International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Antimicrobial Effects of Native California Plants: Grindelia Stricta Platyphylla and Iris Douglasiana

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Abstract: ***The secondary metabolites of traditional medicinal plants are a potential source of new antibiotics (6). Two local plants with a tradition of use by California native tribes are Grindelia stricta platyphylla (coastal gum weed), a remedy used internally for respiratory and skin ailments and Iris douglasiana (Douglas Iris), also used to treat skin sores. Aqueous, ethanolic, acetonic, and ethyl acetate plant extracts were tested for antimicrobial activity, using the disk diffusion method, against gram - positive and gram - negative bacteria to determine the most effective extraction solvent. The ethyl - acetate extracts of both plants (Grindelia and Iris) showed the most antimicrobial activity, inhibiting growth of gram - positive Mycobacterium phlei and Staphylococcus aureus. For Grindelia aerial parts: the MIC was 0.0898 µg/µl against M. phlei and 0.346 µg/µl against S. aureus, the MBC was 0.1796 µg/µl against M. phlei but did not kill S. aureus. For Iris rhizomes: the MIC was 0.1944 µg/µl against M. phlei and 0.0970 µg/µl against S. aureus, the MBC was 0.1944 µg/µl against S. aureus but did not kill M. phlei. Preliminary phytochemical screening was also done on the extracts. Our hypothesis is that the California Native plants, Grindelia stricta platyphylla and Iris douglasiana, will have antimicrobial activity.***

Keywords: medicinal plant constituents, plant extract, ethyl acetate, California native plants, Grindelia stricta platyphylla, Iris douglasiana, antibiotic testing, antimicrobial activity, anti - mycobacteria, phytochemical screening

1. Introduction

There is a need to find new antibiotics to keep up with the development of antibiotic resistance in pathogenic microbes (6). The coastal gum weed, Grindelia is a remedy of the Salish and many native tribes of California who used it as a tea for respiratory ailments, even tuberculosis (Mycobacterium) and externally for skin eruptions such those caused by (Staphylococcus) (1, 2, 4). Iris, *Iris sp.* were also used as a traditional remedy for skin eruptions by many native tribes of the Pacific Coast (1, 3). In previous studies, *Grindelia sp.* and *Iris sp.*, native to Nevada have been identified as traditional medicinal plants with antimicrobial activity (1).

2. Materials and Methods

2.1. Extract Preparation

Grindelia stricta platyphylla aerial parts and *Iris douglasiana* rhizomes (Figure 1) were identified (7) and collected locally on the central California coast. Plants were extracted into four solvents of decreasing polarity (6): water, ethanol, acetone and ethyl acetate.

2.2. Kirby Bauer Disk Diffusion Assay

Mueller - Hinton agar plates were each aseptically inoculated with bacteria: *Staphylococcus aureus* (ATCC 27659), *Mycobacterium phlei* (Ward's 85W1691), *Pseudomonas aeruginosa* (ATCC 10145), *Escherichia coli* (ATCC 11775). Paper disks (10 mm) were inoculated with each extract. Grindelia disks: ethyl acetate (51.0 µg), acetone (51.0 µg), ethanol (10.2 µg), water (5.1µg). Iris disks: ethyl acetate (27.6 µg), acetone (27.6 µg), ethanol (5.52 µg), water (2.76 µg). Positive controls were gentamicin (10 µg), vancomycin (30 µg), ampicillin (10 µg) (Hardy) and rifampin (5 µg, BBL). Negative controls were disks with solvent only. Plates were incubated at 35°C for 24 hours. Zones of inhibition were measured.

2.3. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

Serial dilutions of ethyl acetate extracts of each plant were prepared in nutrient broth. Each dilution was inoculated with 20 µl *S. aureus* or *M. phlei* and incubated at 35°C for 24 hr. Positive controls were solvent, nutrient broth, and bacteria. Negative controls were solvent and nutrient broth. Tubes showing no growth were sub - cultured into nutrient broth and incubated for 24 hr at 35°C. From this subculture, the lowest dilution with growth was the MIC. The lowest dilution that showed no growth was the MBC.

2.4. Phytochemical Screening

Chemical color tests were done for the presence of sterols/terpenoids/resins (Liebermann - Burchard), alkaloids (Dragondorff, Wagner), carbohydrates (Fehling), flavonoids (Shinoda), tannins/phenols (Ferric Chloride). Extracts were tested for the presence of saponin (hemolysis) by the disk diffusion method on 5% blood agar plates. Negative controls were disks with solvent only.

