Research Article

PRELIMINARY SCIENTIFIC INVESTIGATION OF THE EFFECTIVENESS OF THE MEDICINAL PLANTS *PLANTAGO MAJOR* AND *ACHILLEA MILLEFOLIUM* AGAINST THE BACTERIA *PSEUDOMONAS AERUGINOSA* AND *STAPHYLOCOCCUS AUREUS* IN PARTNERSHIP WITH INDIGENOUS ELDERS.

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ABSTRACT

This preliminary investigation was undertaken in partnership with Indigenous elders to investigate the antibacterial effectiveness of common Plantain (*Plantago major* L.) and Yarrow (*Achillea millefolium* L.) against the skin pathogens *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Plants were selected, prepared and antibacterial chemicals were tested from plants harvested according to elders' guidance. Spectrophotometry, Kirby Bauer disc diffusion testing, standard bacterial population counts, and determination of concentrations of the plant antibacterial chemicals, alkaloids and saponins, were conducted. The spectrophotometry method provided results that were ineffective at determining viable bacterial biomass. Kirby Bauer disc diffusion testing and standard bacterial population counts showed that both plants were more consistently effective against the gram positive bacterium, *S. aureus*, versus the gram negative, *P. aeruginosa*. Although not significant, alkaloid concentration in *P. major* was higher at the 7:00 p.m. picking time compared to the 11:30 a.m. picking time, which agreed with the elder's Indigenous science knowledge. Saponin concentration in *P. major*, on the other hand, showed similar results for the 11:30 a.m. and 7:00 p.m. picking times. In addition to determining antibacterial effectiveness against common skin pathogens, the use of local plant species for medicinal preparations also contributes to the discussion of possible alternatives to antibiotic preparations for topical healing of bacterial skin infections.

KEYWORDS: alkaloids, antimicrobial, saponins, traditional medicine in Northern America, antibiotics, *Plantago major* L. and *Achillea millefolium* L.

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INTRODUCTION

The development of resistant bacteria from prolonged exposure to antibacterial agents, such as antibiotics, and harmful effects resulting from the toxicity of antibiotic usage is an increasing public health challenge. Considering these concerns, bioactive chemical agents in plants might be one helpful solution, requiring further investigation. Plant medicines are widely known and continue to make an important contribution to health care for many Indigenous people (Holetz *et al.,* 2002; Ferreira *et al.,* 2012; Alkholy *et al.,* 2013; Ferreira *et al.,* 2013), however less than 10% of higher plant species have been investigated for biological activity, such as antibacterial effectiveness (Fabricant and Farnsworth, 2001).

Two plants historically used by local Indigenous people of British Columbia and Saskatchewan in treating skin and wound infection are the common Plantain (*Plantago major* L.) and Yarrow (*Achillea millefolium* L.). This study works in partnership with First Nations elders to bring Indigenous science knowledge together with Western science knowledge and further the investigation of local plants used as medicines to treat skin & wound infections caused by the bacteria *P. aeruginosa* and *S. aureus*.

Plantago major is a perennial species that grows from a short, thick taproot. It has broad oval dark green basal leaves with green to white small flowers that are borne in a dense spike. This commonly used medicinal plant is an introduced species that grows in disturbed places such as roadsides, trails, and urban areas (Vance *et al.,* 1999). Plantain leaves have been used as a wound healing remedy for centuries in almost all parts of the world and have also been used in the treatment of a number of diseases apart from wound healing (Samuelsen, 2000). For example, this plant is known as nature's "Band-Aid" and is invaluable as a first aid remedy for cuts, scrapes, bee stings, and burns (Keane, 2009). Indigenous elders from British Columbia and Saskatchewan often use *P. major* in the treatment of skin

