Analysis of Floral Sources of a Local Honey Used in Clinical Treatment of Topical Community Acquired Methicillin Resistant *Staphylococcus Aureus*

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ABSTRACT

A study was initiated during the summers of 2015–2019 to characterize the floral and chemical components in a local honey (clinical honey) that was being used in a Food and Drug Administration (FDA) approved clinical study designed to evaluate effectiveness in controlling topical community acquired methicillin resistant *Staphylococcus aureus* (caMRSA) infections. Floral sources were determined by collecting nectar and pollen from plants visited by bees within the area where the local honey is being produced (Study Area). Pollen characteristics were determined by using both light microscopy (LM) and scanning electron microscopy (SEM). This information was compared to pollen collected by a pollen trap on hives within the study area. The nectars and the medical honey were analyzed for biologically active compounds using Solid Phase Micro Extraction (SPME) and Gas Chromatography-Mass Spectrometry (GC-MS). This equipment allows a researcher to extract, separate, and identify chemical components of a honey or nectar sample. Fourteen biologically active compounds were identified from the eighteen floral sources and the clinical honey. Nine of the fourteen

compounds were selected for standard minimum inhibitory concentration (MIC) antibacterial assay using CAMRSA ATCC BAA-44 strain. Phenylethyl alcohol and phenylacetaldehyde were the only compounds exhibiting promising activity against caMRSA. Both exhibited bacteriostatic activity. A variety of antibiotic compounds were unique to clinical honey. This suggests that the various nectars provide a large chemical base for antibiotic compounds.

KEYWORDS: Topical community acquired methicillin resistant *Staphylococcus aureus* caMRSA, Honey, Nectar, Methylglyoxal, Phenylethyl alcohol, Phenylacetaldehyde, Pyruvic aldehyde, Antibacterial assay, Solid Phase Micro Extraction (SPME), Gas chromatography-mass spectrometry (GS-MS), Russian knapweed, Tamarisk, Coyote willow, Scanning Electron Microscopy (SEM).

1 INTRODUCTION

Community acquired methicillin resistant *Staphylococcus aureus* (caMRSA) infections are not uncommon and involve the skin causing abscesses. Currently, these infections have become resistant to antibiotics that were previously effective (Vandamme et al., 2013).

Alternative treatments include the use of honey. Honey has been used for wound healing for over a thousand years (Majno, 1975). In ancient Egypt and Greece, honey was used in various types of wound poultice as early as 1700 BC. Most of the honey currently used in wound care is Manuka® honey (Medi-honey®), which originates only from nectar of a single plant species, *Leptospermum scoparium* (*L. scoparium*) growing in Australia and New Zealand (Adams et al., 2009). The antibacterial activity of Manuka® honey is attributed to the chemical methylglyoxal (pyruvic aldehyde).

When a honey other than Manuka® is used, little is known about the honey in terms of its region of origin, its floral make-up (nectars) or antibacterial compounds. As an example, in vitro studies investigated the potential of natural honey from Northern Ireland and a number of locations in Africa to control bacterial infections (Al-Jabri, 2003). Other in vitro studies demonstrated antibacterial activity against caMRSA isolates and other *Staphylococcal* species but the honey was not analyzed for antibacterial compounds(Maeda et al., 2008).

The following investigation characterizes the nectar sources and chemical components found in a varietal honey (clinical honey) from northwest New Mexico that has demonstrated favorable in-vitro activity against methicillin resistant *S. aureus* (caMRSA) infections (Rankin, 2012). The investigations took place in May to July of 2015–2019 and involved seven undergraduate students participating in a summer undergraduate research program at San Juan College in Farmington, New Mexico. Floral sources were identified through direct observation of bee activity. Nectars and the clinical honey were analyzed as to chemical composition using Solid Phase Micro Extraction (SPME) and Gas Chromatography-Mass Spectrometry (GC-MS). The chemicals occurring in the nectars and clinical honey having potential biological activity were further investigated in vitro for activity against caMRSA.

2 MATERIALS AND METHODS

2.1 The Study Area, Floral Source Identification and Pollen Collection

The study area is located approximately 3 km west of Farmington, New Mexico and adjacent to the north side of the San Juan River (Figure 1). The study area was established by designating an approximately 0.5-km radius centered on the hives. A main irrigation canal and maintenance road are located east to west approximately 30 m north of the hives.

