who used at least 150 plant species for food, medicine and religious practices (Native American Roots, 2015; Timbrook, 1990).

Our sources from California's traditional medicinal knowledge are very limited. From the few interviews available, like the ones conducted by the anthropologist John P. Harrington, that took place at the beginning of the last century, there is evidence of a long list of plants used by native cultures in California. Sometimes the strategies and rituals were shared between tribes. As a great example of this phenomenon, mugwort, an aromatic plant that has been used medicinally and ceremonially for thousands of years. Many California tribes, like the Chumash, burned and inhaled the smoke from the leaves of mugwort to promote dreams, healthy sleep, and to repel bad spirits. Similar rituals were performed miles away by the Paiute people (Reid, 2009).

Despite the historical success of traditional medicinal plants, there are still enormous gaps in the knowledge of the chemical composition and pharmacological properties of many plants that have been used for curative purposes by native tribes in the United States (Fleming, 2018), and in the whole world. Plants can synthesize a wide variety of small-molecular-weight compounds, which are used for example to attract pollinators and defense reactions against herbivory and against viral, fungal and bacterial infections. People have found that these secondary metabolites possess biological effects and could be used for medicinal purposes. For example, different plant compounds, like carvacrol from oregano, thymol from thyme, eugenol from clove, and cinnamaldehyde from cinnamon have been recognized, because of the activity of their components, to have antimicrobial properties being able to work as antiseptics or in the treatment of infections (Burt, 2004; Kon et al., 2016).

According to the World Health Organization bacteria, amongst other microorganisms, could get the ability to nullify the effects of antimicrobial drugs, this ability is commonly known as antimicrobial resistance (Kon et al., 2016). Antimicrobial resistance happens naturally over time. In a colony, a small number of bacteria are naturally resistant to some antibiotics. When taken, antibiotics kill the bacteria that caused the illness, called

pathogens, but they also kill the normal and healthy bacteria that protect our body from external infections. The naturally resistant bacteria survive and multiply, eventually, the infection spreads resistant to the drug taken. Because of the excessive and inappropriate use of antimicrobials the process of antimicrobial resistance acquisition has accelerated and the occurrence of resistant bacteria has increased. For this reason, the treatment of multiple infections has become challenging due to the rapid and increasing development of multidrug resistance microbes. This is a significant public health concern around the globe since some bacterial infections are already very difficult to treat (Coates et al., 2011; Farrell et al. 2003). The prudent use of antimicrobials in addition to the discovery of novel classes of compounds in the development of new antibiotics are essential to mitigate these concerns (Coates et al., 2011; Kon et al., 2016).

Within the context of the inevitable ongoing emergence of bacterial resistance, scientists are searching for novel antimicrobial agents in natural sources (Fleming, 2018; Murphy, 1999). There are four major groups of antimicrobial compounds made by plants: phenolics and polyphenols, terpenoids and essential oils, lectins and polypeptides, and alkaloids (Quave, 2016). Since plants are an effective and environmentally friendly source for antimicrobials researchers all over the world are investigating herbal remedies that were used for thousands of years before the appearance of nowadays antibiotics.

Urinary tract infections (UTIs) are the second most prevalent bacterial infection in humans, affecting 150 million people each year worldwide (Farrell et al., 2003; Flores-Mireles et al., 2015; Mazzariol et al., 2017; Tandogdu, 2013). UTIs are defined as microbial colonization of the normally considered sterile urinary tract. Pathogens colonize the urinary system (urethra, bladder, ureters, and/or kidneys), and cause infection by attachment to the tissue and the host defense mechanism failing to impede said attachment (Flores-Mireles et al., 2015; Lee & Neild, 2007). UTIs are caused by a range of pathogens, both, Gram-negative and Gram-positive bacteria, most commonly by

uropathogenic *Escherichia coli* (UPEC), *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterococcus faecalis*, *Staphylococcus saprophyticus*, *Pseudomonas aeruginosa*, *Enterobacter* spp., and *Staphylococcus aureus*. (Farrell et al. 2003; Flores-Mireles et al., 2015; Mazzariol et al., 2017; Tandogdu, 2013). According to the U.S Department of Health & Human Services, 50% of women are affected by UTIs at least once in their lifetime and 15% of men reported at least one by the age of 35 (see also Foxman, 2013). The indiscriminate and widespread use of antibiotics in the treatment of UTIs has resulted in increased resistance of urinary pathogens to available antibiotics (Farrell et al., 2003; Flores-Mireles et al., 2015; Mazzariol et al., 2017; Tandogdu, 2013). The high recurrence rates, 20% of cases within 6 months, in addition to the increasing antimicrobial resistance is threatening the standardized therapies increasing their risk of failure (Flores-Mireles et al., 2015; Lee J. & Neild, 2007; Lee ,2018; Tandogdu, 2013). The use of antibiotics can long-term adversely affect the normal microbiota in the vaginal and gastrointestinal tract, as most antibacterial compounds, target more than just the uropathogenic bacteria (Flores-Mireles et al., 2015). The development of new strategies to cure UTIs is necessary in addition to antibiotic stewardship to preserve the current treatments (Flores-Mireles, et al., 2015; Lee, 2018).

UTIs are also one of the ailments commonly addressed for their treatment with certain plants by California tribes. Bladder, kidney, and urinary tract disorders were the most common diseases found when researching medicinal plants utilized by Native Americans. Interestingly, most medicinal plants were used primarily as infusions and decoctions. For example, the Costanoan Indians, based along the coast of California, used a decoction of flowers, stems, and leaves of sticky monkeyflower (*Mimulus aurantiacus*) to control bladder and kidney infections (Adam & Garcia, 2006; Bocek, 1984). They also used a decoction of the aerial parts of the horsetail (*Equisetum* spp.) for bladder and kidney ailments, and yerba mansa (*Anemopsis californica*) and mugwort (*Artemisia douglasiana*)

for bladder, kidney, and urinary tract disorders and to wash cuts and wounds to prevent infections. The Chumash at the same time of using yerba mansa, mugwort, and horsetail like the Costanoan, used a tea of yerba del aire (*Baccharis plummerae*) for kidney trouble. They used desert tea (*Ephedra californica*) to induce urination, to cure urinary tract complaints, and for cuts and wounds. They also used a decoction of California juniper (*Juniperus communis* and *J. californica*), manroot (*Marah* spp.), black elderberry (*Sambucus nigra*), and hooker's evening (*Oenothera elata*) for urinary disorders. Table 1 shows a summary of our findings of plants used by these and other native cultures and their uses for urinary disorders.

The search continues for alternatives to current therapies, creating new antibacterials, combining the drugs available, and "reviving" old formal antibacterials to cope with antibacterial resistant bacteria. The traditional use of plants to treat urinary problems suggests that extracts of these plants may possess antibacterial activity. We hypothesized that the plants used by California tribes to treat urinary disorders contain antimicrobial compounds and extracts obtained from these plants will inhibit uropathogenic bacterial growth.

This work describes the chemical composition and/or the antibacterial activity of the oil from five plant species, *Artemisia californica*, *Mimulus aurantiacus, Equisetum telmateia, Equisetum hyemale,* and *Marah fabacea*.

# Chapter 2

# METHODS AND MATERIALS

## *2.1 Literature research*

Literature research was conducted for the search for plants used for treatment of infection by tribes located in California. Herbal treatments related to ailments caused by bacteria, like fevers, urinary tract infections, prevention, disinfectants, et cetera, were listed. For a list of the plants used to treat infections, with the tribe associated, and the method they used to cure these infections please see Table 1.

## *2.2 Plant collection*

Plants were collected from Poly Canyon adjacent to California Polytechnic State University (Cal Poly), San Luis Obispo, California, USA in spring 2019 (March-May). Identification was confirmed by Dr. Jenn Yost, of the Biological Sciences department. The species collected were: *Artemisia californica*, *Mimulus aurantiacus, Equisetum telmateia, Equisetum hyemale,* and *Marah fabacea*. Flowers, leaves, roots, and/or aerial parts (depending on the plant) were separated after collection.

California sagebrush (*Artemisia californica)* is a shrub with thread-like hairy and light green to gray leaves that have a strong sage smell when crushed. Is native to western California, endemic to chaparral and woodlands. It can grow 1.5 to 2.5 m tall. *Artemisia californica* ranges approximately 1000 km from Northern Baja, Mexico to Mendocino County, California at low elevations (<800 m) along the coast (Pratt & Mooney, 2013).

Sticky monkeyflower (*Mimulus aurantiacus)*, native to the West Coast of North America, is a small, perennial, (Rooney-Latham & Blomquist, 2014) Hummingbird-Pollinated (Belisle et al., 2012) shrub with characteristic sticky leaves and orange flowers.