wounds/infection. In British Columbia, plantain is called "Frog's Leaves" by elder Geraldine Manson. Also known as "Frog's Pants", the following is a story told by elder Betty McKenna from Saskatchewan: "Plantain is called frog's pants because of the Woman's medicine wheel. On this medicine wheel, woman is facing north, the fish is facing south and the turtle and the frog are facing the right and left sides, respectively. All these living organisms have their eggs when they are born, so they share the same healing ways. Plantain is called the Frog's Pants because it is believed that the frog came, hopped away and left its pants, which are the plantain's leaves. Women take the frog's pants, chew the leaves and apply them as a compress on the skin to cure certain diseases. As people were living close to the land, plantain was especially useful for soil-borne diseases such as rashes, sty and pink eyes. The compress is also good at drawing the infection out. Chewing plantain is an important step as it is believed that the medicinal properties of plantain are released when combined with saliva. It is important to the woman who is chewing the leaves not to have fillings or gold teeth as these materials change the medicines. Although it is a cure for everyone, it is traditionally the women who would chew it because they were the medicine people in their family. Women would chew several plantain leaves and spit them out in a container to give them to people who would then bring the container home for future uses. Roots were also used once they were boiled" (B. McKenna, personal communication, 2011). Elders Geraldine Manson and Delores Louie of British Columbia agree with elder Betty's shared knowledge, which aligns similarly with their own knowledge.

Achillea millefolium possesses white flower heads that are densely packed in a round topped terminal cluster. Its woolly leaves are divided into many segments that grow from a branched rhizome. *Achillea millefolium* is one of the most abundant white flowers growing across the Canadian prairie and British Columbia. In North America, Indigenous people use it for

healing wounds (Chandler *et al.,* 1982). The traditional knowledge keeper Harvey Alphonse from British Columbia advised on the use of *A. millefolium* as traditional medicine to treat skin infection while reducing inflammation. Elders in Saskatchewan call this plant species porridge-on-a-stick and share that "a tea made using the entire top of the plant helps support the immune system and can be used for chest infections. It can also be boiled in water and used as a rinse to make your hair shiny and get rid of dandruff" (Yuzicapi *et al.,* 2013).

Antibacterial properties associated with many plants are attributed to the biologically active compounds identified as alkaloids and saponins. Alkaloids are a large family of nitrogen-containing secondary metabolites whose main function is to defend against predators (Taiz and Zeiger, 2002). Saponins are glycosides with soap-like properties that act as feeding deterrents against herbivores (Taiz and Zeiger, 2002). Alkaloids and saponins are both found in *P. major* (Cowan 1999; Mojab *et al.,* 2003; Cordeiro *et al.,* 2006) and *A. millefolium* (Chandler *et al.,* 1982; Khan and Gilani, 2011), and have shown marked antibacterial activities against gram positive bacteria (Avato *et al.,* 2006; Khan *et al.,* 2012). Saponins are known to be particularly effective against gram positive bacteria (such as *S. aureus*) compared to gram negative bacteria (such as *P. aeruginosa*) (Pistelli *et al.,* 2002, Avato *et al.,* 2006, Soetan *et al.,* 2006).

Previous studies have also indicated that environmental conditions associated with different geographical locations may influence levels of biologically active plant compounds (Lagalante and Montgomery, 2003). Similarly, Indigenous science knowledge also informs that picking the plant leaves at specific times of the day may provide more or fewer benefits relative to the effectiveness of the plant medicine against bacterial wound infections (elder Geraldine Manson, personal communication, 2011).

This preliminary study has employed selected standard methods including spectrophotometry, Kirby Bauer disc diffusion testing, and bacterial population counts to investigate the effectiveness of local plant medicines. The plants selected for study, *P. major* and *A. millefolium*, are used by Indigenous people' in British Columbia and Saskatchewan against wound infection and will be used for study on the known bacterial skin pathogens *P. aeruginosa* and *S. aureus*. The investigation also includes the Indigenous science knowledge and advisement of local elders by determining the antibacterial effectiveness of plant medicine treatments intended to parallel advised usage by the elders, and determines the levels of alkaloid and saponin concentrations at the advised picking times of 11:30 a.m. and 7:00 p.m.

MATERIALS AND METHODS

Plant material

Plantago major and *Achillea millefolium* were identified by S.N. and F.G. and collected with elders following traditional protocols. In British Columbia, whole plants were picked along the Nanaimo River in the town of Cedar during June 2012. *Plantago major* was picked at different times during the day (11:30 a.m. and 7:00 p.m.) because the elder informed the research team that the late picking time is the most recommended for antibacterial effectiveness in wounds. In Saskatchewan, whole plants were collected from the vicinity of Moose Jaw in July 2011. Plant material was washed in a 10% bleach solution (Kinney *et al.,* 1987) and dried at 40° C (Thakhiew *et al.,* 2014) until constant weight was observed and ground to powder. The powered plant material was used for the Soxhlet extraction procedures. Ground plant material was also exchanged between laboratories in British Columbia and Saskatchewan. Spectrophotometry, Kirby Bauer disc diffusion testing and bacterial population counts occurred in British Columbia, while alkaloid and saponin determinations were conducted in Saskatchewan.