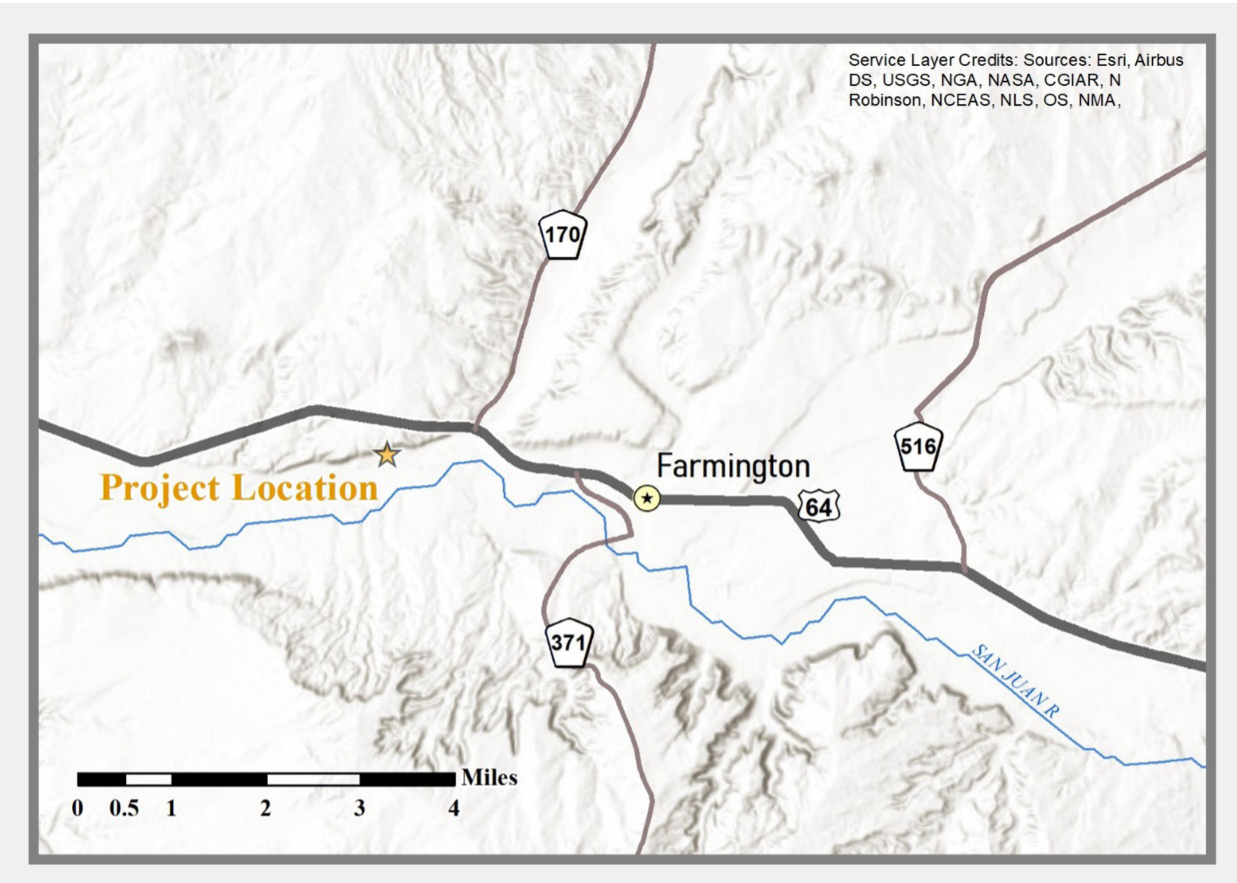

Figure 1: Map of the study area located near Farmington, New Mexico.

A field survey was done within the study area by collecting plants visited by bees during late June and early July. This time period coincides with previous years' spring honey harvest. Floral sources within the study area were identified (Heil, 2013) and visitation by honeybees recorded by systematically walking the study area.

Flowers were clipped from each plant species and transported to the laboratory. Flowers were allowed to dry overnight to collect shed pollen. Pollen grains were either stained with safranin and permanently mounted on glass slides for observation with light microscopy (LM) or critical point dried and sputter coated with gold palladium for observation with Scanning Electron Microscopy (SEM) (Hesse, 2010). Pollen transported by bees to the hives was intercepted by placing a pollen trap on a beehive and collecting pollen packets after a 48-hour period of time. The pollen packets were separated in the lab based on color, each color representing a specific pollen source (Figure 2). An exception was two of the packet colors (white and grayish white, third and fifth from the left

in Figure 2) were subsequently both identified as Coyote willow. Field-collected pollen was used to compare to pollen from the pollen packets. The pollen packets were prepared for observation by crushing and suspending in glycerin with safranin added as a stain. This was then mounted on microscope slides and observed in-situ using LM.

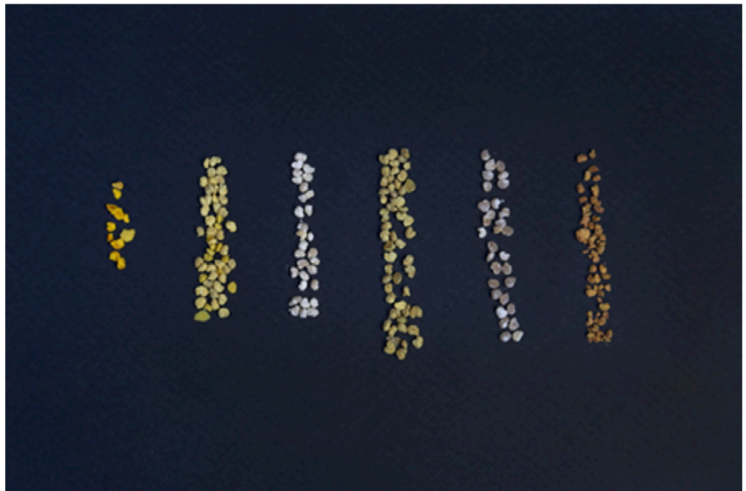

Figure 2: Pollen from pollen packets separated by color. From left to right: dark yellow (*Carduus nutans*), yellow (*Atriplex canescens*), white (*Centaurea repens*), grayish/yellow (*Salix exigua*), grayish/white (*Salix exigua*), rust (*Tamarix parviflora*). The grayish yellow pollen and the grayish white pollen were both identified as Coyote willow.