It grows mainly in wet soil, and it is widely distributed throughout California (Rooney-Latham & Blomquist, 2014). Leaves, stems, and flowers are reported to be edible, where some native Americans ate as salad. (Butler, 2004)

Horsetail (*Equisetum* sp.), a living fossil, is an erect green hollow fern with greatly reduced leaves fused into rings, that grows in moist and sandy areas. *E. telmateia* and *E. hyemale* have a widespread distribution in Europe, Asia, northwest Africa and north America (Yeganegi, 2018).

Manroot (*Marah* spp.) is an herbaceous vine, native and endemic to California, with palmately lobed leaves that climb over scrub shrubs. It has a huge underground root (where its name comes from), star-shaped cream-colored flowers, and bright green and prickly spherical fruits (Ritter, 2018)

Leaves of *M. aurantiacus,* aerial parts of *E. telmateia* and *E. hyemale,* leaves of *A. californica* and *M. fabacea* were stored separately in plastic bags in a freezer at -18ºC. The roots collected from *M. fabacea* were cut and stored in plastic bags in a freezer at -18ºC.

### *2.3 Essential oil production*

A frozen sample from the leaves, flowers, root or aerial parts from the five plants species collected was subjected to steam distillation, ethyl acetate extraction, and rotary evaporation. Briefly, 100 g of frozen plant material was blended with an immersion blender in 750-1000 mL of distilled water. 100 g of frozen plant material was used per cycle obtaining on average 0.3 g of oil each cycle. Cycles were repeated until obtaining 0.9-1.2 g of oil. Two steam distillation apparatus were used simultaneously. Each steam distillation apparatus was composed of a round flask electric heater (heating mantle from Thermowell), a 500 mL round bottom flask with plant material, distilled water and porous boiling chips (Fisher Scientific Co.), a Claisen adapter with its glass stopper, a three-way adapter with a thermometer with its adapter, a condenser with its water outlet and inlet, a

vacuum adapter, and a receiving 250 mL Erlenmeyer flask containing 25 g of solid sodium bromide (Fisher Scientific Co.), in addition to several clamps and spring clamps to set everything in position and a bucket with iced water to feed the condenser. The plant material-water mixture was heated and distillate was collected for an hour after the first drop of distillate fell into the receiver or until 250 mL of distillate were collected.

The distillate in the collection flask was poured into a separatory funnel with ethyl acetate, allowing the layers to separate. A total of 100 mL of ethyl acetate were used divided into three separations using ≈30 mL each separation. The aqueous layer was drained into a 250 mL beaker, and discarded. The organic layer was collected into a separate Erlenmeyer flask. Anhydrous magnesium sulfate ($MgSO_4$) was added to dry the organic layer. $MgSO_4$ was filtered using a fluted filter paper and a funnel and the organic layer was collected into a new round bottom flask.

Lastly the ethyl acetate was removed *in vacuo,* from the desired oil using a Rotovapor ® R II from BÜCHI, Isotemp ® refrigerating circulator from Fisher Scientific Co., and the MaximaDry diaphragm Vacuum pump from Fisher Scientific Co. The resulting oils were weighed and their yields calculated with respect to dry matter mass and then transferred into a glass vial with a solvent. Oils were kept in 3 mL of ethyl acetate or methanol and stored in a freezer at -18ºC until they were analyzed or used in the bioassays. A range of concentrations from 0.3-0.4 g/mL oil to solvent was obtained depending on the oil. The essential oils (EO) obtained and the percent recovery for each plant can be found in Table 2.

### *2.4 Antibacterial Assay*

The disk diffusion method was utilized as preliminary determination of the antibacterial activity of the essential oils of the aerial parts of *E. telmateia* and *E. hyemale*, and the oils from the leaves and root of *M. fabacea*. The essential oils were individually tested against a panel of 22 bacterial species. The bacteria used are detailed in Table 3.

Microorganisms were provided by the Department of Biological Sciences, Cal Poly or came from Dr. Alejandra Yep's laboratory collection.

*Disk Diffusion Assay.* Antibacterial tests were carried out as follows: Bacterial strains were inoculated from single colonies grown in Tryptic Soy Agar (TSA) in 1 mL of Tryptic Soy Broth (TSB) and incubated at 37ºC or 30ºC for 24 h according to the preferred temperature of the bacteria with shaking at 200 rpm. 100 μL of cultivated bacteria were spread on TSA in petri dish and allowed to air dry before the next step. All media plates were prepared according to the instructions of the manufacturer, in 100 mm Petri Dishes (Olympus plastics) with 15 mL of agar, the following brands of TSA were used: remel, BD Bacto TSA, and Fisher BioReagents. Sterile filter paper disks (Whatman 52 Hardened, 6 mm in diameter) were impregnated with 6 μL of essential oil dissolved in a solvent (around 1g/3mL of ethyl acetate) and placed on the inoculated agar. Negative controls were prepared using the pure solvent employed to dissolve the oils (ethyl acetate). The inoculated plates were incubated for 24 h at 37ºC or 30ºC according to the optimum growth temperature of each bacteria strain. *Micrococcus luteus, Pseudomonas fluorescens, Aeromonas hydrophila, Enterobacter aerogenes, and Morganella morganii,* grew at 30ºC, and the rest of the strains were incubated at 37ºC. Antimicrobial activity was evaluated after 24 h by measuring the zone of inhibition (ZOI) diameter in millimeters nearest to the whole millimeter, which represents the absence of bacterial growth, against the organism tested in comparison to the negative control. Each assay was repeated three times. The results expressed by the average and standard deviation of the ZOI in millimeters for *Equisetum telmateia, Equisetum hyemale* and the leaves and root of *Marah fabacea* are presented in Table 3. Oils from *A. Californica* and *M. aurantiacus* were not tested for antibacterial activity.

### *2.5 Statistical analysis*

All the disk diffusion assays were carried out in triplicates, the results are presented as the average with standard deviation in Table 3. Results were submitted to analysis by the Wilcoxon test.

### *2.6 Essential Oil Analysis*

GC-MS analyses were performed using the Agilent 7890B with 5977B MS (Single Quad) Gas Chromatography-Mass Spectrometry (GCMS) System equipped with Agilent DB-5MS capillary column in the oils from *A. californica, M. aurantiacus* and *E. telmateia*. The oven temperature was kept at 50ºC for 3 min and then programmed from 50 to 250 ºC at a rate of 3ºC/min. The relative proportions of the essential oil components were expressed as percentages obtained by GC peak area normalization. The identification of the components was based on the comparison of the relative retention times and database matching of the mass spectra with those of standards of the NIST17.L GC-MS library data, as well as by those reported in the literature. GC-MS analyses were conducted on the EOs of the leaves of *M. aurantiacus,* the aerial parts of *E. telmateia* dissolved in methanol and ethyl acetate, and the GC-MS of the leaves of *A. californica* were ran three times. Results are listed in Tables 4 - 9.

The components' formula, classification and chemical structure for the Tables 4 - 9 were obtained from the National Institute of Standards and Technology (NIST) Chemistry WebBook and PubChem database from the National Center for Biotechnology Information. The chemical structure was drawn using the software ChemDraw 19.1.1.32 (PerkinElmer Informatics, Inc.).

# Chapter 3

# RESULTS

## *3.1 Literature research*

We examined the literature in the search for plants used for the treatment of infections by tribes located in California. A total of 18 plant genera were found to be used as remedies for conditions possibly related to microbial infections. We observed a long list of herbal treatments to cure fevers, urinary tract infections, to prevent infections in wounds and sores, and as disinfectants. Cultures all along California used similar remedies and sometimes used the same plant species to cure the same type of infection. Drinking decoctions or teas, and poultice applications were the most referred form of administration. The plant organs involved were roots, stems, leaves, bark, aerial parts, buds, flowers, seeds, and whole plants. The tribes Chumash, Gabrieliño, Maidu, Shoshone, Costanoan, Achomawi, Cahuilla, Rumsen, Mutsun and Mahuna were mentioned. Detailed results are listed in Table 1.