Spectrophotometry

In accordance with communication with the traditional knowledge keeper H. Alphonse and the elder D. Louie, *P. major* and *A. millefolium* were dried, weighed, and placed into sterile beakers. *P. major* was weighed at 0.5 g, 5.0 g and 10.0 g while *A. millefolium* was weighed at 0.08 g, 5.38 g and 10.76 g. To conduct a combined treatment to test for synergistic effects using a *P. major* / *A. millefolium* combination, each plant was weighed at 0.08 g, 5.38 g and 10.76 g and placed into beakers (triplicate). The weighed plant matter was then soaked in a 10% bleach solution for 15 minutes, followed by rinsing twice with distilled water (Kinney *et al.,* 1987).

Elder D. Louie advised us that chewing *P. major* is an important step for preparation of the plant medicine. The chewing process may help with the release of plant chemical components. Therefore, according to the elder's advisement, the different weights of plant matter for the *P. major* trials and the combined *P. major* / *A. millefolium* trials were placed into individual sterile mortars and saliva (from same individual) was added depending on weight. Based on the elder's knowledge, 1.0, 3.0 and 6.0 ml of saliva were added to the 0.5, 5.0 and 10.0 g of *P. major*, respectively. For the combination study, 6.0 ml of saliva was added to the *P. major* / *A. millefolium* for each of the sample weights investigated. The plant / saliva mixtures were pressed twenty times each using a sterile pestle to conduct a procedure to parallel chewing practices, as recommended by the elder's local Indigenous science knowledge. The plant / saliva mixtures were then aseptically transferred into different sterile beakers, and 40 ml of sterile Trypticase Soy Broth (TSB) were added to each beaker. Each of the three weights of *A. millefolium* was covered with tin foil and steeped using 62.5 ml of TSB for one hour in keeping with the local Indigenous science knowledge of H. Alphonse. Following this procedure, 5 ml of each of the plant/broth solutions were pipetted into spectrophotometry test tubes (triplicates), and each test tube was inoculated with 100 µl of

bacteria *P. aeruginosa* (ATCC 10145) or *S. aureus* (ATCC 25923). Controls were also developed in triplicates. The control for the *P. major* and *P. major* / *A. millefolium* treatments consisted of broth with 3 ml of saliva. The control for the *A. millefolium* treatments paralleled the teachings of H. Alphonse and consisted of TSB broth with no saliva. Immediately following inoculation with bacterial cultures, initial absorbance readings were conducted. The test tubes were then incubated for 18 hours at 37° C, followed by the taking of an absorbance reading using a Spectronic 20 (Milton Roy Company) and the recording of the difference between the two readings. Absorbance readings were taken at an optical density of 600 nm for both *P. aeruginosa* (Davies *et al.,* 1993; Kim *et al.,* 2012) and *S. aureus* (Nychas *et al.,* 1990).

Soxhlet extraction for Kirby Bauer disc diffusion testing

To conduct Soxhlet extractions, 79 g of dried plant material were used to fill Soxhlet thimbles and 150 ml of methanol (ACS Laboratory grade) were used to conduct extraction procedures. The final extracts were then roto-evaporated at 30° C, at 235 RPM, until thick in consistency, but not yet solidified. The extracts were then transferred to sterile glass vials and maintained in dark conditions by wrapping in tin foil.

