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Research Article

Antibacterial Screening of Selected Plants from Southwest USA in Search of Potential Natural Alternatives for Antibacterial Application

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Abstract

The emergence of drug resistant microorganisms has posed important public health issues. The annual cost of treating antibiotic resistant infections in the United States alone has been estimated to be as high as $30 billion. This has led to an urgent need for new antimicrobial drugs, particularly from natural resources. Phytochemicals obtained from medicinal plants have been used widely in the development of novel therapeutics including antimicrobial agents.

Therefore, it is imperative to detect substances which have inhibitory effects on the growth of bacterial species. This paper explores the efficacy of ethanol (80%) extracts of leaves of several plant species from southern Arizona that were screened for their antimicrobial efficacy against *Staphylococcus epidermidis*, *Mycobacterium smegmatis*, and *Streptococcus mutans*. Extracts were prepared by a maceration process and the antibacterial activity of different plants were evaluated and compared by measuring their zones of inhibition. Most notably, *Lagerstroemia*, *Mahonia aquifolium*, and *Punica granatum* expressed the highest antibacterial activity of the thirty-three (33) plant extracts tested.

Keywords: Antimicrobial; Antibacterial and Plants

1. Introduction

The use of medicinal plants as a source for medical relief can be traced back over five millennia to higher-evolved plants in many cultures globally [1, 2]. Because the vast majority of these plants have not been evaluated for medicinal uses, it is imperative to detect substances which have an inhibitory effect on the growth of bacterial species. Angiospermic plants possess a reservoir of these effective therapeutic compounds and constitute an inexhaustible source of harmless protectants against detrimental bioflora [3-5]. Many of these southwestern native plant species are multi-purpose herbal plants which have been used as human food as well as an alternative for medicinal purposes worldwide. Many of these plants have species that are naturally occurring in the Sonoran Desert, and because of the center's location, these species were chosen to be used for experimentation.

With the ultimate goal of offering appropriate and effective antibacterial drugs to patients, plants have proven to be a valuable source of natural products for maintaining human health [6]. The historical use of plant compounds for pharmaceutical purposes has shown considerable support for the role of medicinal plants to become a promising source in creating a variety of drugs [6] as roughly 80% of individuals from developed countries use medicinal compounds derived from medicinal plants [1, 6-8]. These plants' introduction into traditional medicine as antibiotics have become one of many developed countries' most valuable weapon in fighting bacterial infections and have greatly benefitted the health-related quality of human life ever since their discovery [9].

In addition to the rising costs of antibiotic resistant infection treatment, the core of the underlying problem remains that these detrimental floras are becoming increasingly antibiotic resistant to many of the current treatments [6, 9, 10]. In combination with this reality and the growing consumer desire for natural, effective products [1], the experimentation done by this research group has been seeking out potential alternatives for antibacterial application. Current methods of assessment of antibacterial and antimicrobial properties can be often seen through methods such as Kirby-Bauer, minimum inhibitory concentration (MIC), thin layer chromatography (TLC), and various other tests that commonly utilize microbial culture techniques [11]. Positive test results to these various methods have often expressed high correlations to effective means of antibacterial agents. Of these tests, the Kirby-Bauer test was chosen and used as the primary method for experimentation in this research.

The three microbes utilized in this study were *Staphylococcus epidermidis*, *Mycobacterium smegmatis*, and *Streptococcus mutans*. *S. epidermidis* is a bacterium that is typically associated with human skin, and less associated with human mucus, exemplifying anaerobic activity for its function and survival [12]. *M. smegmatis* is a mycobacterium that is considered to be a non-pathogenic microorganism as it exhibits quick regeneration time, and therefore, it is commonly used in laboratories for conducting bacterial experiments such as with this study [12]. Additionally, *S. mutans* is an anaerobic bacterium that is typically found in the oral cavity of humans. It is known to be a key contributor in tooth decay and has been shown to be a contributor to endocarditis [12, 13]. The three microbes were chosen for this study due to their prevalence and universal usage in assessing antimicrobial effectiveness.

