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Research Article
Antimicrobial Activity of Indigowoad (*Isatis indigotica* Fort) and Plains Wild Indigo (*Baptisia bracteata*) Roots

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Abstract

Indigowoad Roots (IR) (*Isatis indigotica* Fort) and Plains Wild Indigo Roots (PWIR) (*Baptisia bracteata*) have been shown to be high in phenolic compounds and plants high in phenolic compounds often have beneficial health effects including antimicrobial activity. Indigowoad is a well-known medicinal plant as well as an edible plant root similar to daikon, sweet potatoes, yam, ginseng and carrots. Plains wild indigo roots, leaves and seeds have traditionally been used by native Americans for medicinal purposes and its extracts are consumed to enhance health. The *in vitro* antimicrobial activity of IR and PWIR was determined on five microbes (*Escherichia coli*, *Pseudomonas aeruginosa*, *Legionella pneumophila* sub sp., *Pneumophila*, *Staphylococcus aureus* and *Streptococcus mutans*), as well as general oral cavity bacteria. The IR2 inhibited cell growth of *S. aureus*, *E. coli*, *S. mutans* and *P. aeruginosa*. The PWIR inhibited cell growth of *S. aureus* and *P. aeruginosa*, which are normal skin flora, *E. coli* is a normal intestinal parasite and *S. mutansis* causes tooth decay and periodontal disease. The best treatment for oral cavity bacteria was IR2 which reduced bacterial counts by 2.63±0.26 log relative to water treatment. The PWIR reduced them by 0.66±0.21 log.

Key words: Antimicrobial activity, indigowoad root (*Isatis indigotica* Fort), plains wild indigo (*Baptisia bracteata*), MIC

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INTRODUCTION

Recently, there has been world-wide interest in plant materials that may improve human health. Phytonutrients such as phenolic compounds are known to be present in most unprocessed whole plants and if these beneficial bioactive constituents are increased in the human diet, they should improve immunity and lead to better overall health status. Phenolic compounds are major contributors to the total antioxidant capacity of vegetables, fruits and grains (Heo *et al*., 2007; Jacobo-Velazquez and Cisneros-Zevallos, 2009) as well as having antimutagenic, antifungal and antiglycemic properties (Friedman, 1997; Canbek *et al*., 2014). Phenolic compounds in the human diet have also been reported to have antimicrobial benefits (Mohamed *et al*., 2010; Puupponen-Pimia *et al*., 2001). Plant based antimicrobials are receiving increasing interest as the effectiveness of modern antibiotics have been reduced with the increased emergence of pathogens that are drug resistant. Although, several plant species have been analyzed for antimicrobial properties, numerous promising plant species remain to be studied (Kaur *et al*., 2016). This study focuses on the roots of two plants with a history of health benefits-indigowoad (*Isatis indigotica* Fort) and plains wild indigo (*Baptisia bracteata*). Chang *et al*. (2016) showed that Indigowoad Roots (IR) and Plains Wild Indigo Roots (PWIR) have high amounts of phenolic compounds, which raises the question of whether these plants may have potential for antimicrobial activity.

Indigowoad root is considered to be a safe medicinal herb, which has been planted and used all over Asia for centuries and has been recorded in ancient Chinese books (Li, 2003). It is believed to have strong activity in removing toxins and eliminating inflammation (Wei *et al*., 2011). It is consumed by millions of people throughout the world, especially in Asian diets, where it is an edible plant root similar to daikon, sweet potatoes, carrots, yam, ginseng, etc. In China, indigowoad is even used as a beverage base (like orange juice or blended juice). Plains wild indigo grows naturally in the plains of the USA and has historically been used to enhance human health. It was mentioned by USDA (Casey and Wynia, 2010) as a "Culturally significant plant" and its roots, leaves and seeds have traditionally been used for medicinal purposes. Native Americans made tea from the dried leaves to treat colds, concentrate bile and aid the liver. It was also used to treat cuts, bruises, sore arms, legs, stomach cramps, open cuts, eye disease and rheumatism (Casey and Wynia, 2010). In addition, tea made from the roots was consumed to aid digestion or used as mouthwash to treat periodontal disease and sore throats. Plains wild indigo seeds have been reported to scavenge free radicals and may have the potential for inhibition of microorganisms (Borchardt *et al*., 2008; Mukku *et al*., 2013). The most common uses for wild indigo are as an anti-bacterial, a fungicide and to treat lymphatic disorders (Foster and Duke, 1999).