2.4.1. Test for sterols/terpenoids/resins (Liebermann - Burchard)

Extract was dissolved in 2 ml of chloroform ($CHCl_3$), then 10 drops of acetic anhydride ($C_4H_6O_3$) were added together with 1 - 3 drops of concentrated sulfuric acid (H_2SO_4). Formation of a green color was positive.

2.4.2. Test for alkaloids (Dragondorff, Wagner)

Dragendorffs reagent was made by adding 2 Pepto - Bismol tablets to 20 ml of deionized water and breaking it up to dissolve.10 ml of concentrated hydrochloric acid (HCL) was added and swirled until it stopped foaming. This mixture was allowed to settle and was decanted to remove solids.7g

of potassium iodide (KI) was then dissolved in 2 ml of water, added to the decanted mixture. This was brought up to a volume of 100 ml with water and filtered through a filter paper by vacuum, resulting in a clear brown liquid which was used for the test. Plant extract was boiled in 10 ml methanol, 1% HCl and 6 drops of Dragendorffs reagent were added. A brown - red precipitate was positive.

2.4.3. Test for carbohydrates (Fehling)

A two - part solution was made. Solution A: 63g of copper sulfate ($CuSO_4$) was dissolved in deionized water and brought up to volume of 1000ml. Solution B: 352g of potassium sodium tartrate (Rochelle Sitt) and 154g of NaOH were dissolved in deionized water and brought up to volume of 1000ml. Equal parts of solution A and B were mixed before using as the mixture will not keep, the mixture turns blue on mixing.2 ml of Fehling's solution A and Fehling's solution B were added to 2ml of extract in a test tube. The test tube was boiled for 10 minutes in a water bath. The formation of red precipitate was positive.

2.4.4. flavonoids (Shinoda)

A few pieces of magnesium ribbon and concentrated hydrochloric acid (HCl) were added to eachplant extract in a test tube. The formation of a red - pink color was positive.

2.4.5. Test for tannins/phenols (Ferric Chloride)

A 0.5 Normal, 2% Ferric chloride solution was made using 135g of hydrated ferric chloride salt ($FeCl_3 \cdot 2H_2O$) added to 20 ml of concentrated hydrochloric acid (HCl) and brought up to volume of 1000ml with deionized water.200 mg of plant extract was boiled in 10 ml of deionized water and a few drops of the above ferric chloride mixture was added. Formation of a blue - black precipitate was positive.

2.4.6. Test for saponin (hemolysis)

Saponins present can hemolyze blood by breaking down red blood cells. Mueller - Hinton 5% blood agar plates (Hardy Diagnostics) were used.30 µl of extract were put onto sterile paper disks on a blood agar plate and incubated for 24 hours. The presence or absence of hemolysis was observed. Zones of clearance indicated the presence of saponins.

3. Results

3.1. Kirby Bauer Disk Diffusion Assay

The extracts in ethyl acetate, the most non - polar solvent, showed the most antimicrobial activity (Figure 2). No extracts inhibited *E. coli*. Compared to commercial antibiotics, the ethyl acetate Grindelia extract was as effective as gentamicin, 18% more effective than vancomycin, 100% more effective than ampicillin and 53% more effective than rifampin against *M. phlei*. The ethyl acetate extract of Iris was 27% more effective than ampicillin and 6% more effective than vancomycin against *S. aureus* (Figure 1).