Table 1. Literature research. The use of plants for the treatment of infections by tribes in California

| Scientific name | Common name | Used as | Part of the plant used | Used for/to | Used by | Reference |
|---|---|---|---|---|---|---|
| *Anemopsis californica* | Yerba mansa, yerba del manso, lizard tail. | Decoction/ tea | The whole plant, roots, stems, and leaves. | Bladder, kidney, and urinary tract disorders and infections; wounds, cuts, sore washing. | Mahuna, Chumash, Costanoan. | Bard 2006; Bussey et al. 2014; Native American Netroot 2015; Timbrook 1990; Timbrook 2007; Walker & Hudson 1993. |
| *Arctostaphylos uva-ursi* | Bearberry, uva-ursi, manzanita. | Unspecified | Bark and leaves. | As astringent and antiseptic for kidney and urinary tract, and relieve bladder ailments. | California Indians, Rumsen, Mutsun. | Reid, Wishingrad and McCabe 2009. |
| *Artemisia californica, A.douglasiana* | California sagebrush, romerillo, mugwort. | Decoction/ tea | Leaves and stems. | Cuts and wounds washing, infections, pain, fever, urinary problems. | Chumash, Costanoan. | Adams 2012; Allison 2017; Eun 2014; Perri 2019; Reid, Wishingrad and McCabe 2009; Timbrook 1990; Violet Sage Walker*, Chumash culture Share 2019. |
| *Asarum caudatum* | Wild Ginger | Soft poultice/ tea | Whole plant, leaves. | Applied to prevent infections, sore washing. | Unspecified | Reid, Wishingrad and McCabe 2009. |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Baccharis plummerae* | Yerba del aire | Tea | Unspecified | Kidney trouble | Chumash | Timbrook 1990 |
| *Ephedra californica, E. viridis* | Desert tea, joint fir, green Mormon tea. | Decoction/ tea | Stems or roots. | Induce urination and treat urinary infections, kidney and bladder disorders, and urinary complaints; cuts and wounds washing. | Chumash, Cahuilla. | Adam & Garcia 2006; Adam, Garcia and Lien 2010; Bard 2006; Native American Netroots. 2015; Timbrook 1990; Timbrook 2007; Violet Sage Walker*, Chumash culture Share 2019. |
| *Equisetum* spp. *E. laevigatum, E. telmateia* | Horsetail, smooth horsetail, great horsetail. | Decoction | Aerial parts | Bladder problems and to treat kidney ailments. | Costanoan, Achomawi, Cahuilla, Chumash by the coast. | Adam & Garcia 2006; Strike 1994; Timbrook 2007. |
| *Grindelia spp.* | Gumplant | Tea | Sticky buds, flowering heads, leaves. | Urinary ailments, kidney problems, bladder infections, cuts and sore washing, pulmonary troubles. | Shoshone, Blackfeet Indians | Reid, Wishingrad and McCabe 2009; Bard 2006. |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Hemizonia congesta* | Hayfield tarweed | Unspecified | Unspecified | Control bladder infections. | Maidu | Adam & Garcia 2006. |
| *Juniperus communis, J. californica* | Juniper berries, California juniper. | Decoction | Unspecified | Genito-urinary disorders. | Chumash | Bard 2006; Timbrook 2007; Walker & Hudson 1993. |
| *Madia elegans* | Madia | Unspecified | Unspecified | Control bladder infections. | Maidu | Adam & Garcia 2006 |
| *Marah* spp., *M. watsonii, M.macrocapus* | Manroot, Wild cucumber, chilicote. | Toasted and crushed | Whole plant, roots, seeds, leaves. | Remedy for urinary disorders. | Indians from Mendocino county, Gabrieleño, Maidu, Chumash. | Adam & Garcia 2006; Strike 1994; Timbrook 2007; Walker & Hudson 1993. |
| *Mimulus aurantiacus* | Sticky monkeyflower | Decoction | Flowers, stems, and leaves. | Control bladder and kidney infections. | Costanoan | Adam & Garcia 2006; Bocek 1984. |
| *Oenothera elata* | Hooker's evening, tall evening primrose. | Unspecified | Unspecified | Bladder and yeast infections. | Chumash | Adam, Garcia, and Lien 2010. |

| | | | | | | |
|---|---|---|---|---|---|---|
| *Plantago* spp. | Lanten | Leaves heated and applied as poultice | Leaves | Infected sores | Chumash | Timbrook 1990 |
| *Quercus agrifolia* | Coast live oak | Unspecified | Bark, acorns and galls, mold that grew on acorns. | Bladder infections; wounds washing. | Luiseño | Reid, Wishingrad and McCabe 2009. |
| *Sambucus nigra, S. mexicana* | Black elder, elderberry, sauco. | Dried/ teas | Flowers | Anti-inflammatory use of the urinary tract, fevers. | Chumash | Allison et al. 2017; Bard 2006; Native American Netroots 2015; Trill et al. 2017; Violet Sage Walker*, Chumash culture Share 2019. |
| *Trichostema lanatum* | Romero | Unspecified | Unspecified | Disinfectant | Chumash | Fleming et al. 2018 |

*Violet Sage Walker vice chairwoman of the Northern Chumash Tribal Council, a Northern Chumash descendant and a local community leader hosted a Chumash Culture Share in October, 2019, at the San Luis Obispo Botanical Garden, where Chumash spirit, art, history and medicinal plants were discussed.

### *3.2 Plant collection*

A total of four different genera, and five species of plants were collected: *Artemisia californica*, *Mimulus aurantiacus, Equisetum telmateia, Equisetum hyemale,* and *Marah fabacea*. Photographs of the plants collected can be seen in Figure 1 (A-F). Samples were subjected to extraction of the volatile compounds through steam distillation, antimicrobial assays, and/or analysis of the chemical constituents via GC-MS.

### *3.3 Essential oil production*

The process known as steam distillation is a technique used to separate one or more compounds from others. Essential oils, most commonly produced by steam distillation, are aromatic nonpolar liquids obtained from plant materials (flower, buds, seeds, leaves, twigs, barks, herbs, wood, fruits, and roots) (Burt, 2004). The benefits of using steam distillation as our extraction method is that it is an easy way to remove water insoluble compounds from non-volatile compounds in a reaction mixture. To detect differences in bioactivity in different plant organs, the essential oils from the leaves and root of *M. fabacea* were obtained separately for further characterization. Only the aerial parts of *E. telmateia* and *E. hyemale,* and leaves of *A. californica* were employed in the isolation of the essential oils. The highest percentage recovery was found in the leaves of *A. californica* (0.9%) and the lowest was *E. hyemale* (0.163%). Detailed plant matter mass, yield obtained and concentrations of the essential oils acquired using steam distillation are found in Table 2.