From the freshly prepared plant extract, final extract solutions of 500 mg/ml, 50 mg/ml, 5 mg/ml and 0.5 mg/ml (10% sterile dimethyl sulfoxide (DMSO)) were filtered using 0.45 microliter filter syringes. Sterile filter discs (6 mm) were then saturated with 40 µL of each plant extract solution, placed into sterile, covered petri dishes and stored at room temperature in the dark and overnight to remove excess methanol (Mistry *et al.,* 2010). Following this time interval, the filter discs (6 mm) were applied to agar plates (in triplicates) previously swabbed with bacterial cultures of *P. aeruginosa* (ATCC 10145) and *S. aureus* (ATCC 25923), using McFarland Standard procedures for conducting the Kirby Bauer disc

diffusion test (Kelly *et al.,* 1999). The selected bacterial populations were cultured at a density adjusted to 0.5 of McFarland scale for uniform swabbing of bacteria onto the surface of the agar plates (Kelly *et al.,* 1999). In addition to plant extract treatments, positive controls using the antibiotics Ciprofloxacin and Gentimicin were tested for effectiveness. Ciprofloxacin is a fluoroquinolone that acts against both gram positive (e.g. *S. aureus*) and gram negative bacteria (e.g. *P. aeruginosa*) (Agrawal *et al.,* 2007). Gentimicin is an aminoglycoside that acts best against gram negative bacteria. Negative controls included methanol and DMSO saturated filter discs.

Bacterial population counts

Following incubation of test tubes containing plant treatments for spectrophotometry procedures, serial dilutions were conducted on randomly selected test tube solutions. Procedures for bacterial population counts were then conducted and recorded as log of colony forming unit per ml (cfu ml^{-1}) (Harley and Prescott, 2002; Nilson and Holley, 2012; Hazan *et al.,* 2012) following a 24 hour incubation period at 37° C. For each bacterial species, controls with broth and saliva (*P. major*) and broth only (*A. milifolium*) were also prepared.

Soxhlet extraction for determination of the plant biological compounds

Chemicals used throughout these procedures were of analytical grade (hexanes, HCL, methanol, acetone (from Fisher, ON, Canada), 95% ethanol and NH$_4$OH (from Sigma, ON, Canada), KOH (from Occidental Chemical Corporation, TX, USA), petroleum ether (from BDH, ON, Canada), and chloroform and CH$_2$Cl$_2$ (from EMD, ON, Canada). The extraction was performed at ambient pressure at the boiling point of the solvent used. A 3.0 g of powered plant material was extracted with 250 ml of hexane on a water bath for 6 h in triplicate using the Soxhlet apparatus (Tarvainen *et al.,* 2010).

The obtained plant extracts were cleaned from oily materials by saponifying (Daruházi *et al.,* 2008). To conduct this procedure, the hexane extracts were concentrated under vacuum. The residues were then saponified with 50 ml of 95% ethanol and 2 g of KOH in 50 ml ethanol solution in a hot water bath. The extracts were diluted with 100 ml of distilled water and were shaken with 75 ml and then 2 × 50 ml portions of petroleum ether. The organic phases were collected and the solution obtained was washed with 2 × 50 ml portions of distilled water until neutral pH then evaporated in a Büchi Rotavapor R-205 under vacuum. The dry extract was weighed and dissolved in 4 ml of chloroform. These unsaponified extracts were stored in the refrigerator at 4° C until analysis.

Determination of alkaloids

Alkaloids were determined following the method of Fazal *et al.* (2011). Ten grams of grounded plant material was extracted with 100 ml of 100% ethanol. Once the ethanol was evaporated, 2 g of dried extract was dissolved in 20 ml of 5% HCL. The mixture was centrifuged for 10 minutes and the aqueous portion was basified with NH$_4$OH. The basic solution was extracted three times with CH$_2$Cl$_2$ and concentrated under reduced pressure by using a Büchi Rotavapor R-205. Once dried, the sample was weighed to determine the amount of alkaloid residues.

Determination of saponins

Saponins were determined following the method of Fazal *et al.* (2011). Ten grams of grounded plant material were defatted with 100 ml of hexane and incubated for 10–15 minutes. Hexane was separated from the plant extract, which was extracted three times with 30 ml of methanol. The resulting solution was concentrated to one third of its original volume and 100 ml cold acetone was added to this extract. The extract and acetone solution was refrigerated for 50 minutes. The extract was then filtered by pressure filtration using pre-weighed filter paper (Whatman No. 1 Qualitative Circles 125 mm). The weight of the

saponins was determined by subtracting the weight of the pre-weighted filter paper to the weight of the filter paper with saponins.