Deserts are commonly known to be home to a wide variety of plant species that are incredibly resilient and impervious to many of the harsh conditions in which their environments pose. Due to these severe conditions, desert plants have evolved to become resistant to many bacterial and fungal species [8, 14], which has become the basis for the initial intrigue of this research. The deserts located in southwestern United States houses many available plants that have historical medicinal and healing properties [1, 15, 16], but have seldom been chemically explored. Many medicinal applications of common desert plants such as treating burns, lowering blood sugar in diabetics, reducing symptoms of allergies, improving immune system, preventing wound infection, etc. [1, 17] have already been identified in certain desert plants such as Prickly Pear, Mesquite, and Ephedra. Industries predominantly interested in exploring similar plants in recent times have been observed by evaluating a diverse array of plant extracts for their antimicrobial properties and possible applications. With the expansive variety and supply of underexplored desert plants nearby, the objective of the experiment was to provide preliminary data to express antibacterial properties that many of these plants possess for potential antibacterial applications in medicine.

2. Materials and Methods

2.1 Plant material

Taking small excursions into the desert outside of the metro-city area of Phoenix, Arizona, 33 samples were collected of plant leaves, seeds, and stems to run extractions on. Eleven separate plant species were collected during this period. Once collected, the plant parts were set aside to dry for a 15-day period following the protocol by Ahmad et al. [18] in the room temperature laboratory to ensure that the extraction would be easy. Following the drying, the plant parts were ground up using a blender, mortar, and pestle. 100 grams of each plant were collected from the grindings that were created using the blender, mortar, and pestle.

2.2 Extract preparations

All 33 extracts were created using the 80/20 mixture of Ethanol to water and Methanol to water (tinctures). The extractions were carried out with slight modification from the methods laid down by Ahmad et al. [18] who used a 70/30 mixture. The use of the 80/20 mixture was to increase the amount of non-polar plant compounds that were extracted. The tinctures were created by taking plant samples from

the capped flasks and were then mixed with the 80/20 mixture of either Ethanol or Methanol. These were then allowed to sit, capped, at 37°C for a 7-day period with periodic shaking from the researchers. These were then filtered using Whatman No. 1 filter paper. This again was done following the protocol described by Ahmad et al [18].

2.3 Commercial antibiotics

Based on the commercial uses, commonality, and prescription uses to combat bacterial diseases in patients within and surrounding the Phoenix area, one commercially available antibiotic medication, Gentamicin, was used in this trial as a control on the effectiveness of the plant extracts. Gentamicin is widely known for its ability to inhibit protein biosynthesis by irreversibly binding the aminoglycoside to the 30S bacterial ribosome subunit.

2.4 Microorganisms

Three bacterial strains were chosen to provide proof of action against the different major groups of bacterial types which cause disease in humans: *S. epidermidis* and *S. mutans*, Gram-Positive bacteria, and *Mycobacterium smegmatis*, an acid-fast bacterium. In analyzing these plants, it was decided that they should be tested against fairly common bacteria that can be easily cultured in a laboratory setting. The laboratory resources were dedicated to these bacteria as the laboratory budget was low and these were the most readily available at the time.

2.5 Inoculum preparation

The microorganisms were cultured in a shaker at room temperature for 2 hours in Lysogeny Broth (LB) before streaking on a nutrient rich LB agar plate. This was chosen because of its nutrient richness ensuring that the experiment would not be confounded by lack of nutrients. To ensure sterility of the experiment, these streaks were performed under a sterile hood with disposable inoculating loops. This was done similarly to the methodology of Grainge and Alvarez [4].

2.6 Chemicals

All chemicals were of analytical grade. Ethanol was from Fisher Scientific (Massachusetts, USA) and the water was from the Deionizer at Grand Canyon University (Arizona, USA).