Whole plant voucher specimens of each species included in the study were collected in the field. The voucher specimens were transported to the laboratory, pressed, and dried. Dried specimens were subsequently mounted on herbarium paper and identified to species. The preserved plants are housed in the San Juan College Herbarium (SJNM).

2.2 Clinical Honey Collection Method

Clinical honey investigated in the study was obtained from hives managed by one of the authors, Stephen Rankin. The bees were not exposed to antibiotics, miticides, or other chemicals. The honey for the study was harvested during the main nectar flow of late June and early July. Wax was separated from honey through filtration and discarded. The honey was not heated at any time but dispensed into 12 mL syringes and frozen in a household freezer at -23 °C degrees. Honey was thawed prior to analysis using SPME and GC-MS.

2.3 Nectar Collection Method

Flowers were collected in 2016 and 2017 from the 18 plant species previously identified during field observations and nectar extracted from floral nectaries and floral tubes in the lab as follows (Morrant, 2009). Approximately 50 nectaries and floral tubes of a given flower type were placed into 0.5 mL deionized water and sealed in a microcentrifuge tube. This mixture was placed in a laboratory refrigerator at 3 °C for 24 hours. The nectaries and floral tubes were centrifuged and the supernatant collected into pre-weighed 5 mL vials fitted with a Polytetrafluoroethylene (PTFE) faced rubber septum. The vials were stored in a -10 °C freezer until used for analysis.

2.4 Chemical Analysis Method

In addition to the 18 nectar samples, chemical analysis included the clinical honey and nectars from all plants identified as nectar sources for the bees. Headspace SPME was used to collect volatiles from each sample before analysis with GC-MS. This method was developed based on the previous work of others and eliminates interference from water prior to analysis with GC-MS. (Daher, 2010). The GC-MS used was a Thermo Trace GC Ultra Polaris Q equipped with a Restek 5xi-55Sil, 30 m, 0.25 mm internal dimension column.

The SPME fiber used was an 85 mm polyacrylate over fused silica fiber (Suppelco™). It was pre-conditioned in a sealed and heated vial at 290 °C under flowing ultra-high purity nitrogen for 1 hour. Prior to injection in the GC-MS, the fiber was conditioned in the GC inlet at 280 °C for 7 minutes with a helium split flow of 60 mL/min. This resulted in minimal and consistent background signals coming from the fiber and septum. Periodic blank injections were performed and used to subtract out these signals from the results and to ensure there was no carry over by the SPME fiber from run to run.

SPME of clinical honey and nectar samples was performed as follows: The mass of the honey or nectar water mixture was measured, typically 0.2 g to 0.5 g. By calculation, 5% sodium chloride was added (about 0.02 g) along with 10 μL of 1.0 M HCl. The vial was then purged with UHP nitrogen for 10 seconds. The vial was then heated to 70 °C for 30 minutes to equilibrate the headspace. The SPME fiber was then exposed to the headspace at this temperature for 30 minutes.

The fiber was then introduced into the GC Injector, splitless for 1 minute at 280 °C. After 1 minute the fiber was withdrawn and the injector swept with split flow of 40 mL/min. The column flow was set to constant flow of 1 mL/min. The temperature program for the oven was to hold at 35 °C for 1 minute, ramp at 20 °C/min to 240 °C, and hot at 240 °C for 1 minute. The GC-MS transfer line was 300 °C. The GC-MS was set to analyze positive ions using electron impact ionization with the ion source at 200 °C. The mass range was 34–300 amu m/z. Mass spectra were searched using the National Institute of Standards and Technology (NIST) library. Individual peaks were identified and silanes were eliminated from further consideration. Silanes were thought to originate from the PTFE septum of the GCMS.

2.5 Bio Assay of Selected Compounds

During the fall of 2018, compounds were selected for bioassay based on probable antimicrobial activity. Possible activity was identified from information published in PubChem. Chemicals thus identified were sent for bioassay to the Biology Department at New Mexico Institute of Mining and Technology, Socorro, New Mexico. Bioassay initially consisted of utilizing the Kirby-Bauer method to directly observe inhibition in vitro. Each chemical was further tested by determining minimal inhibitory concentration (MIC) for BAA-44 caMRSA of each chemical. MIC is the minimal concertation of a specific chemical that will cause inhibition of growth of a specific microorganism (Gullberg, July 2011). Methylglyoxal (pyruvic aldehyde), the active chemical in Medi-honey® (Manuka®) was included in the assay as a positive control.