Statistical analyses

One-way ANOVA and Tukey's HSD were used to analyze the differences between the diameters of zones of inhibition using the Kirby Bauer disc diffusion test with *P. major*. One-way ANOVA and Tukey-Kramer's were used to analyze spectrophotometry data. Alkaloid and saponin mean percentages in *P. major* at different times were compared by using a *t*-test. Statistical analyses were carried out with the statistical analysis softwares R (version 3.0.1) and NCSS and values of *p < 0.05* were noted as statistically significant.

RESULTS

Spectrophotometry

Plantago major / A. millefolium combined treatment vs. *P. aeruginosa*:

Combined *P. major* with *A. millefolium* treatments were used to observe possible synergistic antibacterial effects on bacterial growth. Treatments with *P. major / A. millefolium* at 0.08 and 5.38 g, with 6 ml saliva, showed absorbance results at 0.65 and 0.77, respectively, and were greater than and significantly different than the control result at 0.31 (Table 1). At the 10.76 g *P. major / A. millefolium* treatment, absorbance results at 0.26 showed a lower result, significantly different than the other treatments, but not significantly different than the control treatment.

Plantago major / A. millefolium combined treatment vs. *S. aureus*

When *P. major / A. millefolium* treatments at 0.08, 5.38 and 10.76 g each, with 6 ml of saliva were used, absorbance results at 0.97, 0.75 and 1.05 showed no significant difference between plant medicine treatments (Table 1). All *P. major / A. millefolium* treatments showed greater absorbance readings and a significant difference when compared with the control treatment at 0.37.

Plantago major treatment vs. *P. aeruginosa*

Plantago major treatments of 0.5, 5.0 and 10.0 g results showed no significant differences in absorbance between the plant treatments and were 0.65, 0.71, and 0.71, respectively (Table 2). All plant treatments showed greater and significantly different absorbance results than the control at 0.31.

Plantago major treatment vs. *S. aureus*

Plantago major treatments at 0.5, 5.0 and 10.0 g showed absorbance results at 0.91, 0.81 and 0.91, respectively, and were greater than the control at 0.37, but not significantly different than the control. Results between plant treatments were not significantly different (Table 2).

Table 1. Spectrophotometry results for the different combined *P. major / A. millefolium* plant medicine treatments when testing effectiveness against *P. aeruginosa* and *S. aureus*.

Treatments	*P. major / A. millefolium*	
	P. aeruginosa	*S. aureus*
Control	0.31[a]	0.37[a]
0.08 g	0.65[b]	0.97[b]
5.38 g	0.77[b]	0.75[b]
10.76 g	0.26[a]	1.05[b]

Means within each column with the same letter (a) are not significantly different (*p < 0.05*).

Table 2. Spectrophotometry results for Plantain (*P. major*) treatments when testing effectiveness against *P. aeruginosa* and *S. aureus*.

Treatments	*P. major* P. aeruginosa	*P. major* S. aureus
Control	0.31[a]	0.37[a]
0.5 g	0.65[b]	0.91[a]
5.0 g	0.71[b]	0.81[a]
10.0 g	0.71[b]	0.91[a]

Means within each column with the same letter (a) are not significantly different ($p < 0.05$).

Achillea millefolium treatment vs. *P. aeruginosa*

Absorbance results for *A. millefolium* treatments at concentrations of 0.08, 5.38 and 10.76 g were observed (Table 3). For plant medicine treatments, 0.08 and 5.38 g, absorbance results of 0.97 and 1.24, respectively, were significantly different and greater than the control at −0.17. When the 10.76 g treatment was applied, the absorbance reading at 0.45 showed no significant difference with the control group.

Achillea millefolium treatments vs. *S. aureus*

Achillea millefolium treatments at 0.08 and 5.38 g showed greater and significantly different absorbance values at 1.60 and 0.77 when compared with control at 0.41 (Table 3). *Achillea millefolium* treatment of 10.76 g showed absorbance results at 0.35 and no significant difference when compared with the control group.

Table 3. Spectrophotometry results for Yarrow (*A. millefolium*) treatments when testing effectiveness against *P. aeruginosa* and *S. aureus*.

Treatments	*A. millefolium* P. aeruginosa	*A. millefolium* S. aureus
Control	-0.17[a]	0.41[a]
0.08 g	0.97[b]	1.60[b]
5.38 g	1.24[b]	0.77[b]
10.76 g	0.45[a]	0.35[a]

Means within each column with the same letter (a) are not significantly different ($p < 0.05$).