Streptococcus mutans is a serious source of periodontal disease. Oral health problems are primarily a result of either dental caries or periodontal disease and both are greatly affected by the foods in the human diet (WHO., 1987). Essentially every adult in the world has at least one of these diseases. All periodontal diseases are bacterial in origin and the initial inflammation occurs from bacteria in plaque (WHO., 1987). Ouhayoun (2003) studied the effects of mouthwashes containing essential oil from plants on oral bacteria and found that essential oil in mouthwashes are effective at penetrating the protective biofilm called plaque and inhibiting oral bacteria. According to a critical review of oral health conducted by Takahashi and Nyvad (2011), dental biofilms produce acids from carbohydrates that result in caries. After prolonged acidic exposure, the more aciduric bacteria such as *S. mutans* become dominant. *Staphylococcus aureus* is a major concern because it causes airborne infections in medical facilities (Lu *et al*., 2012; Bode *et al*., 2010; Camarinha-Silva *et al*., 2012). Antibiotics are used extensively to combat these infections, which is leading to antibiotic resistance. According to Siegel *et al*. (2006), about 60% of the *S. aureus* organisms obtained from patients in hospital intensive care units were methicillin-resistant. Moore and Flaws (2011) found that *Pseudomonas aeruginosa* is frequently found in hospital patients. *Pseudomonas aeruginosa* causes a variety of diseases and can be acquired from the general community or in a healthcare facility. Typical community-acquired infections include skin and soft tissue. Nosocomial infections are likely to be invasive and include infections of the bloodstream, urinary tract, surgical sites and respiratory tract. Around 11-13.8% of all nosocomial infections are due to *P. aeruginosa* and an even higher rate (13.2-22.6%) of nosocomial infections occur among patients in an Intensive Care Unit (ICU). Infections caused by *P. aeruginosa* are difficult to treat and it is becoming increasingly resistant to antimicrobial agents (Moore and Flaws, 2011). *Legionella pneumophila* is commonly found indoors and can be life threatening. Ishimatsu *et al*. (2001) detected legionellae in air surrounding a cooling tower that was contaminated with *L. pneumophila.* Blatny *et al*. (2008) reported that *L. pneumophila* was found as far away as 200 m downwind from biological treatment ponds. Most isolates of *E. coli* are harmless and don't cause disease in healthy people but some virulent types exist that can cause serious illness

(Eisenstein and Jones, 1988). *Escherichia coli* is an important cause of urinary tract, bloodstream and surgical site infections as well as pneumonia (Weinstein *et al*., 2005).

Based on this past research, *Escherichia coli*, *Pseudomonas aeruginosa*, *Legionella pneumophila* subsp., *Pneumophila*, *Staphylococcus aureus* and *Streptococcus mutans* were selected as the targeted microbes to investigate in this study. The objectives of this study were to determine the *in vitro* antimicrobial activity of water extracts from four sources of commercial indigowoad roots and one source of plains wild indigo root on these five targeted microbes and on general oral cavity bacteria.

MATERIALS AND METHODS

Plant test materials: The plant materials studied were Indigowoad Root (IR) and Plains Wild Indigo Roots (PWIR). All samples were prepared and analyzed in triplicate. Four different samples of the indigowoad (*Isatis indigotica* Fort) roots were analyzed separately. They were commercial products that were purchased from four different stores located in Luodong city of Yilan County in Taiwan. Because they were commercial products, there was no knowledge over how they were grown or handled. Consequently, four different samples were purchased to determine the level of variation in the tested responses from these commercially available products. They were already dried and clean when purchased from the stores. Those samples were then freeze-dried, milled to a powder (18 mesh sieve, 1000 μm) and stored at 4°C until the chemical tests were conducted. The plains wild indigo roots (*Baptisia bracteata* var. leucophaea) were harvested in the summer of 2013 from 10 plants that grew in a pasture in central Texas. These plants grew in the wild and there were no fertilizers, pesticides or other chemicals applied to the plants. These root samples were cleaned and dried in the sun for four days and taken to the laboratory at night time. The samples were freeze-dried, blended together, milled to a powder (18 mesh sieve, 1000 μm) and stored at 4°C until the tests were conducted.

Chemicals: The standards for antibiotics, ampicillin trihydrate and tetracycline hydrochloride, were purchased from Sigma Chemical Co. (St. Louis, MO, USA) and their purity was over 95%. Iron(III) pyrophosphate, L-cysteine·HCl·H_2O, agar-agar (granulated, purified and free from inhibitors for microbiology), nutrient broth and tryptic soy broth (casein-peptone soymeal-peptone broth for microbiology) were all obtained from Merck Chemical Co. (Darmstadt, Germany). Bacto™ Brain Heart Infusion (BHI), Difco™ Legionella Agar Base, Difco™ Plate Count Agar (PCA) and Mueller Hinton Broth (Base for antimicrobial susceptibility testing of aerobic microorganisms by the Broth Dilution Methods) were obtained from Becton, Dickinson and Company (Sparks, MD USA).

Preparation of plant extracts for antimicrobial research: The powder samples of the Plains Wild Indigo Root (PWIR) and the four different samples of indigowoad roots (IR1, IR2, IR3 and IR4) were prepared for the antimicrobial research as follows. For the Water Extract (WE) preparation, the plant samples were prepared by macerating 15 g of each of the powdered plant materials individually in separate containers with 90 mL of distilled water. After sterilizing in an autoclave at 121°C for 30 min, the mixture was filtered with Whatman filter paper (No. 1). The filtrate was concentrated by using a freeze dryer and the residue was stored at 4°C. When needed for the antimicrobial tests, each of these prepared samples were weighed and mixed with sterile distilled water to dilute the stock concentration to 200 mg mL^{-1}.