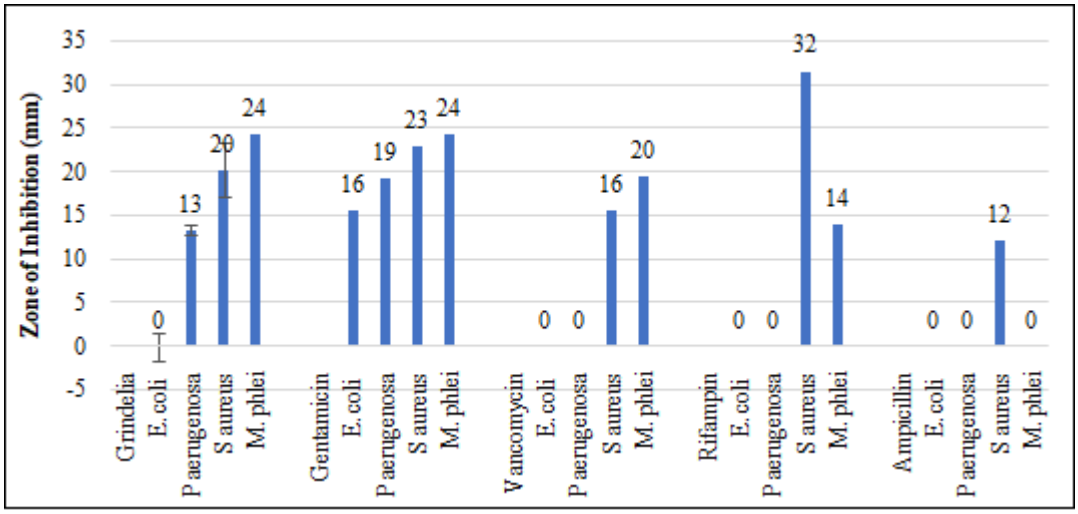

Figure 1: Ethyl acetate extracts were most effective against gram - positive bacteria.

3.2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The ethyl acetate extract of Grindelia was bactericidal against *M. phlei* but only inhibitory against*S. aureus*. The ethyl acetate extract of Iris was bactericidal against *S. aureus* but only inhibitory against *M. phlei* (Table 1).

Table 1: MIC and MBC of ethyl alcohol extracts Grindelia aerial parts and Iris

	MIC (µg/µl)		MBC (µg/µl)	
	Grindelia	*Iris*	*Grindelia*	*Iris*
M. phlei	0.09	0.19	0.18	0.00
S. aureus	0.35	0.10	0.00	0.19

3.3. Phytochemical Screening

The ethyl acetate Grindelia extract tested positive for saponins, sterols/terpenoids/resins, and carbohydrates. The ethyl acetate Iris extract did not test positive for any of the phytochemical screening tests. The acetone Grindelia extract tested positive for saponins, sterols/terpenoids/resins, carbohydrates, and tannins. The acetone Iris extract tested positive for every test except saponins. The ethanol Grindelia extract tested positive for saponins, sterols, flavonoids and tannins. The ethanol Iris extract tested positive for sterols/terpenoids/resins, carbohydrates, and tannins/phenols. The aqueous Grindelia extract tested positive for alkaloids and tannins. The aqueous Iris extract tested negative in all of the tests (Table 2).

Table 2: Phytochemical Screening

Grindelia	Ethyl Acetate	Acetone	Ethanol	Water
saponins	+	+	+	ND
sterols/terpenoids/resins	+	+	+	-
alkaloids	-	-	-	+
carbohydrates	+	+	-	-
flavonoids	-	-	+	-
tannins/phenols	-	+	+	+
Iris	Ethyl Acetate	Acetone	Ethanol	Water
saponins	0	0	0	0
sterols/terpenoids/resins	-	+	+	-
alkaloids	-	+	-	-
carbohydrates	-	+	+	-
flavonoids	-	+	-	-
tannins/phenols	-	+	+	-
+ present. - absent. ND = not determined				

4. Discussion & Conclusion

Grindelia stricta platyphylla aerial parts and *Iris douglasiana* rhizomes, do have some antibiotic activity against acid – fast *Mycobacterium phlei* and gram - positive *Staphylococcus aureus*. Less polar solvents such as acetone and ethyl acetate are most effective at extracting the antimicrobial substances in Grindelia and Iris. The ethyl acetate Grindelia extract may have saponins, sterols/terpenoids/resins, and carbohydrates, the composition of the ethyl acetate Iris extract was inconclusive. In conclusion, the substances in the ethyl acetate extracts of *Grindelia stricta platyphylla* aerial parts and *Iris douglasiana* rhizomes may be useful to develop into new antibiotics. Further studies could include, chemical isolation of the ethyl acetate extract into acid, base, neutral and phenolic fractions (6) and a paper chromatography experiment, in combination with disk diffusion assay to further classify the active substances in the extract, are being considered. The antimicrobial compounds in the ethyl acetate extracts need to be identified.

Acknowledgements
I would like to thank Dr. Christine Case of Skyline College, for guiding me in this experiment in her lab, and for all the time, advice and help she gave. I would also like to thank my microbiology professor Milena Lilles for her inspiration and encouragement. Many thanks to microbiology technicians, Kylin Johnson and Gary Cheang who made all of the lab work possible.

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