Determination of MIC consisted of preparing each compound into a final concentration of 40 mM for testing with the exception of N-à, N-ê-Di-cbz-L-arginine, which was prepared at 10 mM in propylene glycol (PG). All chemicals were compared to pyruvic aldehyde. Each of the compounds

was made to a stock concentration of 100 mM in Dimethyl sulfoxide (DMSO) and tested for activity at 1mM 1% DMSO in liquid culture. All organisms were incubated at 37 °C. Two-fold serial dilutions were made as well as appropriate controls (positive control 100 mM PAO, negative control and vehicle control at 1% DMSO). Results were analyzed by determining spectrophotometric absorbance values at 595 nm.

3 RESULTS AND DISCUSSION

3.1 Description of Study Area

Historically, the study area could be described as a cottonwood gallery positioned along both sides of the San Juan River. By the early 1900s the area was converted to agricultural use, including fruit crops, grain crops, permanent pasture mixes and Alfalfa (Furman, 1977). The area presently is not heavily farmed and exhibits a variety of native and agricultural species. Weedy and invasive plant species include Musk thistle (*Carduus nutans*), Canada thistle, (*Cirsium arvense)*, Showy milkweed*, (Asclepias speciosa*)*,* Tamarisk, (*Tamarix ramosissima)*, Russian olive, (*Elaeagnus angustifolia*) and Russian knapweed (*Centaurea repens*). Native vegetation within the study area includes Four-wing saltbush (*Atriplex canescens),* Greasewood (*Sarcobatus vermiculatus*), Coyote willow (*Salix exigua*) and Rio Grande cottonwood (*Populus angustifolia*). The ditch bank is extensively populated with Coyote willow, Tamarisk, and Russian knapweed.

3.2 Floral Sources

Eighteen species of plants (Table 1) were identified (Heil, 2013; USDA, NRCS, 2023) within the study area as pollen and nectar sources. Identification was based on observing visitation by bees. During this time period, bees were observed most frequently visiting Russian knapweed, Tamarisk, Four-wing saltbush, and Coyote willow that were in bloom along the ditch bank. Using LM, five pollens were positively identified from the pollen packets and from the clinical honey (Table 2). Pollen positively identified from the clinical honey was Russian knapweed and Coyote willow with a much smaller amount of Musk thistle and Tamarisk. These results indicate that the clinical honey is a mixed floral source honey containing a significant contribution of pollen from both Russian knapweed and Coyote willow. Clinical honey was collected immediately following the late spring nectar flow late June through early July. Other plants listed in Table 1 were infrequently visited by bees and are believed to add very little to the final chemical makeup of the clinical honey. Four-wing saltbush pollen was not observed in the clinical honey but was found in the pollen packets. Four-wing saltbush is a dioecious, wind pollinated plant, producing male and female flowers on different plants. Pollen from the male plants was shed in great amounts and was observed occurring commonly on all vegetation in the study area. Only male flowers were visited by bees and pollen was collected only from male flowers.

Scientific Name	Common Name	Pollination Status
Asclepias speciosa	Showy milkweed	Insect (Bees) By Pollanaria [2]
Atriplex canescens [3]	Four-wing saltbush	Wind
Carduus nutans	Musk thistle	Insect
Chaetopappa ericoides	Rose heath	Unknown [1]
Convolvulus arvense	Morning glory	Insect
Delphinium scaposum	Blue larkspur	Insect
Eleagnus angustifolia [3]	Russian olive	Insect and Wind
Erigeron spp.	Fleabane	Unknown/Probably Insect [1]
Mentzelia albicalus	Sticky leaf	Insect
Opuntia polycantha	Prickly pear cacti	Insect
Phacelia crenulata	Scorpionweed	Unknown/Probably Insect [1]
Centaurea repens [3]	Russian knapweed	Insect (Bees)
Salix exigua [3]	Coyote willow	Insect/Wind
Senecio spp.	Groundsel	Unknown/Probably Insect [1]
Sisymbrium altissimum	Tumble mustard	Wind
Sphaeralcea leptophylla	Scaly globemallow	Insect
Stanleya pinnata	Princess plume	Wind
Tamarix ramosissima [3]	Tamarisk	Insect
Tragopogon dubius	Goats beard	Unknown/Probably Insect [1]

Table 1: Plants visited by honeybees within the study area, 0.5 km radius. 2016/2017.
[1] Plants listed as Unknown for pollination status were however observed hosting bees and or ants.
[2] Pollinaria: A packet containing multiple pollen grains. Characteristic of plants belonging to the genus *Asclepias*.
[3] In bold: Plant species most visited by bees.

Scientific Name	Common Name	Pollen Grain Size (µm) Polar Axis	Pollen Trap (color and % per 20 ml sample of pollen packets)	Pollen Identified by Light Microscopy in Clinical Honey
Centaurea repens	Russian knapweed	40 µm	White/37% (2)	(1)
Atriplex canescens	Four-wing saltbush	29 µm	Yellow/14% (2)	
Tamarix parviflora	Tamarisk	45.1 µm	Rust/2% (2)	(1)
Salix exigua	Coyote willow	31.9 µm	Grayish Yellow and Grayish White/32% (2)	(1)
Carduus nutans	Musk thistle	33.9 µm	Dark Yellow/15% (2)	(1)

Table 2: (1) Pollen identified in the clinical honey. (2) Pollen identified from the pollen packets.