Bacterial strains and growth conditions: Five bacterial strains (three gram-negative and two gram-positive) were used for antibacterial testing. These five pathogenic microorganisms were obtained from the Bioresource Collection and Research Center (BCRC) of the Food Industry Research and Development Institute (FIRDI), Hsinchu, Taiwan. The three gram-negative bacterial strains were: *Escherichia coli* (BCRC 11634), *Pseudomonas aeruginosa* (BCRC 11633) and *Legionella pneumophila* subsp., *Pneumophila* (BCRC 17854). The two gram-positive bacterial strains were: *Staphylococcus aureus* (BCRC 10451) and *Streptococcus mutans* (BCRC10793). Details on these strains and their cultivation conditions are given in Table 1.

The cultivation and assay medium for *S. aureus* was tryptone soy broth/agar. The cultivation and assay medium for *E. coli* was nutrient broth/agar. The cultivation and assay medium for *S. mutans* was brain heart infusion broth/agar. The cultivation and assay medium for *P. aeruginosa* was tryptone soy broth/agar. The cultivation and assay medium for *L. pneumophila* was Buffered Charcoal Yeast Extract (BCYE) agar. The BCYE agar was made from Difco™ L-cysteine·HCl·H_2O- 0.4 g, Iron(III) pyrophosphate-0.25 g, agar (as needed)-17.0 g, distilled water-1.0 L, yeast extract (Difco 0127)-10.0 g, charcoal (Sigma C5510)-2.0 g. Autoclave at 121°C for 15 min. Prepare fresh solution of L-cysteine·HCl·H_2O (0.40 g in 10 mL distilled water) and soluble ferric

Table 1: Bacterial strains and their growth conditions

BCRC NO.	Strain	Oxygen requirement	Growth conditions (°C)	Biosafety level	Medium
10451	*Staphylococcus aureus*	Aerobic	37	2	Tryptic soy broth/agar
11634	*Escherichia coli*	Aerobic	37	2	Nutrient broth/agar
10793	*Streptococcus mutans*	Aerobic	37	1	Brain heart infusion broth/agar
11633	*Pseudomonas aeruginosa*	Aerobic	37	2	Tryptic soy broth/agar
17854	*Legionella pneumophila* subsp., *pneumophila*	Microaerophilic 5% CO_2	37	2	BCYE agar

pyrophosphate (0.250 g in 10 mL distilled water). Member filter and sterilize each solution separately. Add L-cysteine·HCl·H_2O to basal medium first (Table 1).

The procedure for culturing the bacteria is as follows. Each of the pure cultures of strains was streaked onto their appropriate nutrient agar plate and all were incubated at 37°C for 18-24 h. Next, the well-isolated colonies were aseptically transferred to their appropriate nutrient broth and incubated at 37°C in an incubator with rotating plates at 100 rpm for 18-24 h. The inoculum for each tested strain was adjusted by using the 0.5 McFarland standard for visual comparison to obtain a suspension density equivalent to around 1.5×10^8 CFU mL^{-1}. The Optical Densities (OD) of the incubated bacteria were measured using an UV spectrophotometer (Spectroquant® Pharo 300, Merck) at 600 nm wavelength. If the OD values indicated CFU mL^{-1} densities that were higher than the desired level, then it was diluted. If the OD values were less than desired, then the incubation was done again (Gursoy *et al*., 2009; Khanam *et al*., 2015).

Anti-microbial study: Two different types of antimicrobial activity tests were conducted on the five bacteria strains selected for this study. For the first test, the agar dilution method was used to determine the Minimal Inhibitory Concentration (MIC) of the antimicrobial agents, which is the lowest concentration of the antimicrobial agent that will inhibit the visible growth of the microorganism in question. The second test was the disk diffusion method which is a measurement of the diameter of a ring that forms around a colony after treatment. No ring means that there was no antimicrobial activity and if a ring exists, the size of the ring indicates the degree of antimicrobial activity. The agar dilution method is considered to be a reference method for other antimicrobial susceptibility tests because of its accuracy (European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), 2000). The disk diffusion method is a common method of determining the susceptibility of certain bacteria to antimicrobial agents.

Agar dilution method for determining Minimal Inhibitory Concentration (MIC): The agar dilution method was used to determine the Minimum Inhibitory Concentrations (MICs) of the antimicrobial agents. The agar dilution method used was based on Clinical and Laboratory Standards Institute (2007), which is an international standard procedure for this method and it was developed through the Clinical and Laboratory Standards Institute consensus process.

For the agar dilution method, two-fold serial dilutions of the plant extracts developed earlier were made in molten MHA medium that was cooled down to 45°C to obtain the desired final concentrations (Klancnik *et al*., 2010). The MIC of the extracts was tested at various concentrations ranging from 0.391-200.00 mg mL^{-1}. The stock solution was utilized to get the desired extract concentrations of 200.0, 100.0, 50.0, 25.0, 12.5, 6.25, 3.125, 1.563, 0.781, 0.391 mg mL^{-1} by the two-fold serial dilutions method. The stock solutions were mixed with 19 mL of agar to get eleven different levels of final plant extract concentrations in the medium that ranged from 0-10 mg mL^{-1}. The agar plate number and the corresponding final plant extract concentration in mg mL^{-1} were: 0 = 10, 1 = 5, 2 = 2.5, 3 = 1.25, 4 = 0.625, 5 = 0.313, 6 = 0.156, 7 = 0.078, 8 = 0.039, 9 = 0.019 and 10 = 0.