Kirby Bauer disc diffusion test with *Plantago major*

Plantago major extract vs. *P. aeruginosa*

According to Nascimento *et al.* (2000), zones of inhibition measurements that measure 1 mm greater than the 6 mm filter discs indicate effectiveness against the bacterial populations tested. The undiluted *P. major* extract (500 mg/ml) against *P. aeruginosa* showed zones of inhibition at 8.10 mm for picking times at 11:30 a.m. and 6 mm zone of inhibition for picking times at 7:00 p.m. (Table 4). The zone of inhibition in the morning was statistically higher than the negative controls DMSO and methanol. Results for the more dilute extract treatments showed a lack of antibacterial effectiveness against *P. aeruginosa*, showing no zones of inhibition for both picking times. The antibiotics Ciprofloxacin and Gentimicin resulted in zones of inhibition greater than 17 mm. In summary, the undiluted *P. major* extract (500 mg/ml) treatment picked in the morning showed antibacterial effectiveness against *P. aeruginosa*.

Table 4. The diameters (mm) of inhibition zones using the Kirby Bauer disc diffusion test with *P. major* at different times during the day with the bacteria *P. aeruginosa* and *S. aureus*.

Treatments	11:30 a.m. *P. aeruginosa*	11:30 a.m. *S. aureus*	7:00 p.m. *P. aeruginosa*	7:00 p.m. *S. aureus*
Plant Extraction				
500 mg/ml	8.10^b	9.05^b	6.0^a	7.95^b
50 mg/ml	6.0^a	6.86^{ab}	6.0^a	6.19^a
5 mg/ml	6.0^a	6.0^a	6.0^a	6.0^a
0.5 mg/ml	6.0^a	6.24^{ab}	6.0^a	6.0^a
0.05 mg/ml	6.0^a	6.0^a	6.0^a	6.0^a
DMSO	6.0^a	6.0^a	N/A	6.0^a
Methanol	6.0^a	6.0^a	6.0^a	6.0^a
Ciprofloxacin	28.81^e	24.67^d	29.33^c	23.38^d
Gentimicin	19.90^d	20.14^c	17.52^b	19.38^c

Means within each column with different letters (a–e) differ significantly ($p < 0.05$)

Plantago major extract vs. *S. aureus*

Plantago major extract (500 mg/ml) against *S. aureus* showed zones of inhibition significantly greater than the negative controls in both morning and evening picking times (Table 4). Results for the more diluted extract treatments used on *S. aureus* showed weak or no antibacterial activity. The antibiotics Ciprofloxacin and Gentimicin resulted in zones of inhibition greater than 19 mm in both morning and evening and showed a significant difference vs. *P. major* extract at 500 mg/ml. In summary, the undiluted *P. major* extract at 500 mg/ml showed effectiveness against *S. aureus* for treatments using plants picked during both morning and evening picking times.

Bacterial population counts

Plantago major vs. *P. aeruginosa*

The *P. aeruginosa* population counts for *P. major* treatments conducted at 0.5 and 10 g were similar to the control at too numerous to count (TNTC) (Table 5). Therefore, when considering effectiveness of *P. major* against *P. aeruginosa*, *P. major* showed little effectiveness against *P. aeruginosa*.

Plantago major vs. *S. aureus*

Bacterial population counts for *S. aureus*, following application of *P. major* treatments of 0.5 and 10.0 g showed viable bacterial cell counts of 1.20 log cfu ml^{-1} and too few to count (TFTC), respectively, while the control treatments showed bacterial cell counts at TNTC (Table 5). These results indicate that both *P. major* treatments were effective at reducing the number of viable *S. aureus* bacterial cells.

Achillea millefolium vs. *P. aeruginosa*

The plant *A. millefolium* treatment at 0.08 g showed *P. aeruginosa* viable bacterial cell counts at TNTC, while the *A. millefolium* treatment of 10.57g showed 2.44 log cfu ml^{-1} (Table 6). Control results were TFTC. Results indicate that *A. millefolium* treatment at 10.57 g is more effective against *P. aeruginosa* when compared to the 0.08 g treatment. However, both of these treatments showed less effectiveness when compared to the control, and thus indicate a lack of effectiveness against *P. aeruginosa*.

