# Probing medicinal plants for novel antimicrobial compounds

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## INTRODUCTION & OBJECTIVE

According to the World Health Organization, infectious diseases account for three of the top ten global causes of death [1]. Therefore, the isolation, or creation, of novel antimicrobial agents is currently at the forefront of modern healthcare. This is an especially important topic due to the stark decrease in antimicrobial drug development in recent years [2] and due to the rise of superbugs, or microorganisms that are resistant to more than one type of antimicrobial treatment, which are predicted by 2050 to cause 10 million deaths per year [3]. Plants naturally produce a robust supply of novel metabolic compounds that can be used to treat a variety of human diseases, including many antimicrobial agents, such as phenolics, terpenoids, alkaloids, polyacetylenes, lectins and polypeptides [4]. However, with the advent of modern antibiotic drugs mainly of bacterial, fungal and synthetic sources, many of these natural plant-derived antibiotic sources have been left unexplored.

Therefore, our work is focused on screening poorly explored medicinal plants in the hopes of discovering novel antimicrobial drugs. To do this, we have been testing extracts of various plants found in the Valpo medicinal garden for their effects against twelve bacterial and fungal microbes of interest. To date, methanolic and hexane extracts of aerial, reproductive, and root portions of 13 plants have been screened (Fig. 1), with five showing promising activities (Fig 3).

| Scientific Name | Common Name | Part(s) Extracted | Solvent(s) Used at 0.05 mg/uL | Activity Against Gram-Positive Bacteria? | Activity Against Gram-Negative Bacteria? | Activity Against Fungi? |
|---|---|---|---|---|---|---|
| *Achillea terracotta* | Terracotta Yarrow | Flowers & Roots | MeOH Only | Yes for flower/root in MeOH | Yes for flower in MeOH | No in MeOH |
| *Agastache foeniculum* | Anise Hyssop | Leaves & Flowers | MeOH Only | No in MeOH | No in MeOH | No in MeOH |
| *Allium schoenoprasum* | Culinary Chives | Leaves | MeOH & Hexane | No in MeOH & Hexane | No in MeOH & Hexane | No in MeOH & Hexane |
| *Artemisia dracunculus* | French Tarragon | Leaves | MeOH Only | No in MeOH | No in MeOH | No in MeOH |
| *Rumex sanguineus* | Red-Veined Sorrel | Leaves | MeOH & Hexane | No in MeOH & Hexane | No in MeOH & Hexane | No in MeOH & Hexane |
| *Aronia melanocarpa* | Low Scape Mound Chokeberry | Leaves, Roots & Berries | MeOH & Hexane | Yes: Leaves/Roots in MeOH | Yes: Leaves in MeOH | No in MeOH |
| *Rubus idaeus* | Heritage Red Raspberry | Leaves & Roots | MeOH & Hexane | Yes: Leaves/Roots in MeOH & Hexane | Yes: Leaves/Roots in MeOH & Hexane | Yes: Only Root in MeOH |
| *Arabidopsis thaliana* | Thale Cress or Mouse-ear Cress | Aerial Portions & Roots | MeOH & Hexane | No in MeOH & Hexane | No in MeOH & Hexane | No in MeOH & Hexane |
| *Origanum vulgare* | Oregano | Flowers | MeOH | No in MeOH | No in MeOH | No in MeOH |
| *Anethum graveolens* | Dill | Flowers, Seeds & Roots | MeOH | Yes: Seeds in MeOH | No in MeOH | No in MeOH |
| *Lavandula angustifolia* | Lavender | Flowers, Leaves & Roots | MeOH | Yes: Stems and Roots in MeOH | Yes: Roots in MeOH | No in MeOH |
| *Agastache foeniculum* | Anise Hyssop | Seeds | MeOH | Yes: Seeds in MeOH | No in MeOH | No in MeOH |
| *Calendula officinalis* | Flashback Calendula | Roots & Seeds | MeOH | Yes: Root in MeOH | No in MeOH | No in MeOH |

**Figure 1:** Thirteen plant species that underwent antibiotic screening with parts, solvents and results summarized.

**OBJECTIVE => Screen medicinal plants for antimicrobial activity with the goal of discovering novel bioactive compounds (using analytical chemistry techniques).**