The bacterial suspensions developed previously were diluted to 10^6 CFU mL^{-1}. Then 0.1 mL of this diluted bacterial suspension was inoculated on the solid MHA medium. The agar plates were incubated aerobically at 37°C for 18-24 h for all tested bacterial cultures. The MIC was defined as the lowest concentration of plant extract in the solid media where no growth was observed (Klancnik *et al*., 2010).

The BCYE agar that was used for *Legionella pneumophila* subsp., *Pneumophila*, naturally has suspended black particles and these dark particles greatly limited the optical density readings, thus the determination of minimal inhibitory concentration could not be done for this organism.

Disk diffusion method: The disk diffusion test was based on Clinical and Laboratory Standards Institute (2011), which is an international standard procedure for this method that was developed through the Clinical and Laboratory Standards

Institute consensus process. The disk diffusion method to determine antimicrobial activity was done for the five plant extracts and the five bacteria strains that were selected for this study.

The inoculum for each tested strain developed earlier was diluted around 100 times to obtain a bacterial suspension of around 1.5×10^6 CFU mL^{-1} (Alviano *et al*., 2008). Four of the inoculum suspensions were spread evenly over the entire nutrient agar surface (Mueller-Hinton Agar, MHA) and incubated at 37°C (Shaker Incubator, COCONO LM-590) for 48 h. However, the *Legionella pneumophila* inoculum suspension was spread on the specific BCYE agar surface and incubated at 37°C for 48 h at 5% CO_2 (CO_2 incubator, NAPCO series 5400).

The disks (8 mm diameter) were sterilized prior to use at 121°C for 15 min in an autoclave. The individual disks were then loaded with 0.05 mL of one of the antimicrobial test materials. The test materials included the five plant extracts, two positive controls (Ampicillin trihydrate and tetracycline hydrochloride) and one negative control (sterile water) (Jaberian *et al*., 2013; Khanam *et al*., 2015). The concentration of the plant extracts was 200 mg mL^{-1} and the concentration of the positive controls were 20 mg mL^{-1}.

The impregnated disks were dried for 5 min and dispensed onto the surface of the inoculated plates with sterile forceps. Each disk was pressed down firmly to ensure complete contact with the agar surface. The disks were placed apart a suitable distance and not relocated once having contact with the agar surface. The plates were labelled and incubated at 37°C for 24 h. The diameter of the Zone of Inhibition (ZI) of bacterial growth around each disk was measured in millimeters (Jaberian *et al*., 2013; Khanam *et al*., 2015).

Plant extracts antibacterial activity test on oral cavity bacteria (using the total colony count method): Studies were also conducted to determine if the plant extracts from this study have potential to treat general oral health problems. To study this, bacterial samples were obtained from volunteers about 1-2 h after meals by wiping the buccal mucosa with sterilized cotton swabs. The samples were placed in transport fluid (Nutrient broth) for 5 min. Then 1.5 mL of the specimens and 0.5 mL of either sterilized water or plant extract sample were placed in a tube and incubated at 37°C and 100 rpm for 24 h. After 24 h, a set of serial dilutions was made, a sample of each was placed into a non-selective liquefied agar medium and the medium was immediately poured into petri dishes. For complete mixing of the dilution and agar, the plates were rotated to disperse the sample sufficiently and allow the agar to solidify at room temperature. This procedure produced a set of pour plates from the different dilutions to allow accurate counting of the microorganisms. The agar plates were inverted and incubated at 37°C for 48-72 h. After the incubation period, the plates were examined for growth colonies and all visible colonies were counted. Only the plates with 30-300 colonies were investigated and CFU mL^{-1} was calculated according to the standard formula (a) as presented in Lubrizol Advanced Materials Inc. (2009) and Reynolds (2013). Standard equation is:

$$\text{CFU mL}^{-1} = \frac{\text{Colony forming units, CFUs, on an agar plate}}{\text{Total dilution of tube}} \times \text{Volume plated}$$

Statistical analysis: All samples were prepared and analyzed in triplicate. Significant differences between means of the different treatments were analyzed by one-way analysis of variance (ANOVA) and further analyzed with Tukey HSD test and Duncan's multiple range test to determine significant differences between means ($p<0.05$) using SAS (SAS 9.3, SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Agar dilution method for Minimal Inhibitory Concentration (MIC): Table 2 presents results from the dilution method as MIC values for each bacteria type and treatment. For

Table 2: Antibacterial activity from four different samples of indigowoad roots (IR) and plains wild indigo root (PWIR) as determined by two different methods

	Staphylococcus aureus 10451		*Escherichia coli* 11634		*Streptococcus mutans* 10793		*Pseudomonas aeruginosa* 11633		*Legionella pneumophila* subsp., *pneumophila* 17854	
Plant sample	ZI (mm)	MIC (mg mL^{-1})	ZI (mm)	MIC (mg mL^{-1})	ZI (mm)	MIC (mg mL^{-1})	ZI (mm)	MIC (mg mL^{-1})	ZI (mm)	MIC (mg mL^{-1})
IR1	-	-	-	-	-	-	-	-	-	NA*
IR2	25	2.5	-	10	-	10	9	10	-	NA
IR3	14	-	-	-	-	-	-	-	-	NA
IR4	12	-	-	-	-	-	-	-	-	NA
PWIR	9	10	-	-	-	-	10	10	-	NA