Achillea millefolium vs. *S. aureus*

When *A. millefolium* treatment at 0.08 g was used for *S. aureus,* 0.90 log cfu ml^{-1} were recorded (Table 6). The *A. millefolium* treatment at 10.57 g yielded results at TFTC. Both of these treatment results showed a lower number of viable *S. aureus* bacterial cells when compared to the control, at 2.28 log cfu ml^{-1}. This indicates that both *A. millefolium* treatments were effective at reducing the number of viable *S. aureus* bacterial cells.

Table 5. Numbers of bacteria grown on TSA (log cfu ml⁻¹) for Plantain (*P. major*) versus *P. aeruginosa* and *S. aureus*.

Treatments	Log cfu ml⁻¹ *P. aeruginosa*	Log cfu ml⁻¹ *S. aureus*
Control	TNTC	TNTC
0.5 g	TNTC	1.20
10.0 g	TNTC	TFTC

Abbreviations: TFTC= too few to count, TNTC= too numerous to count.

Table 6. Numbers of bacteria grown on TSA (log cfu ml⁻¹) for Yarrow (*A. millefolium*) treatments versus *P. aeruginosa* and *S. aureus*

Treatments	Log cfu ml⁻¹	Log cfu ml⁻¹
	P. aeruginosa	*S. aureus*
Control	TFTC	2.28
0.08 g	TNTC	0.90
10.57 g	2.44	TFTC

Abbreviations: TFTC= too few to count, TNTC= too numerous to count.

Alkaloids and saponins

Results from British Columbia *P. major* analyses show low alkaloid levels for plants picked during 11:30 a.m., at 0.07% (Table 7). Alkaloid levels for plants picked at 7:00 p.m. were recorded at 0.24%. The difference was not statistically different ($p = 0.2742$). Saponins showed similar results for the 11:30 a.m. and 7:00 p.m. picking times at 0.18% and 0.13%, respectively ($p = 0.1776$). Although values for alkaloids and saponins were not significantly different, the increase in % for alkaloids at the 7:00 p.m. picking time coincided with the elder's Indigenous science knowledge.

Table 7. Alkaloid and saponin mean percentage (%) in Plantain (*P. major*) with t-test comparisons.

Sample	Alkaloids (%)	Saponins (%)
Plantain, BC, 2012, 11:30 a.m.	0.0656 ± 0.0223^a	0.1807 ± 0.0663^a
Plantain, BC, 2012, 7:00 p.m.	0.2373 ± 0.3771^a	0.1337 ± 0.0327^a

Values are means ± SD of three or more measurements. Means within each column with the same letter (a) are not significantly different ($p < 0.05$).

DISCUSSION

Spectrophotometry

Our spectrophotometry results for the two most diluted plant treatments (in plant treatment combinations or on their own) indicate that an increase in bacterial biomass occurred and thus these results show ineffectiveness against both *P. aeruginosa* and *S. aureus*. The more concentrated plant treatments often showed absorbance results that were lower than the less concentrated plant extracts, indicating a lower bacterial biomass in those cultures and possible effectiveness against the bacterial populations. For example, *A. millefolium* appeared more effective against both bacterial species at the most concentrated plant treatment. The controls, however, consistently showed lower absorbance results, which indicates that the plant treatments initially allowed bacterial growth to occur. This also indicates that specific time frames may be required before specific plant treatments effectively kill bacterial cells. The resulting higher absorbance values recorded for plant treated samples are attributed to dead and

viable bacterial cells (Hazan *et al.,* 2012) with the most effective plant treatments further determined by conducting bacterial population counts. Therefore, for the plant treatments performed on the selected bacteria, this method provided results that were ineffective at determining viable bacterial biomass.

The effect of plant picking times on antibacterial effectiveness and plant biological compounds

Plants were picked at two different times (11:30 a.m. and 7:00 p.m.) to follow the elder's recommendations that late plant picking times increase effectiveness of the plant medicine when using *P. major*. Results for the Kirby Bauer disc diffusion method showed that *P. major* treatment resulted in a greater and more consistent level of antibacterial effectiveness against the known skin pathogen *S. aureus*, when compared with results for *P. aeruginosa*. Our results also indicate that the highest *P. major* concentration was effective at reducing the growth of *S. aureus* at both morning and evening plant picking times. Results showed that *P. major* was less effective against *P. aeruginosa* with measured zones of inhibition observed for the 11:30 a.m. plant picking time only. Lack of zones of inhibition may also occur if some plant biochemical components are unable to effectively diffuse through the agar medium (in spite of addition of DMSO to facilitate movement).