3.3 Pollen Identification

Pollen was characterized based on physical characteristics identified both in LM and SEM, (Hesse, 2010). Those characteristics are listed in Table 3. Pollen ranged in size from 21.4 µm–45.1 µm along the polar axis and 18.3 µm–32.4 µm on the equatorial axis. The equatorial plane is located through the pollen grains center and the polar axis is a plane running perpendicular to the equatorial plane. Russian knapweed pollen was notably the smallest pollen and Tamarisk the largest pollen characterized. The pollens, Figures 3–12 below, are characterized by three apertures most notable in Russian knapweed, Tamarisk, and Coyote willow. The exception is Four-wing saltbush, Figures 13–14. With Four-wing saltbush, the apertures are circular and are distributed globally. Dry Four-wing saltbush pollen was characteristically concave on one side giving the pollen the appearance of a bowl. Surface ornamentation included the terms echinate, psilate, verrucate, reticulate, heterobrochate, and perforate.

Species	Polar Axis (µm)	Equatorial Axis (µm)	Pollen Shape	Aperture Number	Surface Ornamentation
Centaurea repens	21.4	20.4	Spheroidal	3	Echinate
Eleagnus angustifolia	29.8	32.4	Spheroidal	3	Psilate, Verrucate
Salix exigua	31. 9	18.3	Prolate	3	Reticulate, Heterobrochate
Tamarix ramosissima	45.1	22.1	Prolate	3	Reticulate
Carduus nutans	33.9	27.5	Spheroidal	3	Echinate
Atriplex canescens	29.1	28.1	Spheroidal	>6, Porus	Perforate

Table: 3: Pollen Characteristics as defined in Hesse et al., 2010.

3.2.1 Selected Pollen Microscopy

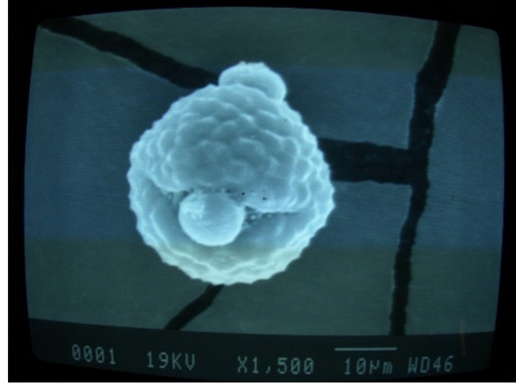

Figure 3: SEM of Russian knapweed (*Centaurea repens*) 1500X magnification.

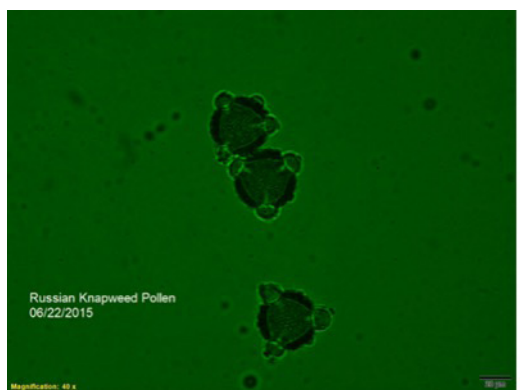

Figure 4: LM of Russian knapweed (*Centaurea repens*).

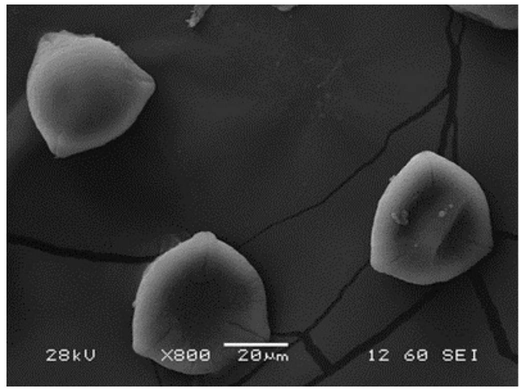

Figure 5: SEM of Russian olive (*Eleagnus angustifolia*) 800X magnification.

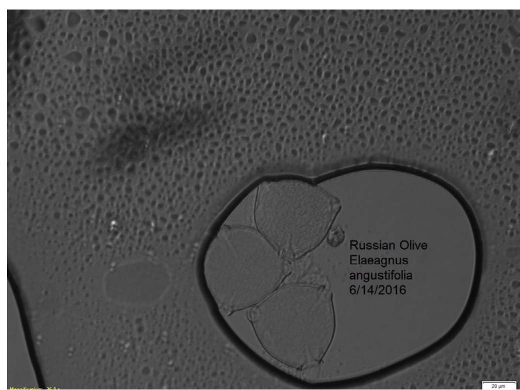

Figure 6: LM of Russian olive (*Eleagnus angustifolia*).

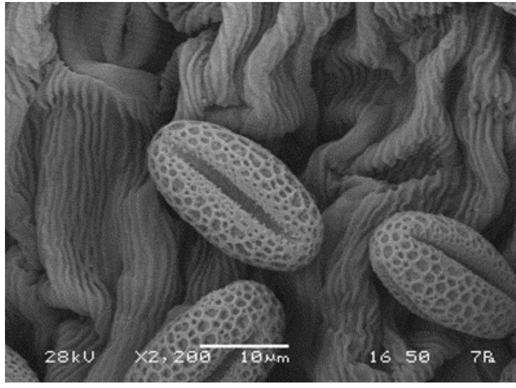

Figure 7: SEM of Coyote willow (*Salix exigua*) 2,200X magnification.