-: Not achieved, ZI: Zone of inhibitions as determined by the disk diffusion method, MIC: Microbial inhibition concentration as determined by the agar dilution method, *BCYE agar naturally has black suspended particles and these dark particles prevented us from obtaining minimum inhibitory concentration values for *Legionella pneumophila* sub sp., *Pneumophila*

Staphylococcus aureus, the MIC for IR2 was 2.5 mg mL^{-1} and the MIC for PWIR was 10 mg mL^{-1}. There was no MIC for IR1, IR3 and IR4 for the extract concentrations studied. For *Escherichia coli*, the MIC for IR2 was 10 mg mL^{-1}. There was no MIC for PWIR, IR1, IR3 and IR4 for the extract concentrations studied. For *Streptococcus mutans*, the MIC for IR2 was 10 mg mL^{-1}. There was no MIC for PWIR, IR1, IR3 and IR4 for the extract concentrations studied. Ocheng *et al*. (2014) reported that the pulp juice of *Helichrysum odoratissimum* showed good antimicrobial activities on *Streptococcus mutans*. They found the MIC of *Helichrysum odoratissimum* from hexane extraction was 0.25 mg mL^{-1} and with methanol extraction, it was 1 mg mL^{-1}. For *Pseudomonas aeruginosa*, the MIC for IR2 and for PWIR was 10 mg mL^{-1}. There was no MIC for IR1, IR3 and IR4 for the extract concentrations studied.

These results showed that the water extracts from IR2 and PWIR were effective in inhibiting some bacteria growth at some of the higher extract concentrations studied. The four plant samples from the indigowoad root (IR1-4) were all from the same plant species but from different sources, yet their antimicrobial activities were very different. These variations are similar to those found by Samuelsson and Bohlin (2010), who reported that the active constituents in plants can be affected by where the plant grows, the time when harvested and the storage conditions, which may explain some of the contrasting results.

Disk diffusion method for Zone of Inhibition (ZI): The zone of inhibition for each bacteria type and treatment is given in Table 2. As a point of reference, the negative control (water) had no restraint on the growth of any of the bacteria. For *Staphylococcus aureus*, the two positive controls (Ampicillin trihydrate and tetracycline hydrochloride) had zones of inhibition (ZI) of 37 mm and 26 mm, respectively. For the plant extract treatments, IR2 had the best response with a ZI of 25 mm, followed by IR3 with 14 mm, IR4 with 12 mm, PWIR with 9 mm and IR1 with 0 mm.

For *Escherichia coli*, the positive controls showed some inhibition of cell growth (for ampicillin trihydrate the ZI = 15 mm and for tetracycline hydrochloride the ZI = 19 mm) but none of the plant extracts restrained cell growth. For *Streptococcus mutans,* the positive controls showed some inhibition of cell growth (for ampicillin trihydrate the ZI = 11 mm and for tetracycline hydrochloride the ZI = 17 mm) but none of the plant extracts restrained cell growth.

For *Pseudomonas aeruginosa*, one positive control showed some inhibition of cell growth (Tetracycline hydrochloride where ZI = 13 mm) and the other (Ampicillin trihydrate) had no apparent zone of inhibition. For the plant extract treatments, only IR2 and PWIR showed some restrained cell growth. The ZI for PWIR was 10 mm while the ZI for IR2 was 9 mm. None of the plant extracts or control treatments showed any restraint of cell growth for the *Legionella pneumophila* subsp., *pneumophila*.

General discussion of antimicrobial activity: Table 2 presents a summary of antimicrobial activity results for both the agar dilution method and the disk diffusion method. The IR2 was shown to inhibit *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus mutans* and *Pseudomonas aeruginosa* growth. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are normal skin flora, *Escherichia coli* is a normal intestinal parasite and *Streptococcus mutansis* is the most important bacterial cause of tooth decay and periodontal disease. Thus, IR2 could potentially have beneficial health effects for epithelial tissue infections, diarrhea and teeth gum inflammation.

The PWIR was shown to inhibit cell growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* which are two types of normal skin flora. Thus, PWIR could potentially have beneficial health effects for infected epithelial tissue. The book titled "Culturally significant plants" (Casey and Wynia, 2010) stated that *Baptisia bracteata* had beneficial effects on cuts and sore throats and it has been used as a mouthwash and eye wash. Borchardt *et al*. (2008) reported that crude seed extract from *baptisia bracteata* gave a ZI for *Staphylococcus aureus* that was 10 mm. Mukku *et al*. (2013) reported that *baptisia bracteata* seeds had a ZI for *Staphylococcus aureus* of 7 mm. These values are similar to the ZI of 9 mm found in this study for the roots of the same plant.