2.7 Kirby-Bauer test

Using nutrient-rich agar plates, Kirby-Bauer tests were run to determine if the plant extracts had any effects on the various bacterial species that were selected as test subjects. The Kirby-Bauer test method requires that a small disk is submerged in the solution and then allowed to dry in a sterile environment. Following this, the disks were applied to the surface of an agar plate with bacteria covering the surface of the plate and allowed to incubate for 48 hours to allow for enough bacterial growth that the results could be seen with the naked eye. The Kirby-Bauer tests were run according to the protocol described by Digrak et al. [7]. If the compounds in the plants were effective, it was easy to observe a small dead space without bacterial growth around the disks, or the zone of inhibition. These zones' diameters were then measured and recorded. This information is included in the data section of this document. This method was chosen as a simple test run as it is an effective and efficient test run before

continuing to a Thin Layer Chromatography to isolate specific compounds unique to these Sonoran Desert plants for further testing.

3. Results

Staphylococcus epidermidis, *Mycobacterium smegmatis*, and *Streptococcus mutans* were found to be resistant against various plant extracts with only 5 extracts showing moderate to high activity on each. Each of the extracts, with few exceptions due to limitations in availability, were measured out to 6 grams of dry weight in order to maintain a consistent concentration throughout each extract. *Mycobacterium smegmatis* was found to be the least resistant, overall, against many of these extracts, having only 5 that had not been able to produce any visible zone of inhibition. In contrast, *Streptococcus mutans* had expressed the highest level of resistance against the extracts, only having 9 of the 33 plants producing a zone of inhibition of any kind. *Staphylococcus epidermidis* expressed slightly less resistance to these extracts, having 13 plants produce zones of inhibitions (ZOI) of any size. Notable results were found in five extracts that exhibited moderate to high antimicrobial activity against the three bacterial strains in comparison to the control of Gentamicin. In order of *Staphylococcus epidermidis*, *Mycobacterium smegmatis*, and *Streptococcus mutans*, each sample exhibited a mean average of Zone of Inhibition (ZOI) measured in millimeters (mm); *Lagerstroemia* (5, 6, 5.5), *Mahonia aquifolium* (6, 6, 5), *Punica granatum* (6.5, 8, 7.5), *Myrtus communis* (3, 4, 4), *Prosopis glandulosa* (5, 1, 3). These results can be located in Table 1, which also exhibits the mean average of Gentamicin for Zone of Inhibition against the three bacterial strain.

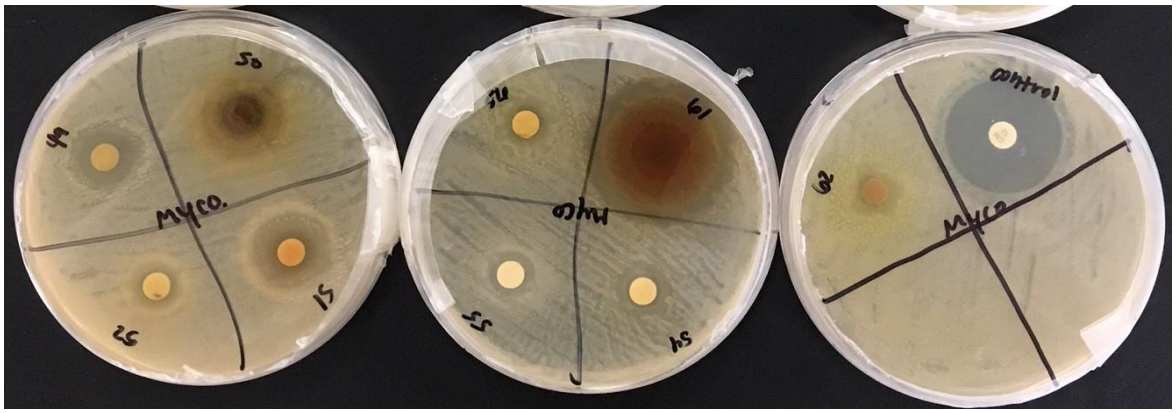

Figure 1: This project aimed to investigate the effect of identified plant extracts against three common target bacterial pathogens, to establish antimicrobial response through detection of inhibition zones to further in the hunt for new natural methods for antimicrobial resistance in reducing microbial and fungal activity.