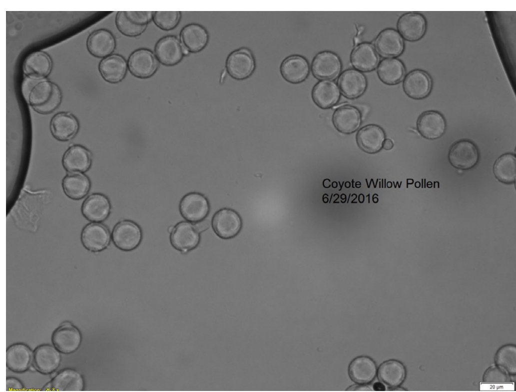

Figure 8: LM of Coyote willow (*Salix exigua*).

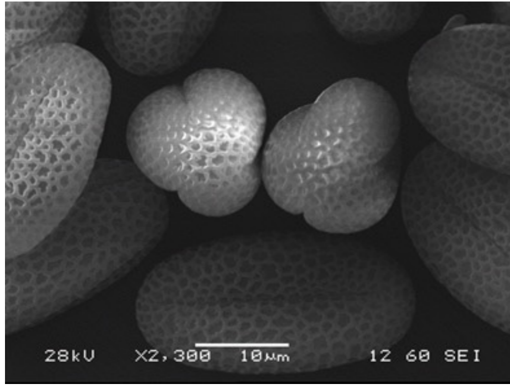

Figure 9: SEM of Tamarisk (*Tamarix ramosissima*) 2,300X magnification.

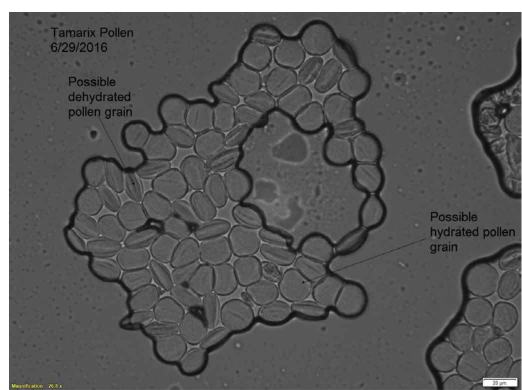

Figure 10: LM of Tamarisk (*Tamarix ramosissima*).

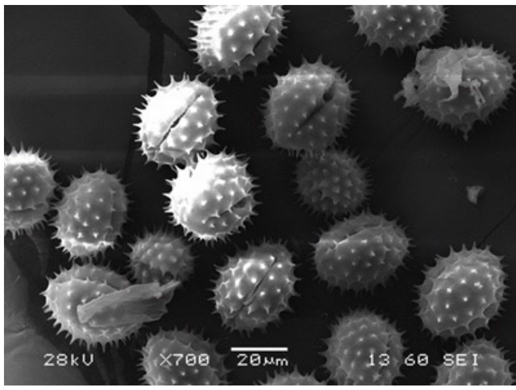

Figure 11: SEM of Musk thistle (*Carduus nutans*) 700X magnification.

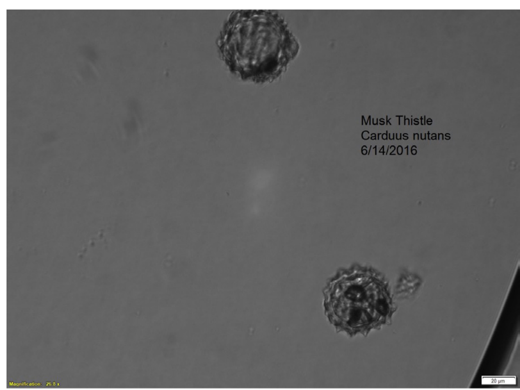

Figure 12: LM of Musk thistle (*Carduus nutans*).

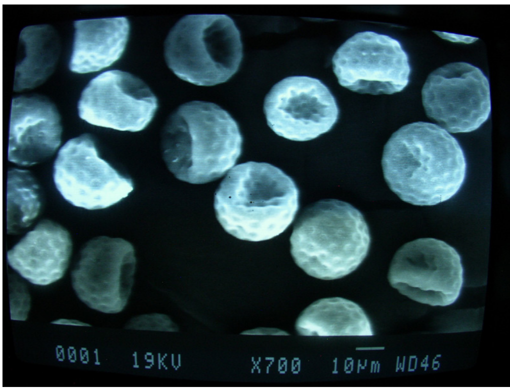

Figure 13: SEM of Four-wing saltbush (*Atriplex canescens*) 700X magnification.