In accordance with Indigenous science knowledge, the concentration of alkaloids was greater at the 7:00 p.m. picking time compared to the 11:30 a.m. picking time in *P. major*. This was not the case for the saponins, which showed similar, yet slightly higher, saponin levels for *P. major* at the 11:30 a.m. picking time. The higher alkaloid levels determined for *P. major* suggest that picking plant material later in the day for use as plant medicines may improve the effectiveness of *P. major* against specific bacterial species. Thus, for the alkaloid results, although not significant, the higher alkaloid levels at the 7:00 p.m. picking time coincide with local Indigenous science knowledge. Plant picking times for this study

were arranged on a day when the weather was cloudy and rainy at the 11:30 a.m. hour, with similar conditions at 7:00 p.m. Future studies that focus on the elder's guidance relative to picking times should happen at mid-day when conditions are distinctly hotter vs. a cooler evening hour to contribute further to result outcomes for alkaloids and saponins which may lead to correlations with zones of inhibition.

Plant / antibiotic treatments and antibacterial effectiveness

The plant antibacterial compounds associated with *P. major* and *A. millefolium* appear to be more effective against the gram positive *S. aureus* bacteria tested in this study. This is supported by previous studies (Pistelli *et al.,* 2002, Avato *et al.,* 2006, Soetan *et al.,* 2006). This indicates that the medicinal plants selected for study may show stronger antibacterial effectiveness against gram positive bacteria, possibly due to lack of an outer cell membrane. Results for the commercial antibiotics used as treatments showed strong antibacterial effectiveness against both the gram positive and gram negative bacteria used during this study**.**

It may also be important to consider the heightened awareness of the medical community to increasing patterns of resistance to antibiotics by bacterial populations. When also considering antibiotic treatment of skin infections, topical applications of antibiotics have been shown to cause contact dermatitis (Sasseville, 2011) and contribute to drug-resistant gram negative strains of skin microflora. Some bacterial strains are known to cause gram-negative folliculitis following topical application of the antibiotic Clindamycin (Worret and Fluhr, 2006), while according to Blumenthal and colleagues (1998), *P. major* shows a lack of toxicity on the human body.

The plant treatments tested may show antibacterial effectiveness while additionally contributing to wound healing activity. For example, to fight bacterial infection, the

immune system increases levels of one type of leukocyte, the neutrophils, at the wound site. While neutrophils phagocytize bacteria, reactive oxygen species form, often leading to damaging effects on tissues. *Plantago major* extracts significantly inhibit the production of reactive oxygen species by human neutrophils, limiting potential damage to tissues (Reina *et al.,* 2013). *Plantago major* has also been shown to contribute to the healing process of wounded tissues. One study showed that *P. major* water and ethanol leaf extracts stimulated proliferation and migration of cells during wound healing (Zubair *et al.,* 2012) indicating that during the healing of wounds, *P. major* may effectively contribute to fighting infection while serving to limit tissue damage and by contributing to the repair of skin tissue.

CONCLUSION: INDIGENOUS SCIENCE KNOWLEDGE AND WESTERN SCIENCE KNOWLEDGE COMING TOGETHER

The coming together of Indigenous science knowledge with Western science knowledge may highlight a responsible, positive mechanism for effectively treating skin infections. The issue of antibacterial effectiveness of plant medicines also further contributes to discussion on the need to reduce antibiotic usage resulting from the documented increase in bacterial resistance to a wide spectrum of antibiotics frequently used today. Like antibiotics, plant medicines must be respected for their potentially powerful medicinal abilities and individuals should talk

with elders or others who have an understanding of Indigenous science knowledge prior to preparing and using plant medicines to heal wounds.

Future collaborative studies between Indigenous science knowledge and Western science knowledge need to be further expanded in partnership with First Nations elders / traditional knowledge keepers (Nilson *et al.,* 2008; Ferreira and Gendron, 2011; Gendron *et al.,* 2013) with a continued focus on picking times, plant species, antibacterial plant chemical components and an increase in bacterial species studied to further investigate the natural abilities of plants to fight infection.

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