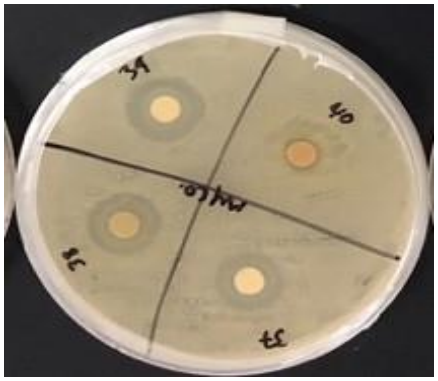

Figure 2: *Mycobacterium smegmatis* can be seen with a moderate to high level of activity with *F. paradoxa, H. patens, Thelesperma, and V. rigidula.*

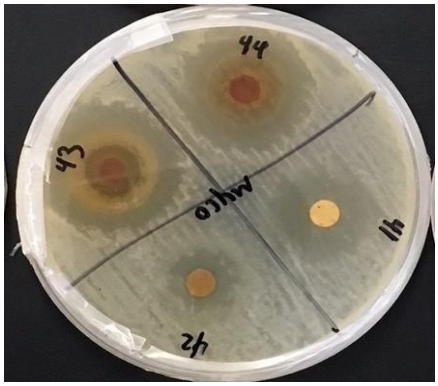

Figure 3: Looking at the bacterial culture of *Mycobacterium smegmatis*, one can see that the *Gaura, C. californica, Lagerstroemia, and M. aquifolium*, respectively, had a relatively large effect on the bacteria with antibacterial activity in the range of a 3 as it is comparable to those of smaller zones expressed.

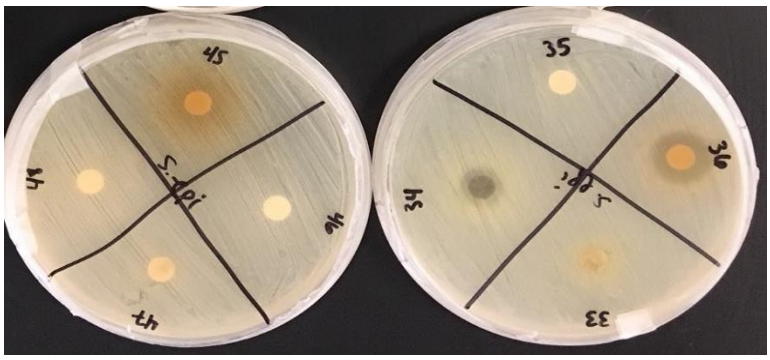

Figure 4: The plates were divided into four (4) regions to test four (4) different Sonoran Desert Plant species on each plate as seen above with a plate of cultured *S. epidermidis*. These plates were run using the standardized Kirby-Bauer method.

Botanical Name	Weight (Dry)	*M. smegmatis*	*S. mutans*	*S. epidermidis*
Tribulus terrestris L.	6g	1.0 mm	0 mm	0 mm
Acacia linifolia	6g	2.5 mm	1.0 mm	0 mm
Nerium oleander	6g	2.5 mm	1.5 mm	0 mm
Pedilanthus macrocarpus	6g	0.5 mm	0 mm	0 mm
Celtis ehrenbergiana	6g	1.0 mm	0 mm	0 mm
Olneya tesota	6g	0.3 mm	0 mm	0 mm
Cercidium floridum	6g	3.0 mm	0 mm	0.5 mm
Parkinsonia hybrid	6g	2.5 mm	0 mm	4.5 mm
Celtis occidentalis	6g	0.5 mm	0 mm	0 mm
Simmondsia chinensis	6g	0 mm	0 mm	0.5 mm
Shrub verbenas	6g	0.3 mm	0 mm	0 mm
Myrtus communis	6g	4.0 mm	4.0 mm	0.3 mm
Fallugia paradoxa	2.7g	0 mm	0 mm	0.5 mm
Hamelia patens	6g	0 mm	0 mm	0 mm
Thelesperma	3.2g	0.3 mm	0 mm	1.0 mm
Vachellia rigidula	5.5g	0 mm	0 mm	0 mm
Gaura	6g	3.0 mm	2.0 mm	1.5 mm
Calliandra californica	6g	2.0 mm	0 mm	5.5 mm
Lagerstroemia	6g	6.0 mm	5.5 mm	5.0 mm
Mahonia aquifolium	6g	6.0 mm	5.0 mm	6.0 mm
Berberis nevinii	6g	2.5 mm	0 mm	0 mm
Echinocereus engelmannii	6g	2.5 mm	0 mm	0 mm
Pachycereus marginatus	6g	2.0 mm	0 mm	0 mm
Opuntia basilaris	6g	0.3 mm	0 mm	0 mm
Olea europaea	6g	0 mm	0 mm	0 mm
Chilopsis	6g	1.0 mm	0 mm	0 mm
Condea emoryi	6g	0 mm	0 mm	0 mm
Encelia farinosa	6g	0.5 mm	0 mm	0 mm
Justicia californica	6g	0.3 mm	0.5 mm	0 mm
Asclepias erosa	6g	0.5 mm	0 mm	2.0 mm
Dyssodia tenuisecta	6g	0.3 mm	0 mm	0 mm
Punica granatum	6g	8.0 mm	7.5 mm	6.5 mm
Prosopis glandulosa	6g	1.0 mm	3.0 mm	0.5 mm
Gentamicin (Control)		11 mm	11 mm	14 mm