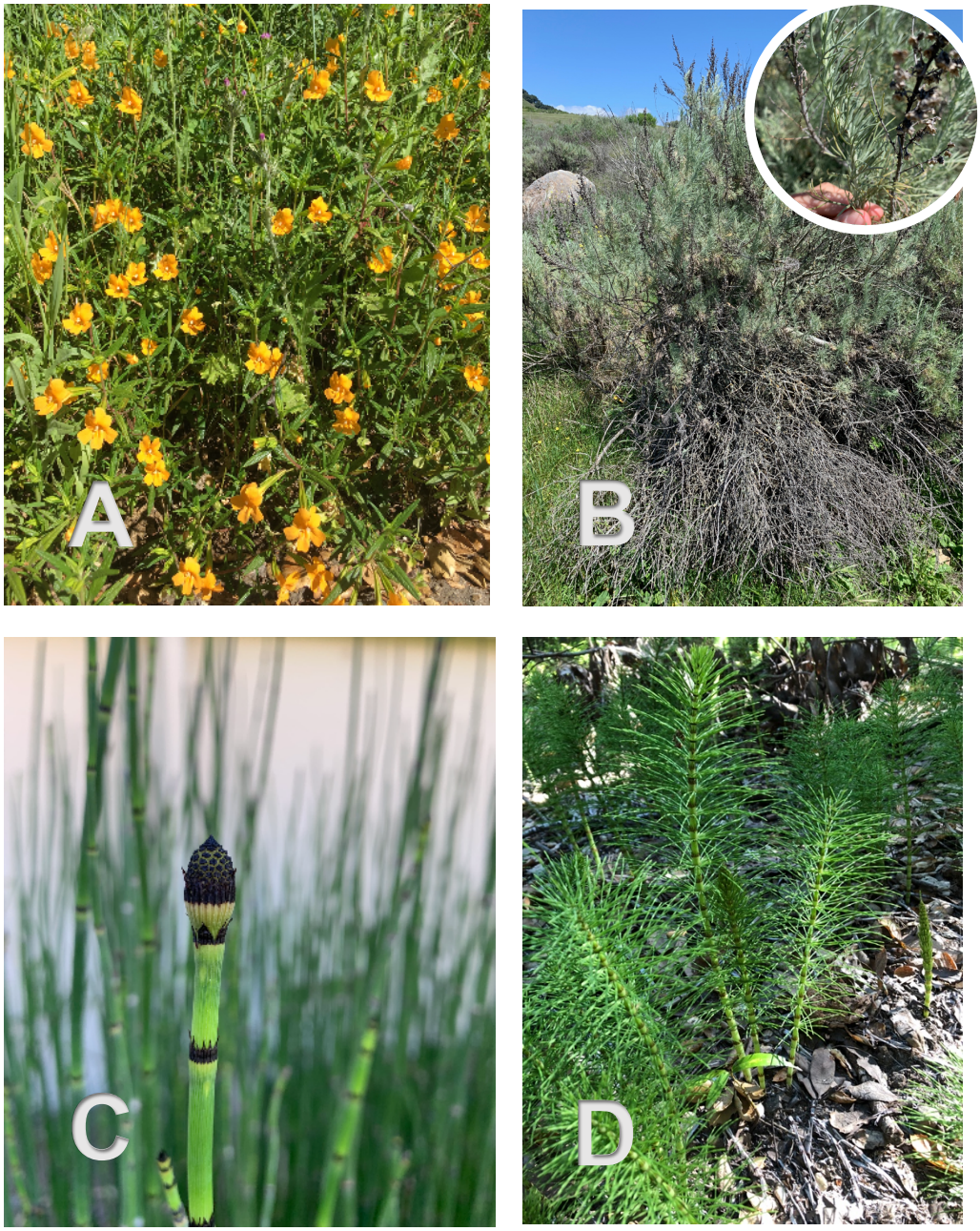



Figure 1. Photographs of the plants examined in this work. A. *Mimulus aurantiacus* B. *Artemisia californica* C. *Equisetum hyemale* D. *Equisetum telmateia*


Figure 1. *Cont*. F. *Marah fabacea.* G. *Marah fabacea* root

Table 2. Essential oils, plant material used, yield and concentrations obtained using steam distillation.

| Plant/Part | Plant material (grams) | Yield obtained (grams) | Percent recovery (%) | Concentration in 3 ml of ethyl acetate (g/mL) | Tested in Antibacterial assay | Chemical composition analysis |
|---|---|---|---|---|---|---|
| *Artemisia californica* leaves | 100 g | 0.9 g | 0.9% | 0.3 g/mL | NO | YES |
| *Mimulus aurantiacus* leaves | 550 g | 1.2 g | 0.218% | 0.4 g/mL | NO | YES |
| *Equisetum telmateia* | 400 g | 1.1 g | 0.275% | 0.37 g/mL | YES | YES |
| *Equisetum telmateia** | 100 g | 0.2 g | 0.2% | 0.067 g/mL* (methanol) | NO | YES |
| *Equisetum hyemale* | 550 g | 0.9 g | 0.163% | 0.3 g/mL | YES | NO |
| *Marah fabacea* leaves | 500 g | 1.1 g | 0.22% | 0.37 g/mL | YES | NO |
| *Marah fabacea* root | 500 g | 1.2 g | 0.24% | 0.4 g/mL | YES | NO |

* Dissolved in methanol

### *3.4 Antibacterial Assay*

For an assessment of the antibacterial activity of the essential oils was tested through a Kirby-Bauer disk diffusion method, in which a paper disk is soaked with the oil and is laid on top of an inoculated agar. The essential oils of *E. telmateia*, *E. hyemale*, the root and leaves of *M. fabacea* were tested against a list of Gram-positive and Gram-negative bacteria. The findings are displayed in Table 3.

The essential oil of *E. telmateia* inhibited the growth of all tested bacteria except for both *Listeria monocytogenes* strains, but not all of them were significantly different to the ZOI obtained with their solvent ethyl acetate. The Gram-positive *Enterococcus faecalis and Bacillus subtilis;* and Gram-negative *Escherichia coli* CFT073, *Escherichia coli* (C), *Escherichia coli* 536, *Klebsiella pneumoniae, Klebsiella aerogenes, Proteus mirabilis* H14320*, Morganella morganii, Acinetobacter baumanni, Pseudomonas fluorscenes, Aeromonas hydrophila, Citrobacter freundii and Salmonella* serovar montevideo were the ones that displayed significant difference between the ZOIs of the EO and the solvent.

*E. coli* 0157:H7, *E. coli* 536, and *Proteus mirabilis* H14320 were the only ones with ZOI significantly different using the oil obtained from the other horsetail, *E. hyemale.*

Results indicate some antibacterial activity for the volatile oils produced by the root of manroot against *E. coli* 0157:H7, *E. coli* K12, *Salmonella* serovar montevideo, but *Bacillus subtilis* was the only with significant difference. The essential oil from the leaves did exhibit activity against *E. coli* 0157:H7 and *Salmonella* serovar montevideo, but none of them was significantly different to the ZOI of the solvent.

Table 3. Antibacterial activity of *E. telmateia, E. hyemale, M. fabacea* root and leaf essential oils using disk diffusion method.

| Gram | | Diameter of inhibition zone (mm) | | | | |
|---|---|---|---|---|---|---|
| | | *E. telmateia* EO | *E. hyemale* EO | *Marah* Root EO | *Marah* Leaf EO | Ethyl acetate (control) |
| - | *Escherichia coli* CFT073* | **10.67±2.08** | 0 | 3±5.2 | 3±5.2 | 0 |
| | *Escherichia coli* 0157:H7 | 9.33±0.58 | **10.67±1.15** | 6.67±5.86 | 7.33±6.43 | 0 |
| | *Escherichia coli* (C) | **12±1** | 3±5.2 | 3.33±5.77 | 0 | 0 |
| | *Escherichia coli* K12 | 6±5.2 | 0 | 7.33±6.43 | 3.67±6.35 | 3.33±5.2 |
| | *Escherichia coli* 536* | **9.67±0.58** | **9.67±0.58** | 3.67±6.35 | 0 | 0 |
| | *Klebsiella pneumoniae* | **9.67±1.15** | 3±5.2 | 3.67±6.35 | 2.67±4.62 | 0 |
| | *Klebsiella aerogenes* | **9±0** | 0 | 3.67±6.35 | 3.67±6.35 | 0 |
| | *Proteus mirabilis* H14320* | **9.67±1.15** | **9.33±0.58** | 0 | 0 | 0 |
| | *Morganella morganii** | **9±0** | 0 | 0 | 0 | 1.33±3.27 |
| | *Providencia stuartii** | 6±5.2 | 0 | 3±5.2 | 3±5.2 | 0 |
| | *Acinetobacter baumanni** | **11.33±0.58** | 0 | 3.67±6.35 | 0 | 0 |
| | *Pseudomonas fluorscenes* | **9.33±0.58** | 0 | 0 | 0 | 0 |
| | *Aeromonas hydrophila* | **9.67±0.58** | 0 | 3.67±6.35 | 3±5.2 | 0 |
| | *Citrobacter freundii* | **9±0** | 0 | 0 | 3.67±6.35 | 0 |
| | *Salmonella* serovar montevideo | **9±0** | 0 | 6.67±5.86 | 6.33±5.51 | 0 |
| | *Serratia marcescens* | 6±5.2 | 0 | 0 | 0 | 0 |
| + | *Enterococcus faecalis* | **9.33±0.58** | 0 | 4±6.93 | 3.33±5.77 | 1.5±3.67 |
| | *Staphylococcus aureus* | 2.67±4.62 | 0 | 0 | 6±5.2 | 0 |
| | *Bacillus subtilis* | **9±0** | 0 | **10±1.73** | 3.33±5.77 | 0 |
| | *Micrococcus luteus* | 3.33±5.77 | 0 | 0 | 0 | 0 |
| | *Listeria monocytogenes* 6301 | 0 | 0 | 0 | 0 | 0 |
| | *Listeria monocytogenes* 6306 | 0 | 0 | 0 | 0 | 0 |

Values are presented as the average ± standard deviation. Bold values are significantly different ($p<0.05$) to the control according to Wilcoxon Rank Sum Test; EO= essential oil, *=UTI pathogens

### *3.5 Essential Oil Analysis*

To further characterize the obtained oils, and see if their components have been previously recognized as having antibacterial properties, we carried out a detailed compositional analysis through GC-MS. The chemical composition of the essential oils obtained from leaves of *Mimulus aurantiacus*, aerial parts of *Equisetum telmateia,* and leaves of *Artemisia californica* were analyzed by GC-MS. There are many types of compounds found in essential oils, including aldehydes, alcohols, and terpenes. The composition of essential oils from a particular plant species can vary both temporally and geographically. In addition, the composition from different parts of the same plant can also widely differ. Eighty-five (85) compounds were identified via GC-MS in the oil of *Equisetum telmateia* dissolved in ethyl acetate, the main components of which can be found in Table 6 and six (6) compounds were identified in the oil of the same plant dissolved in methanol found in Table 5. One hundred and forty-eight (148) constituents were recognized in the oil of the leaves of Sticky Monkey Flower (summary on Table 4). One hundred and twenty-nine (129), one hundred and twenty-four (124), and one hundred and eighty-eight (188) compounds were identified in the three analyses of the oil from *Artemisia californica*, results are summarized in Tables 7 – 9. Figures 2 and 3 represent the GC-MS chromatogram of the essential oils from *Marah fabacea* and *E. telmateia* respectively.