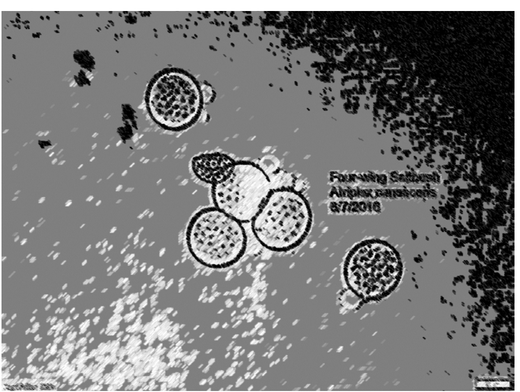

Figure 14: LM of Four-wing saltbush (*Atriplex canescens*).

3.4 Headspace SPME and GC-MS Analyses

The following, summarized in Table 4, shows results of the chemical investigation. With few exceptions, chemicals identified in the floral nectars did not transfer to the clinical honey. Bees certainly metabolize the various chemicals in the nectar and the chemical components of the honey is the result. Only two compounds were common to both the nectars and the clinical honey. Oxime-methoxyphenyl (Racemic-4-aminopentan-1-ol) and phenylethyl alcohol. Both are known to show antibiotic and antifungal qualities (PubChem. 2017, June). In addition to the clinical honey, the oxime-methoxyphenyl was identified in Tamarisk, Russian olive, Russian knapweed, and Musk thistle nectars while Tamarisk was the only source for the phenylethyl alcohol. A third compound, Ethane, 1,1'-oxybis [2-ethoxy-] imparts aroma to Russian olive nectar and was also found in the clinical honey. Interestingly, only Coyote willow nectar and the resulting clinical honey showed one biologically active compound, Benzyl alcohol. Benzyl alcohol is known as an antipyretic, a drug used to reduce fever (PubChem, 2017).

The clinical honey has a unique set of compounds showing possible antibacterial and antifungal qualities that differ from those found in honey in other investigations (Daher, 2010; Negut I., Grumezescu V., Grumezescu A., 2018) (Table 4). It was initially thought that pyruvic aldehyde might be identified in the clinical honey or in one of the nectars. Pyruvic aldehyde was not identified in any of the GC-MS analyses. The nectar sources however provide a wide array of biologically active compounds that determine the unique chemical make-up of the clinical honey. As noted above, two of those chemicals were common in both the clinical honey and the nectars.

Compound Name	**Chemical Structure**	**Source[1]**	**Biological Activity**
Oxime-, methoxy -phenyl (Racemic 4-amino-pentan-1-ol)		Russian olive, Russian knapweed, Tamarisk, Musk thistle, *Clinical Honey*	Antibacterial, Antifungal
Phenylethyl Alcohol		Tamarisk, *Clinical Honey*	Antibacterial, Inhibits the growth of Gram- Bacteria, Aroma
2Ethane, 1,1”-oxybis [2-ethoxy-]		*Russian olive, Clinical Honey*	Aroma
Cyclobutanol		*Clinical Honey*	Antibacterial, Antifungal
1-Pentanol, 4-amino		*Clinical Honey*	Antibacterial, Antifungal
N-a,N-e-Di-cbz-L-arginine		*Clinical Honey*	Antibacterial
Phenylacetaldehyde		*Clinical Honey*	Antifungal, Inhibits the growth of Gram + bacteria, Antioxidant
2,5-Furandicarboxaldehyde		*Clinical Honey*	Antifungal, Aroma, Flavor
3,7-Octadiene-2,6-dimethyl-		*Clinical Honey*	Aroma

1-decene, 10-bromo		*Clinical Honey*	Possible Antibacterial
7-Methyl-Z-tetradecene-1-ol-acetate		*Clinical Honey*	Antibiotic
2-Furancarboxaldehyde, -5-(hydroxymethyl)-		*Clinical Honey*	Antifungal
Z-(13,14-Epoxy) tetradic-11-en-1-ol acetate		*Clinical Honey*	Antibacterial
Delsulphosinigrin		*Clinical Honey*	Antibacterial, Antifungal

Table: 4: Biologically active compounds found in Floral nectars, and *Clinical Honey* SPME and GC-MS. [1]Source indicates identification of the compounds in either the floral nectars, the clinical honey or both.

3.5 Bioassay of Antibacterial and Antifungal Compounds Identified by SPME/ GC-MS

Table 5 summarizes the results of the bioassays. Pyruvic aldehyde (control) was inhibitory to MRSA BAA-44 at micromolar concentrations while the other compounds, including phenylacetaldehyde, were active at millimolar or higher concentrations.

	MRSA BAA-44	
Compound	**Stock Concentration**	**MIC**
Pyruvic aldehyde (Methylglyoxal)	5 molar	400 µM
Phenylethyl alcohol	8 molar	40 mM
N-à,N-ê-Di-cbz-L-arginine	10 mM	> 100 µM
Cyclobutanol	12.3 molar	> 10 mM
Phenylacetaldehyde	7.9 molar	20 mM
(R)-4-aminopentan-1-ol*	100 mM	> 1 mM
(S)-4-aminopentan-1-ol	100 mM	> 100 µM
Racemic 4-aminopentan-1-ol*	100 mM	> 1 mM
5-(Hydroxymethyl-furfural)	1 molar	20 mM

Table 5: Summary of compounds from clinical honey on MRSA ATCC BAA-44. The most active compounds are highlighted in red. Note that many of the compounds are only active in millimolar concentrations, with the exception being pyruvic aldehyde on MRSA ATCC BAA-44 at 400 µM. The asterisk denotes a racemic compound that was synthesized in the lab as this chemical was not available commercially.