Total bacterium count from the oral cavity specimens after treatments: Figure 1 presents the log of the CFU mL^{-1} values with the different treatments for the oral cavity bacteria. For the sterile water treatment, the log of CFU mL^{-1} was 6.82±0.06 log. For the plant extract treatments, IR1 had 5.78±0.52 log, IR2 had 4.19±0.23 log, IR3 had 5.09±0.11 log, IR4 had 6.62±0.14 log and PWIR had 6.15±0.17 log. The best treatment was IR2 which reduced the oral bacteria count by 2.63±0.26 log relative to water treatment. The IR3 was the second best with a 1.73±0.14 log reduction, then IR1 with a 1.04±0.56 log reduction. The PWIR and IR4 had 0.66±0.21 log and 0.20±0.17 log reductions, respectively.

The IR2 may inhibit *Streptococcus mutans* from forming protective biofilms because it has been shown that essential oils can inhibit the *Streptococcus mutans* enzyme activities.

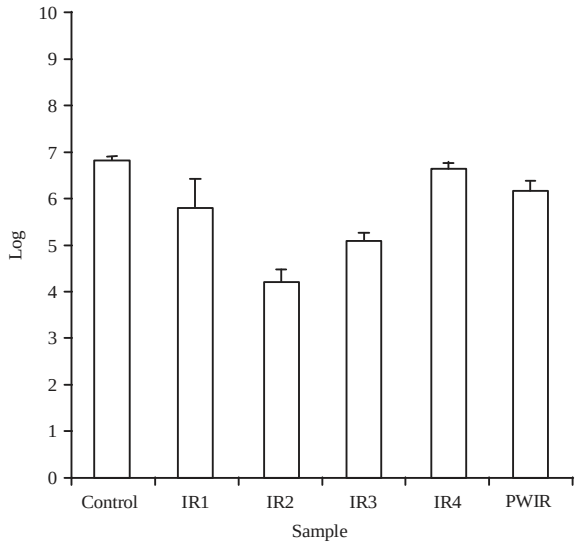

Fig. 1: Log of the total CFU mL^{-1} counts with the different treatments for the oral cavity bacteria study

Gursoy *et al*. (2009) found that *Streptococcus mutans* creates biofilms between the teeth and gums which lead to tooth decay and eventually to periodontal disease. Thus, inhibiting the enzyme activity of *Streptococcus mutans* helps prevent tooth decay and periodontal disease.

Overall, the results of this study showed that water extracts from indigo roots and plains wild indigo roots may show some antimicrobial potential. The IR and PWIR have been reported to have relatively high phenolic compound contents (Chang *et al*., 2016). Phenolic compounds have been reported to have a number of benefits in the human diet including antimicrobial and antioxidant benefits (Puupponen-Pimia *et al*., 2001; Nahak *et al*., 2014). However, other chemicals in the plants may also contribute to antimicrobial activity and more research is needed to determine chemicals or combinations of chemicals that contribute the most to this response. Researchers have shown that the seeds of plains wild indigo have some antimicrobial activity against *S. aureus* which could potentially have beneficial health effects related to infected epithelial tissue (Borchardt *et al*., 2008; Mukku *et al*., 2013). Based on the findings of this study, further research on PWIR and IR may be warranted to better determine their antimicrobial potential for a variety of microorganisms. Also, this research was for water extracts from IR and PWIR and it is likely that using other methods of extraction would yield a stronger effect on antimicrobial activity. Kaur *et al*. (2016) showed that methanol, acetone, ethyl acetate, petroleum ether and chloroform extracts from the medicinal plant, *Parthenium hysterophorus* L., usually had much higher antimicrobial inhibition on *S. aureus*, *E. coli* and *P. aeruginosa* than did the distilled water extract. More research is also needed on how to extract the antimicrobial constituents of the plants and how to apply them to achieve human health benefits.

The antimicrobial response found in this study for IR and PWIR are only for a limited set of conditions for which the sample plants were grown and handled prior to analysis. The variation of antimicrobial responses found for the four different samples of IR in this research demonstrate that growth and handling conditions may have a significant effect on the levels of chemicals in plant tissues that provide antimicrobial activity. Samuelsson and Bohlin (2010) reported that the active constituents in plants can be affected by where the plant grows, the time when harvested and the storage conditions, which may explain some of the contrasting results. Nahak *et al*. (2014) stated that environmental factors (light intensity, season, climate and temperature) during plant growth and the extraction methods used may contribute to the wide variation in total phenols and antioxidant activities found in plants by various researchers. Chang *et al*. (2013a, b) found that the time of day that plant tissue is collected may significantly affect levels of some chemicals. Rezazadeh *et al*. (2012) indicated that soil conditions may affect phenolic levels and antioxidant activity of plants. More research is needed to identify factors that will affect chemical levels in plant tissue so plants can be grown and harvested under optimum conditions to enhance desirable chemical levels. More research is also needed on possible negative aspects of PWIR and IR, such as potential toxicity at certain levels.