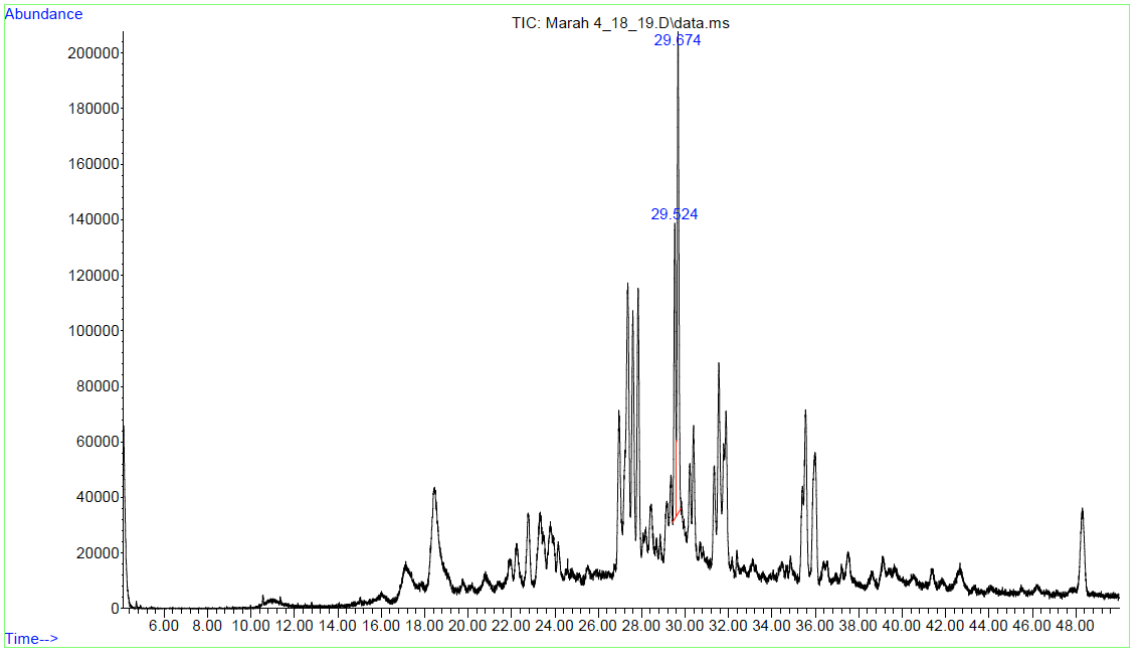



Fig 2. Representation of GC-MS from *Marah fabacea* essential oil

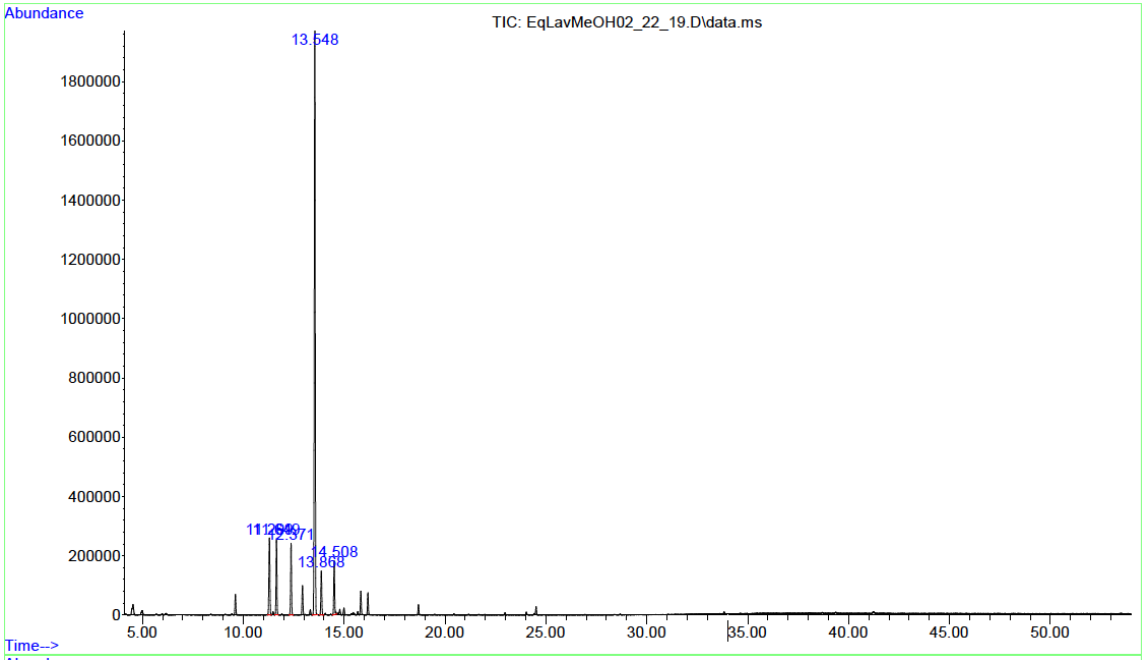
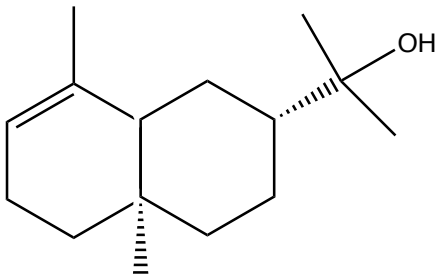
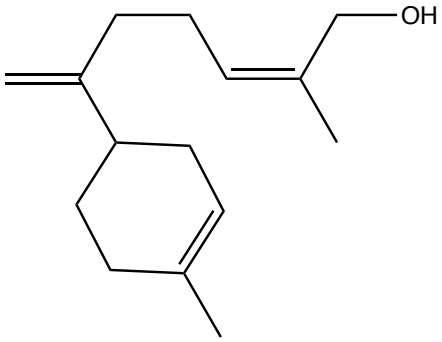
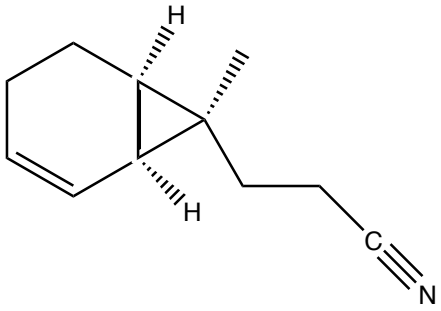
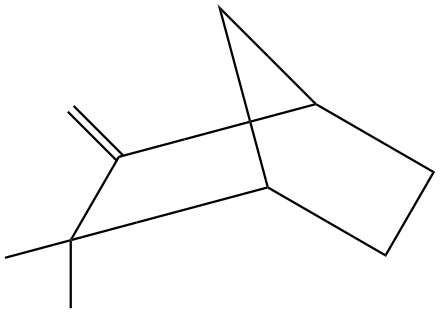



Fig 3. Representation of GC-MS from *E. telmateia* essential oil in MeOH.

Table 4. Summary of the chemical composition of the essential oil from leaves of *Mimulus aurantiacus*

| Component | Classification | Composition (%) | Chemical structure |
|---|---|---|---|
| α-Eudesmol $C_{15}H_{26}O$ | Polycyclic sesquiterpene hydrocarbon | 11.48 |   |
| Lanceol, cis $C_{15}H_{24}O$ | Monocyclic sesquiterpene hydrocarbon | 9.61 |   |
| Exo-7-methyl bicyclo (4.1.0)hept-2-en-endo-7-propanenitrile $C_{11}H_{15}N$ | Not found | 4.54 |   |
| Camphene $C_{10}H_{16}$ | Bicyclic monoterpene hydrocarbon | 4.24 |  |

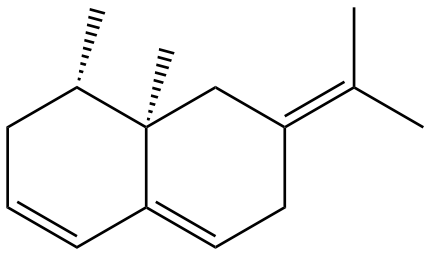
| | | | |
|---|---|---|---|
| β-Vetispirene<br>$C_{15}H_{22}$ | Sesquiterpene hydrocarbon | 3.45 |  |

Table 5 shows a summary of the identified components and the respective relative peak area of *E. telmateia* extract obtained by steam distillation and dissolved in methanol. Table 6 shows the same information but the extract was dissolved in ethyl acetate. We observed no coinciding results between GC-MS analyses. The main components found with the oil dissolved in methanol were thujone (69.67%) and eucalyptol (9.18%) and the main components found in the oil dissolved in ethyl acetate were eugenol (82.85%) and caryophyllene (3.37%). The main components of the oil dissolved in methanol, eucalyptol and thujone, were found in the oil dissolved in ethyl acetate in low concentration, 0.33% and 0.48% respectively. A summary of the components found in both solutions are listed on Tables 5 and 6.