Table 1: Mean zone of inhibition (mm) of all extracts through 3 trials for each bacterium.

4. Discussion

As there are many different pathogens that become prevalent and more antibacterial- or antimicrobial-resistant, there has been research done in various fields to find new methods for combating these pathogens and the new strains that arise. Based on numerous other research projects and studies, desert plants have expressed high potentials for new antibacterial properties that have yet to be fully discovered [15]. expressed many of the potential antimicrobial properties of desert plants in the southwest United States [15], however, minimal research studies have since explored these potentials as well as many Middle Eastern and African desert plants [18-26]. There are hundreds of known desert plants in existence, many which have not yet been comprehensively explored, assessing the potential effectiveness of many of these plants as means for natural methods of antibacterial products must be investigated. Antibacterial efficacy is often determined by methods of Kirby-Bauer plate diffusion techniques [11]. In the present study, nutrient-rich agar was used to perform these tests, offering several advantages to identifying potential antibacterial properties each plant may possess.

In the current study, 33 unique plants that had been acquired from various regions of the Arizona state had been tested against 3 common bacterial pathogens, *Staphylococcus epidermidis*, *Mycobacterium smegmatis*, and *Streptococcus mutans*, had been grown on nutrient-rich agar plates. These tinctures were then placed in the agar by method of Kirby-Bauer and monitored over a 48-hour period. Once Kirby-Bauer for each tincture had been completed, each zone of inhibition (ZOI) was inspected and recorded. After, averaging and compiling the data into one comprehensive table, it was made clear that certain extracts had performed better than others as their ZOI were larger. The zones were assessed on both relative activity as well as by measurement in mm. Upon inspection of the data, only 5 extracts had expressed a zone of moderate to high activity on all 3 bacterial cultures: *M. communis*, *Gaura*, *Lagerstroemia*, *P. granatum*, and *P. glandulosa*. It has been made clear that *M. smegmatis* showed the least antibacterial-resistant activity as 27 of the 33 extracts averaged to express a clear ZOI. This was highly comparable to that of *Staphylococcus epidermidis* and *Streptococcus mutans*, which had 13 and 9 extracts which expressed clear ZOI, respectively. Based on these findings, many of these plant extracts, particularly the 5 which expressed ZOI in all 3 bacterial cultures, have potential to be utilized as natural methods for antibacterial application.

In looking ahead to future directions, the group plans to further explore the composition of the more successful plant species from the experiment by performing MIC, TLC, and NMR tests in an attempt to identify any specific antimicrobial compounds. Additionally, these tests would be performed in hopes to identify any common compounds shared between the various plant species to aid in further understanding antimicrobial resistance.

5. Conclusion

M. communis, *Gaura*, *Lagerstroemia*, *P. granatum*, and *P. glandulosa* extract were the only samples to

show moderate to high antibacterial activity against *Staphylococcus epidermidis, Mycobacterium smegmatis,* and *Streptococcus mutans.* Overall, this project has yielded results helpful to the search of antimicrobial compounds. Further screening and characterization of antimicrobial principles are in progress.

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Disclosures

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