CONCLUSION

Under the conditions of this study, it was found that IR2 can inhibit cell growth of *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus mutans* and *Pseudomonas aeruginosa*. *Staphylococcus aureus* and *Pseudomonas aeruginosa* are normal skin flora, *Escherichia coli* is a normal intestinal parasite and *Streptococcus mutansis* is the most important bacterial cause of tooth decay and periodontal disease. Thus, IR2 could potentially have beneficial effects on epithelial tissue infections, diarrhea and teeth gum inflammation. The PWIR was found to inhibit growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa* which are two types of normal skin flora. Thus, PWIR could potentially have beneficial health effects related to infected epithelial tissue. The best treatment for oral cavity bacteria was IR2 which reduced the bacteria count by 2.63±0.26 log relative to water treatment. The IR3 was the second best with a 1.73±0.14 log reduction, then IR1 with a 1.04±0.56 log reduction. The PWIR and IR4 had 0.66±0.21 and 0.20±0.17 log reductions, respectively.

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REFERENCES

Alviano, W.S., D.S. Alviano, C.G. Diniz, A.R. Antoniolli and C.S. Alviano *et al*., 2008. *In vitro* antioxidant potential of medicinal plant extracts and their activities against oral bacteria based on Brazilian folk medicine. Arch. Oral Biol., 53: 545-552.

Blatny, J.M., B.A.P. Reif, G. Skogan, O. Andreassen and E.A. Hoiby *et al*., 2008. Tracking airborne *Legionella* and *Legionella pneumophila* at a biological treatment plant. Environ. Sci. Technol., 42: 7360-7367.

Bode, L.G.M., J.A.J.W. Kluytmans, H.F.L. Wertheim, D. Bogaers and C.M.J.E. Vandenbroucke-Grauls *et al*., 2010. Preventing surgical-site infections in nasal carriers of *Staphylococcus aureus*. N. Engl. J. Med., 362: 9-17.

Borchardt, J.R., D.L. Wyse, C.C. Sheaffer, K.L. Kauppi and R.G. Fulcher *et al*., 2008. Antioxidant and antimicrobial activity of seed from plants of the Mississippi river basin. J. Med. Plants Res., 2: 81-93.

Camarinha-Silva, A., R. Jauregui, D.H. Pieper and M.L. Wos-Oxley, 2012. The temporal dynamics of bacterial communities across human anterior nares. Environ. Microbiol. Rep., 4: 126-132.

Canbek, M., G. Bayramoglu, H. Senturk, V.A. Oztopcu and M. Uyanoglu *et al*., 2014. The examination of protective effects of gallic acid against damage of oxidative stress during induced-experimental renal ischemia-reperfusion in experiment. Bratislavske Lekarske Listy, 115: 557-562.

Casey, P.A. and R.L. Wynia, 2010. Culturally significant plants. United States Department of Agriculture Natural Resources Conservation Service, USA. http://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/stelprdb1043638.pdf.

Chang, A.C., T.Y. Yang and G.L. Riskowski, 2013a. Ascorbic acid, nitrate and nitrite concentration relationship to the 24 h light/dark cycle for spinach grown in different conditions. Food Chem., 138: 382-388.

Chang, A.C., T.Y. Yang and G.L. Riskowski, 2013b. Changes in nitrate and nitrite concentrations over 24 h for sweet basil and scallions. Food Chem., 136: 955-960.

Chang, A.C., G.L. Riskowski, Y.C. Chang and W.K. Chan, 2016. Contents of important phenolic compounds in indigowoad (*Isatis indigotica* Fort.) and plains wild indigo (*Baptisia bracteata*) roots. Res. J. Med. Plant.

Clinical and Laboratory Standards Institute, 2007. Methods for antimicrobial susceptibility testing of anaerobic bacteria. Approved Standard, 7th Edn. CLSI Document M11-A7. Clinical and Laboratory Standards Institute, Wayne, PA.

Clinical and Laboratory Standards Institute, 2011. Performance standards for antimicrobial susceptibility testing, 21st informational supplement. CLSI Document M100-S21, Clinical and Laboratory Standards Institute, Wayne, PA.

Eisenstein, B.I. and G.W. Jones, 1988. The spectrum of infections and pathogenic mechanisms of *Escherichia coli*. Adv. Internal Med., 33: 231-252.

European Committee for Antimicrobial Susceptibility Testing (EUCAST) of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID), 2000. Determination of Minimum Inhibitory Concentrations (MICs) of antibacterial agents by agar dilution. Clin. Microb. Infect., 6: 509-515.

Foster, S. and J.A. Duke, 1999. A Field Guide to Medicinal Plants and Herbs: Of Eastern and Central North America (Peterson Field Guides). 2nd Edn., Houghton Mifflin Harcourt, USA., ISBN-13: 978-0395988145, Pages: 432.

Friedman, M.N., 1997. Chemistry, biochemistry and dietary role of potato polyphenols. A review. J. Agric Food Chem., 45: 1523-1540.

Gursoy, U.K., M. Gursoy, O.V. Gursoy, L. Cakmakci, E. Kononen and V.J. Uitto, 2009. Anti-biofilm properties of *Satureja hortensis* L. essential oil against periodontal pathogens. Anaerobe, 15: 164-167.

Heo, H.J., Y.J. Kim, D. Chung and D.O. Kim, 2007. Antioxidant capacities of individual and combined phenolics in a model system. Food Chem., 104: 87-92.