Table 5. Summary of the chemical composition of the essential oil from the aerial parts of *Equisetum telmateia* with methanol as solvent

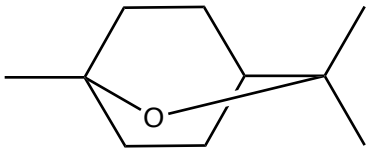
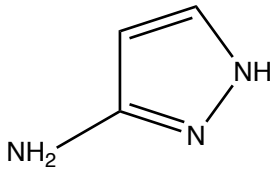
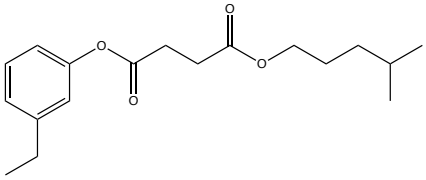
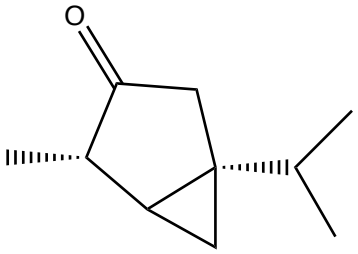
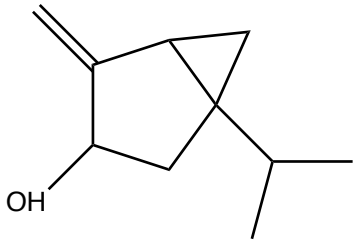
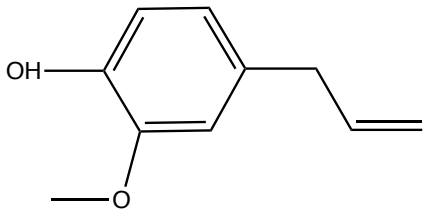
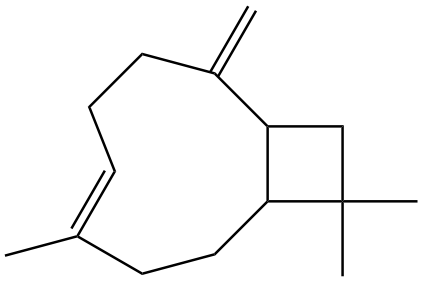
| Component | Classification | Composition (%) | Chemical structure |
|---|---|---|---|
| Eucalyptol $C_{10}H_{18}O$ | Oxygenated monoterpenoid | 9.18 |  |
| 3-Aminopyrazole $C_3H_5N_3$ | Heterocyclic pyrazole | 7.56 |  |
| Succinic acid, 3-ethylphenyl isohexyl ester $C_{18}H_{26}O_4$ | Not found | 7.37 |  |
| Thujone $C_{10}H_{16}O$ | Bicyclic monoterpene hydrocarbon | 69.67 |  |
| Sabinol $C_{10}H_{16}O$ | Bicyclic monoterpene hydrocarbon | 5.60 |  |

Table 6. Summary of the chemical composition of the essential oil from the aerial parts of *Equisetum telmateia* with ethyl acetate as solvent

| Component | Classification | Composition (%) | Chemical structure |
|---|---|---|---|
| Eugenol $C_{10}H_{12}O_2$ | Phenolic compound | 82.85 |  |
| Caryophyllene $C_{15}H_{24}$ | Polycyclic sesquiterpene hydrocarbon | 3.37 |  |

To test the reproducibility of the GC-MS analysis, we ran the analysis of the oil obtained from *Artemisia californica* leaves in triplicate, the results of which indicated identical chemical constituents. Thujone was found to be 10.43% of the total composition of the oil on the first trial (Table 7), 9.47% on the second trial (Table 8) and 8.94% on the third (Table 9). Eucalyptol was also found in the three attempts with a 6.2%, 6.59%, and 4.85% respectively. The last major compound found on all the trials was Germacrene D with 4.42%, 3.92%, and 4.16% respectively. The components were comparable in all trials with consistent percentages, consequently, we did not replicate for the analysis of the rest of the oils.

Table 7. Summary of the chemical composition of the essential oil from leaves of *Artemisia californica* (07/12/19)

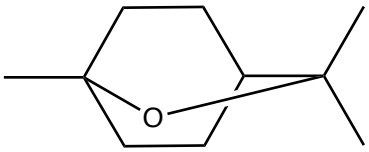
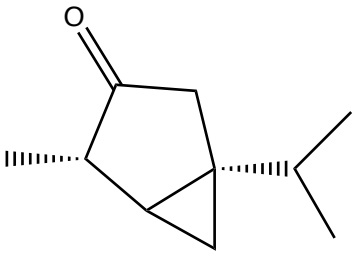
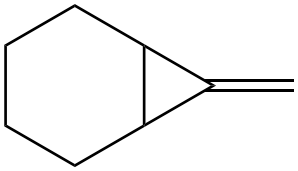
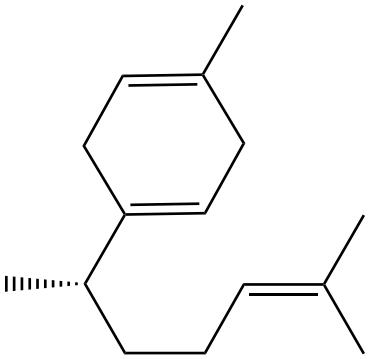
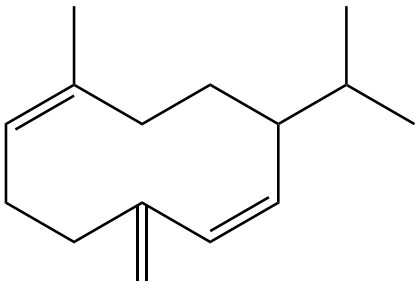
| Component | Classification | Composition (%) | Chemical structure |
|---|---|---|---|
| Eucalyptol $C_{10}H_{18}O$ | Oxygenated monoterpenoid | 6.20 |  |
| Thujone $C_{10}H_{16}O$ | Bicyclic monoterpene hydrocarbon | 10.43 |  |
| Bicyclo[4.1.0]heptane, 7-methylene $C_8H_{12}$ | Not found | 19.91 |  |
| β-Curcumene $C_{15}H_{24}$ | Sesquiterpene hydrocarbon | 7.66 |  |
| Germacrene D $C_{15}H_{24}$ | Sesquiterpene hydrocarbon | 4.42 |  |

Table 8. Summary of the chemical composition of the essential oil from leaves of *Artemisia californica* (08/07/19)