## REFERENCES

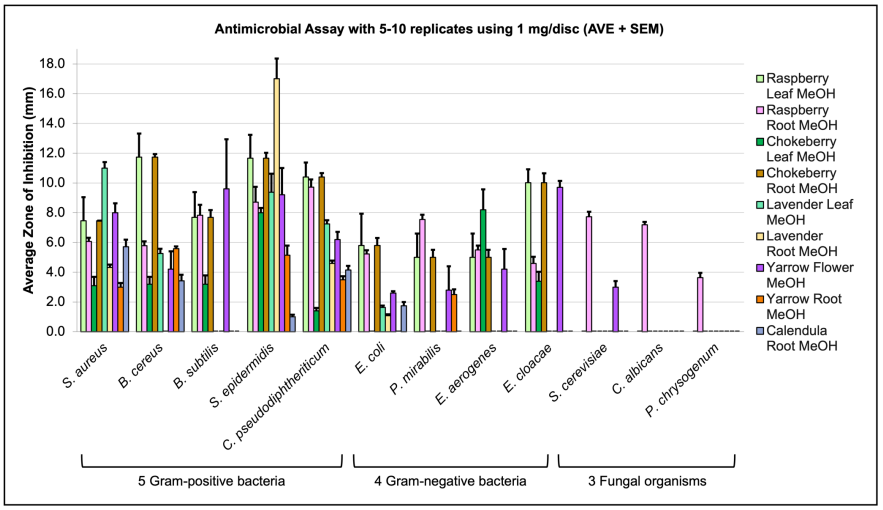
1. World Health Organization. (2020). The top 10 causes of death [Internet]. WHO; [cited 2023 April 13]. Available from: https://www.who.int/news-room/fact-sheets/detail/the-top-10-causes-of-death.
2. Clatworthy *et al.* (2007). Targeting virulence: a new paradigm for antimicrobial therapy. *Nature Chem Biol*. 3: 541–548.
3. Global Health Estimates 2016: Disease burden by Cause, Age, Sex, by Country and by Region, 2000–2016. Geneva, World Health Organization; 2018.
4. Newman and Cragg (2012). Natural Products as Sources of New Drugs over the 30 Years from 1981 to 2010. *J Nat Prod*. 75(3): 311-335.

## METHODS & RESULTS

### Figure 2: Extraction Procedure

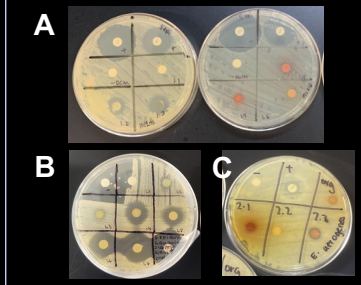
**Extract Preparation and Medicinal Garden Layout.** Plant species were separated into various parts (such as roots, leaves, stems, and reproductive parts) and allowed to dry at 22°C. 4 grams of each dried sample was homogenized using a mortar and pestle. The powdered sample was then macerated in solvent using a 1:10 (plant material:solvent) ratio at 200 rpm, 35°C for 48 hours. The mixture was centrifuged at 5,000 x g for 5 minutes, and the supernatant was filtered through a 0.2 uM membrane. The filtrate was then dehydrated, quantified and tested for antimicrobial activity using the Kirby-Bauer disc diffusion assay. A layout of the Valpo medical garden is shown on the bottom left.

### Figure 3: Antimicrobial Experiments




**Kirby-Bauer Antimicrobial Disc Diffusion Assay.** Blank antibiotic sensitivity discs were impregnated with 1 mg of extract. Once the solvent evaporated, discs were placed onto a media plate with a lawn of the appropriate microorganism (12 unique microbial lines were used). After 48 hours of growth, zones of inhibition were measured in millimeters using a ruler. The average zone of inhibition for five biological replicates is shown above with associated standard error of the mean (SEM). Antibiotics (vancomycin, streptomycin) or antifungal (clotrimazole) compounds were used as positive controls. The solvent (methanol) alone was used for the negative control and showed no zone.

### Figure 4: Preliminary Extract Separation




After column chromatography of the crude extracts of chokeberry leaf MeOH (A), lavender aerial MeOH (B), and yarrow flower MeOH, sub-fractions were re-tested for activity against susceptible microorganisms, resulting in several bioactive sub-fractions. Vancomycin or streptomycin served as positive controls, and MeOH was the negative. Thin-layer chromatography was used to monitor subfraction purity.

## CONCLUSIONS & ON-GOING WORK

- All extracts in Fig. 3 show promising activity against Gram-positive organisms, while raspberry leaf/root, chokeberry root, and yarrow flower/root methanol extracts demonstrate broader-spectrum activity.
- Raspberry root methanol extract inhibited all three fungal organisms tested.
- Several extracts have been preliminarily separated (Fig. 4) and require further sub-fractionation before employing analytical chemistry techniques (*i.e.* mass spec and NMR) to determine the specific compounds responsible for their biological activity.
- These data highlight the importance of plants as an invaluable pharmaceutical resource at a time when antimicrobial drug discovery has plateaued.

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