Ishimatsu, S., H. Miyamoto, H. Hori, I. Tanaka and S.I. Yoshida, 2001. Sampling and detection of *Legionella pneumophila* aerosols generated from an industrial cooling tower. Ann. Occup. Hyg., 45: 421-427.

Jaberian, H., K. Piri and J. Nazari, 2013. Phytochemical composition and *in vitro* antimicrobial and antioxidant activities of some medicinal plants. Food Chem., 136: 237-244.

Jacobo-Velazquez, D.A. and L. Cisneros-Zevallos, 2009. Correlations of antioxidant activity against phenolic content revisited: A new approach in data analysis for food and medicinal plants. J. Food Sci., 74: R107-R113.

Kaur, M., N.K. Aggarwal and R. Dhiman, 2016. Antimicrobial activity of medicinal plant: *Parthenium hysterophorus* L. Res. J. Med. Plant, 10: 106-112.

Khanam, Z., C.S. Wen and I.U.H. Bhat, 2015. Phytochemical screening and antimicrobial activity of root and stem extracts of wild *Eurycoma longifolia* Jack (Tongkat Ali). J. King Saud Univ.-Sci., 27: 23-30.

Klancnik, A., S. Piskernik, B. Jersek and S.S. Mozina, 2010. Evaluation of diffusion and dilution methods to determine the antibacterial activity of plant extracts. J. Microbiol. Methods, 81: 121-126.

Li, S., 2003. Compendium of Materia Medica: (Bencao Gangmu). Foreign Languages Press, Beijing, China.

Lu, Z., J. Wang and Y. Zhang, 2012. Quantitative real-time PCR detection of airborne *Staphylococcus aureus* in hospital indoor atmosphere. Mod. Applied Sci., 6: 22-26.

Lubrizol Advanced Materials Inc., 2009. Total viable count with enrichment microbiological procedure. Lubrizol Test Procedure No. TP-6TV0001, August, 2009, New York.

Mohamed, A.A., A.A. Khalil and H.E.S. El-Beltagi, 2010. Antioxidant and antimicrobial properties of kaff maryam (*Anastatica hierochuntica*) and doum palm (*Hyphaene thebaica*). Grasas Y Aceites, 61: 67-75.

Moore, N.M. and M.L. Flaws, 2011. Introduction: *Pseudomonas aeruginosa*. Clin. Lab. Sci., 24: 41-42.

Mukku, V.J., S. Friedland, N.E. Sorlie, H.S. Donati-Lewis and B. Dingmann, 2013. Antimicrobial activity of selected Native American seeds. J. Med. Plants Res., 7: 2928-2932.

Nahak, G., M. Suar and R.K. Sahu, 2014. Antioxidant potential and nutritional values of vegetables: A review. Res. J. Med. Plant, 8: 50-81.

Ocheng, F., F. Bwanga, M. Joloba, A.K. Borg-Karlson, A. Gustafsson and C. Obua, 2014. Antibacterial activities of extracts from Ugandan medicinal plants used for oral care. J. Ethnopharmacol., 155: 852-855.

Ouhayoun, J.P., 2003. Penetrating the plaque biofilm: Impact of essential oil mouthwash. J. Clin. Periodontol., 30: 10-12.

Puupponen-Pimia, R., L. Nohynek, C. Meier, M. Kahkonen, M. Heinonen, A. Hopia and K.M. Oksman-Caldentey, 2001. Antimicrobial properties of phenolic compounds from berries. J. Applied Microbiol., 90: 494-507.

Reynolds, J., 2013. Serial dilution protocols. American Society for Microbiology, April 2013, http://www.microbelibrary.org/component/resource/ laboratory-test/2884-se.

Rezazadeh, A., A. Ghasemnezhad, M. Barani and T. Telmadarrehei, 2012. Effect of salinity on phenolic composition and antioxidant activity of artichoke (*Cynara scolymus* L.) leaves. Res. J. Med. Plant, 6: 245-252.

Samuelsson, G. and L. Bohlin, 2010. Drugs of Natural Origin: A Treatise of Pharmacognosy. 6th Revised Edn., Swedish Pharmaceutical Press, Stockholm, ISBN 9781439838570, Pages: 776.

Siegel, J.D., E. Rhinehart, M. Jackson, L. Chiarello and the Healthcare Infection Control Practices Advisory Committee, 2006. Management of multidrug-resistant organisms in healthcare settings. Am. J. Infect. Control, 35: 165-193.

Takahashi, N. and B. Nyvad, 2011. The role of bacteria in the caries process: Ecological perspectives. J. Dental Res., 90: 294-303.

WHO., 1987. Prevention of Oral Diseases. World Health Organization, Geneva, Switzerland, ISBN-13: 9789241701037, Pages: 84.

Wei, Z.Y., X.B. Wang, H.Y. Zhang, C.H. Yang and Y.B. Wang *et al*., 2011. Inhibitory effects of indigowoad root polysaccharides on porcine reproductive and respiratory syndrome virus replication *in vitro*. Antiviral Therapy, 16: 357-363.

Weinstein, R.A., R. Gaynes, J.R. Edwards and National Nosocomial Infections Surveillance System, 2005. Overview of nosocomial infections caused by Gram-negative bacilli. Clin. Infect. Dis., 41: 848-854.