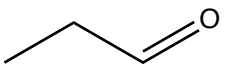
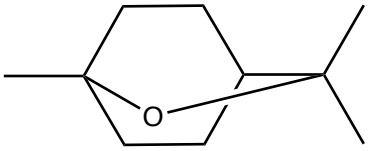
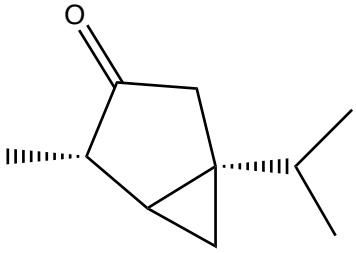
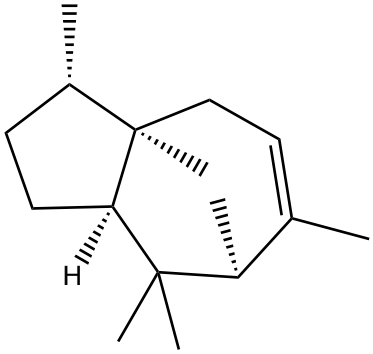
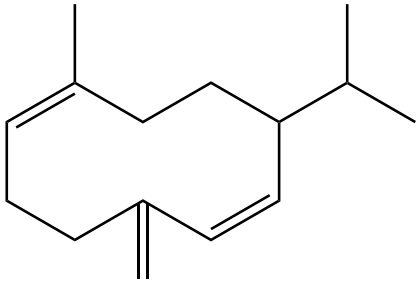
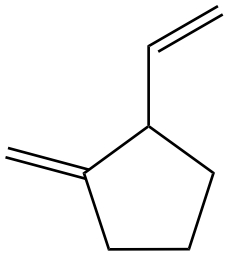
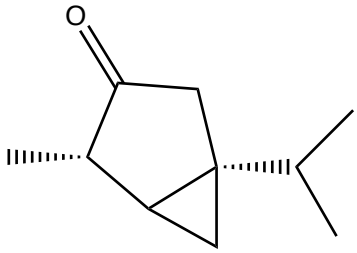
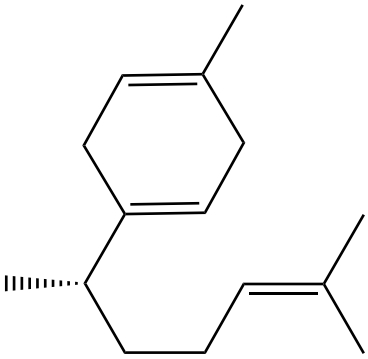
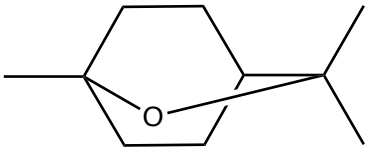
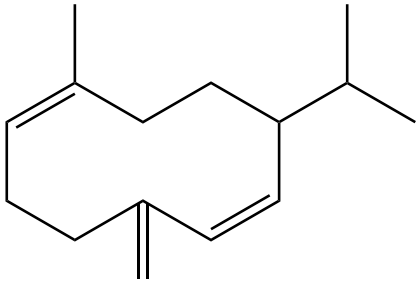
| Component | Classification | Composition (%) | Chemical structure |
|---|---|---|---|
| Propanal $C_3H_6O$ | Aldehyde | 6.67 |  |
| Eucalyptol $C_{10}H_{18}O$ | Oxygenated monoterpenoid | 6.59 |  |
| Thujone $C_{10}H_{16}O$ | Bicyclic monoterpene hydrocarbon | 9.47 |  |
| 1(2H)-Naphthalenone, 3,4,4a,5,8,8a-hexahydro-8a-methyl-, trans- | Not found | 20.08 | Not found |
| α-Cedrene $C_{15}H_{24}$ | Polycyclic sesquiterpene hydrocarbon | 8.07 |  |
| Germacrene D $C_{15}H_{24}$ | Sesquiterpene hydrocarbon | 3.92 |  |

Table 9. Summary of the chemical composition of the essential oil from leaves of *Artemisia californica* (10/29/19)

| Component | Classification | Composition (%) | Chemical structure |
|---|---|---|---|
| 1-Methylene-2-vinylcyclopentane $C_8H_{12}$ | Not found | 12.42 |  |
| Thujone $C_{10}H_{16}O$ | Bicyclic monoterpene hydrocarbon | 8.94 |  |
| β-Curcumene $C_{15}H_{24}$ | Sesquiterpene hydrocarbon | 7.28 |  |
| Eucalyptol $C_{10}H_{18}O$ | Oxygenated monoterpenoid | 4.85 |  |

| | | | |
|---|---|---|---|
| Germacrene D<br>$C_{15}H_{24}$ | Sesquiterpene hydrocarbon | 4.16 |  |

# Chapter 4

# DISCUSSION

The prudent use of antimicrobials and the development of new therapies or alternative treatments are needed to minimize antibiotic resistance (Flores-Mireles et al., 2015). Scientists are “reviving” old antibacterials, creating new antibacterials, and combining the drugs available (Flores-Mireles et al., 2015; Giamerellow & Poulakou 2009; Mazzariol et al., 2017). Here, we studied the utility of plants used by the Chumash and other tribes against infections.

Our findings suggest promising antibacterial activity by *E. telmateia* and its components, eugenol and caryophyllene, against several bacterial strains including urinary pathogens. *E. hyemale* and *M. fabacea* showed antibacterial activity to fewer strains but the horsetail included uropathogens. The components found in *M. aurantiacus* (α-eudesmol) and the ones found in *A. californica* (thujone, eucalyptol, germacrene D and propanal) have been found of having antibacterial activity as pure compounds or as part of essential oils by other studies.

It has long been recognized that some EOs have antimicrobial properties because of their large number of components. The major groups of plant-derived antimicrobials include polyphenols, alkaloids, flavonoids, terpenoids, lectins, tannins, and saponins (Kon et al., 2016). Today we know that phenols, terpenes and aldehydes antibacterial effect is due to their action against the cell cytoplasmic membrane (García-Salinas, 2018).

In the present study, we were able to recognize and analyze 19 different major compounds, where the majority were terpenes, secondary metabolites responsible for the fragrance of plants. Terpenes’ general chemical structure is $C_{10}H_{16}$ but they also occur as sesquiterpenes ($C_{15}$) or with additional elements like oxygen (called terpenoids). Several studies have demonstrated them as active against bacteria by the disruption of the cell

membrane (Cowan, 1999). Eleven of the nineteen compounds identified in this work were terpenes or terpenoids, from which seven were sesquiterpene hydrocarbons, three were monoterpene hydrocarbons and one oxygenated monoterpenoid. The phenolic compound eugenol and the aldehyde propanal were also found in this work (refer to Tables 4 – 9).

Even though Gram-negative organisms are known as slightly less susceptible to EOs than Gram-positive bacteria (Burt, 2004) the essential oil of *E. telmateia* inhibited the growth of two of the six Gram-positive bacteria tested: *Enterococcus faecalis and Bacillus subtilis;* and twelve of the sixteen Gram-negative tested: *Escherichia coli* CFT073, *Escherichia coli* (C), *Escherichia coli* 536, *Klebsiella pneumoniae, Klebsiella aerogenes, Proteus mirabilis* H14320*, Morganella morganii, Acinetobacter baumanni, Pseudomonas fluorscenes, Aeromonas hydrophila, Citrobacter freundii and Salmonella* serovar montevideo (Table 3). This could be due to the high concentration of eugenol found in the GC-MS analysis with the oil dissolved in ethyl acetate (82.85%, Table 6). Multiple studies have demonstrated eugenol antibacterial action, that can be ascribed to the presence of a free hydroxyl group in the molecule (Cowan, 1999).

The review from Marchese et al. in 2017 mentions that eugenol has been demonstrated to act by the disruption of the cytoplasmic membrane, the alteration of bacterial cell membrane permeability by making it hyper permeable, its alteration of the membrane fatty acid composition, it affects the transport of ions and ATP, triggers cell cytotoxicity by the production of intracellular reactive oxygen species (ROS), and inhibits the action of enzymes like protease, amylase, histidine carboxylase, and ATPase. Cell wall deterioration and a high degree of cell lysis were also noted by Thoroski et al., in 1989. As a result, despite the fact that EOs could affect Gram-negatives less than Gram-positives, eugenol has the potential to have a substantial action in both.

Even though eugenol has been demonstrated to have antibacterial activity against *E. coli*, *P. fluorescens, Salmonella typhimurium*, *S. aureus, K. aerogenes,* and *Listeria*

*monocytogenes,* and more bacterial strains (Marchese et al., 2017), in the present study as being part of the oil of *E. telmateia* was not always active against all *E. coli* strains*, S. aureus,* or *L. monocytogenes.* This could be due to the concentration of eugenol in our oil, giving that eugenol was 82.85% in a 0.37 g/mL oil/ethyl acetate concentration, the oil used for the antibacterial assay had 0.306 g of eugenol, higher concentrations might be needed to inhibit said strains.

Eucalyptol, the major component of the leaf extracts from eucalyptus, 24% according to Yang et al. in 2011, and possess antibacterial activity against *S. aureus* by degrading bacterial cell membrane, as reported by Yang. Eucalyptol was also found to be one of the major components of eucalyptus (13.73%) and myrtle (50.13%) by Akin M. et al in 2010, they demonstrated eucalyptus and myrtle EOs to have some antimicrobial activity on Gram-positive and Gram-negative bacteria and responsibilized the high proportions of eucalyptol as one of the responsible for said activity. Karlovic et al. in 2000 demonstrated some antibacterial effects from eucalyptol against *S. aureus*. The EO of *Callistemon citrus* found to be rich in eucalyptol (32.4%) showed antibacterial activity against *E. faecalis* (An et al. 2020). The oil dissolved in methanol, in which eucalyptol was identified to be 9.18% (Table 5) was not tested for antibacterial activity. Eucalyptol was also found to be a major component of *A. californica* (4.85-6.59%, Tables 7 – 9) also not tested for antibacterial activity.

Thujone was the major component found in the analysis done in the oil of *E. telmateia* dissolved in methanol (69.67%, Table 5) and was found to be 8.94-10.43% of the constituents of *A. californica* (Tables 7 – 9). The antimicrobial activity of thujone was demonstrated by Sivropoulou in 1997 against *E. coli, P. aeruginosa, S. aureus, Bacillus subtilis,* and low activity against *S. typhimurium.* The activity of eucalyptol was demonstrated to the same bacteria in the study. Sabinol, one of the precursors of thujone, was also found to be a component of *E. telmateia* dissolved in methanol (5.60%, Table 5).