Neither chemical was as effective as Pyruvic aldehyde, the control. As previously noted, phenylacetaldehyde was identified only in the clinical honey. Phenylacetaldehyde has been identified as an antibacterial component involved in clinical maggot therapy (Heuer, 2011). Phenylethyl alcohol was identified from Tamarisk nectar and from the clinical honey. The other compounds listed in Table 5 did not exhibit notable toxicity to caMRSA.

4 CONCLUSIONS

This investigation characterized a locally produced honey used to treat caMRSA infection. It is not clear which biologically active chemicals or combination of chemicals accounts for the success of the clinical honey in treating caMRSA infections. However, it is evident that a number of floral nectars are involved and that the resulting honey has a significant number of unique antibiotic components. None of the components appears to singly account for successful treatment. The multiple floral makeup of the clinical honey provides a large number of biologically active components that appear to result in the successful use of this honey to treat caMRSA topical infections. Future studies should include investigation of interactions among the antibacterial components identified in the clinical honey and the possible contribution of pH and glucose concentration.

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REFERENCES

Adams, C.; Manley-Harris, M.; Molan, P. The Origin of Methylglyoxal in New Zealand (*Leptospermum scoparium*) Honey. *Carbohydrate Research* **2009**, *344* 1050–1053.

Al-Jabri A.; Nzeako B.; Al Mahrooqi Z.; Al Naqdy, A.; Nsanze, H. In Vitro

Antibacterial Activity of Omani and African Honey. *British Journal of Biomedical Science*, **2003**, *60* (1), 1–4.

Daher, S.; Gülaçar, F. O. Identification of New Aromatic Compounds in the New Zealand Manuka Honey by Gas Chromatography-Mass Spectrometry. *E-Journal of Chemistry*, **2010**, *7* (SI), S7–S14.

Furman, A. *Totha, An Early Day History of the Settlement of Farmington and San Juan County, New Mexico 1875-1900*. Nortex Press. 1977.

Gullberg E.; Cao, S; Berg, O.; Ilbäck, C.; Sandegren, L.; Hughes, D.; Anderson, D. Selection of Resistant Bacteria at Very Low Antibiotic Concentrations. *PLOS Pathogens*. **2011**, *7* (7): e1002158. PMC 3141051, PMID 21811410.

Heil, K.; O'Kane Jr., S.; Reeves, L; Clifford, A. *Flora of the Four Corners Region, Vascular Plants of the San Juan River Drainage Arizona, Colorado, New Mexico and Utah*; Missouri Botanical Garden Press, 2013.

Hesse, M.; Zetter, R.; Halbritter, H.; Weber, M.; Buchner, R.; Frosch A-R.; Ulrich, S. *Pollen Terminology, An Illustrated Handbook*, Springer/Wein, 2010.

Heuer, H.; Heuer, L. *Blowfly Strike and Maggot Therapy: From Parasitology to Medical Treatment*. Parasitology Research Monographs, 2011; pp. 301–323.

Maeda, Y.; Loughrey, A.; Earle, J.; Millar, B.; Rao, J.; Kearns, A.; McConville, O.; Goldsmith, C.; Rooney, P.; Dooley, J.; Lowery, C.; Snelling, W.; McMahon, A.; McDowell, D.; Moore J. Antibacterial activity of honey against community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA). *Complement Ther. Clin. Pract.* **2008**, *14* (2), 77–82. doi: 10.1016/j.ctcp.2007.11.004. Epub 2008 Mar 4. PMID: 18396250.

Majno, G. *The Healing Hand: Man, and Wound Care in the Ancient World*; Harvard University Press. 1975.

Morrant, D.; Schumannand, R.; Petit, S. Field Methods for Sampling and Storing Nectar From Flowers with Low Nectar Volumes. *Annals of Botany*, **2009**. *103* (3), 533–542.

National Institute of Standards and Technology. *Chemical compounds library*. www.nist.gov/nist-research-library (accessed 2017-06-30).

Negut I.; Grumezescu, V.; Grumezescu, A. Treatment Strategies for Infected Wounds. *Molecules* **2018,** *23* (9), 2392. DOI 10.3390/molecules23092392. PMID: 30231567; PMCID: PMC6225154.

PubChem. Chemical Compounds Library. Chemical Compound Information https://pubchem.ncbi.nlm.nih.gov (accessed 2017-06-30).

Rankin S.; Rankin, H. Regional Honey and CA-MRSA (Community Acquired Methicillin Resistant Staphylococcus Aureus). *American Bee Journal* **2012**, *152* (4), 293–294.

USDA, NRCS. 2024. The PLANTS Database http://plants.usda.gov (accessed 2024-01-15).

Vandamme, L.; Heyneman, A.; Hoeksema, H.; Verbelen, J.; Monstrey, S. Honey In Modern Wound Care: A Systematic Review. *Burns* **2013,** *39* (8), 1514–25. DOI: 10.1016/j.burns.2013.06.014. Epub 2013 Jul 26. PMID: 23896128.