The oil dissolved in methanol, in which thujone and sabinol were identified was not tested for antibacterial activity, neither the oil from *A. californica*.

Caryophyllene, also a component in the oil of *E. telmateia* dissolved in ethyl acetate (3.37%, Table 6), was also found to be a major component of eucalyptus, in the study by Akin et al. in 2010 mentioned above, found to be 11.55% of the EO and was mentioned as a possible responsible for the antibacterial activity that the EO showed. E-caryophyllene was also found to be a constituent of some species of the Verbenaceae family, in a study where the plant species were found to have some activity against *E. coli, B. cereus* and *S. aureus* (Montanari et al., 2011). E-caryophyllene was also was found to be a major part of the composition of *Melaleuca leucadendra* by Nguyen Thi Giang An et al. in 2020, the EO of the plant showed antibacterial activity against *E. faecalis*. β-caryophyllene was determined to have antimicrobial activity against *E. coli* and *S. aureus* in high concentrations in a minimal inhibitory concentration (MIC) study (García-Salinas et al., 2018).

The Gram-negatives: *E. coli* 0157:H7, *E. coli* K12, and *Proteus mirabilis* H14320 were the only ones with ZOI significantly different using the oil obtained from the other horsetail, *E. hyemale,* but the chemical composition could not be carried out and chemical composition performed by GC-MS in other investigations were not found.

A study by Radulovic et al. in 2006 studied the composition and antimicrobial activity of a different species of horsetail, *Equisetum arvense*, they found its main constituents to be hexahydrofarnesyl acetone (18.34%), cis-geranyl acetone (13.74%), thymol (12.09%), and trans-phytol (10.06%). The components eucalyptol, found also in the present study in *E. telmateia* using methanol as solvent (9.18%, Table 5) and caryophyllene found in *E. telmateia* using ethyl acetate as solvent (3.37%, Table 6) were found in the Radulovic study being 5.19% and 5.47% respectively. The authors also found *E. arvense* to have antibacterial activity against *S. aureus, E. coli, K. pneumoniae, P. aeruginosa* and

*Salmonella enteriditis. E. coli* inhibition is the only shared by all three horsetails and *E. telmateia* and *E. arvense* showed antibacterial activity against *K. pneumoniae*.

The chemical composition of the manroot could not be completed in this investigation. Studies have described this plant as toxic, producing many poisonings, and containing ribosome-inactivating proteins (RIPs) (Adams & Garcia, 2006). RIPs are widely found in various plant species. It has been demonstrated that RIPs have been connected to antibacterial activities by the alteration of the structure of prokaryotic rRNA (Zhu et al., 2018). As part of this study, the EO from the root of the manroot showed positive results in the antibacterial activity against *Bacillus subtilis*. The EO from the leaves did not exhibit significant activity against any of the strains tested.

Sticky monkeyflower (SMF) antibacterial activity assay could not be completed and studies that have conducted similar assays could not be found, but other plants with the same major constituents have demonstrated having some activity. α-Eudesmol (11.48% in SMF) was also found to be a major component of *Melaleuca leucadendra* oil mentioned of having antibacterial activity against *E. faecalis* in the study cited above. α-Eudesmol was also 10.6% of the EO of *Curcuma zedoaria* that showed antibacterial activity against *S. aureus* and *Corynebacterium amycolatum* (Yonzon et al., 2005). The other components found on *M. aurantiacus*, lanceol, cis; camphene and β-Vetispirene were not found specifically as antimicrobials but all of them are terpenes known to have antibacterial activity.

*Artemisia californica* antibacterial activity assay was not conducted in this study but has shown *E. coli* K12 growth inhibition in other investigations (Allison et al., 2017). A recent analysis by GC-MS of the chemistry of *A. californica* found 15 monoterpenoids in its composition: camphene, mentha-diene, β-pinene, eucalyptol, isopropenylmethylcyclohexanol, trimethylheptadienol, isopropylmethyl-bicyclohexanol, thujanone, thujone, chrysanthenone, camphor, borneol, carene, menthenol and

menthadienol where the major monoterpenoids were eucalyptol (24%), camphor (18%), carene (14%), and menthadienol (9%). (Adams, 2012). In the present study, we found the components of *A. californica* to be monoterpenoids: eucalyptol (4.85-6.67%) and thujone (8.94-10.43%), sesquiterpene hydrocarbons: β-Curcumene (7.28-7.66%), Germacrane D (3.92-4.42%), and α-Cedrene (8.07%) and other components like the aldehyde propanal (6.67%).

Germacrene D and eucalyptol also shown to be constituents of some species of the Verbenaceae family mentioned having activity against *E. coli, B. cereus* and *S. aureus* in the 2011 study by Montanari. Eucalyptol antimicrobial activity was mentioned above.

Propanal has been found to have antibacterial activity against *S. typhimirium* in high concentrations (Lamba, 2007). Has been also identified in ginger (*Zingiber officinale*) by Shareef et al. in 2016, in the same study the antibacterial capacity of ginger has been evaluated, concluding that ginger possesses remarkable antimicrobial activity but they attributed it to its naphthalenamine, decanal, and alfa-copaene composition for their known antimicrobial action. Other aldehydes have been tested for antimicrobial activity finding that they can cause the perturbation of the lipidic fraction of the plasma membrane and are able to penetrate into bacterial cells (Trombetta et al., 2002).

Components that were not mentioned in this discussion were not found in the literature. One could infer that if they have antibacterial activity the mechanism of action would be shared with other components from the same class, terpenes would have an action like other terpenes, aldehydes as other aldehydes, and so on.

Variability on the chemical composition and/or antibacterial activity between EOs from the same plant species between this and other studies could be due to different methods utilized. There are numerous alternatives for extraction methods, chemical composition analysis, and antimicrobial activity assays, using one technique or another

would lead to differences in the chemical constitution, resulting in different concentrations of the chemical contents and therefore different results for antibacterial activity.

Burt explains in his work in 2004 that EOs produced from herbs harvested during or immediately after flowering possess the strongest antimicrobial activity. In the present study, the plants were collected during the Spring season, from March through May without being concerned if the plants made these conditions or not. If the plants had been collected during or immediately after their flowering and could explain the variability on chemical composition and/or antibacterial activity between EOs from the same plant species between this and other studies.

Different harvesting season, developmental phase, geographical sources and plant organ studied (Burt, 2004) could also lead to different results between studies. The differing components found in the EO from *E. telmateia* using methanol or ethyl acetate as solvent could be attributed to the different collection dates, thereby, different developmental phase in the plants collected and different collection sites and not attributed exclusively to the solvent used.

Even though we have information about the plants that were used by California tribes, we do not have details about how long the treatments were, when in the process of getting a urinary infection did they take the herbal tea, whether they used it to prevent an infection or to cure the disease. A remedy must have been effective to some degree for it to stay up to date through generations of oral tradition but a lot could have been lost through the years. If a tea of any of the plants in the list could prevent the infections from taking place, instead of curing an infection already existing, it is possible that such therapy would not greatly impact the normal microbiota. The plants that did not show significant antibacterial potential, like manroot, could still have an effect on the urinary system that does help with the treatment of UTIs without directly affecting bacteria, for example, by increasing urination frequency, or reducing the symptoms by reducing the inflammatory reaction of

the immune system. Patients that today suffer from UTIs are accustomed to having a clean routine, for example, they know to always urinate after a sexual encounter, and consume a sufficient amount of water every day, if a tea of the plants like manroot would prevent these infections they would have an alternative treatment that would not affect their beneficial flora. For this, further experiments would be required.

Because *E. telmateia* inhibited the growth of all uropathogens tested with the exception of *Providencia stuartii*, and the components found on *E. telmateia*, eucalyptol, thujone, eugenol and caryophyllene have been found to have antibacterial activity but no information was found about the antimicrobial activity of 3-aminopyrazole, succinic acid 3-ethylphenyl isohexyl ester and sabinol, our future research will be focused on further characterizing this horsetail EO and its components. The disk diffusion method is used as a preliminary check for antibacterial activity prior to more detailed studies more precise results could be obtained exploring other methods like minimum inhibitory concentration or kill curve assays.

The unexplored tests on this study are expected in the future, analysis of the chemical composition of *E. hyemale* and *M. fabacea*, and antibacterial assays on *A. californica* and *M. aurantiacus* will be performed, as well as further characterization of their components.

These experiments will be continued in the future as we keep searching for alternative compounds to contribute to the antibacterial resistance emergency.

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