Short Communication

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2023.50.008025

Introducing Chemistry Students to Experiments Using the Antimicrobial Herbal Garden Cress Seeds

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ARTICLE INFO

Received: May 25, 2023

Published: June 07, 2023

Citation: Moges Abebe. Introducing Chemistry Students to Experiments Using the Antimicrobial Herbal Garden Cress Seeds. Biomed J Sci & Tech Res 50(5)-2023. BJSTR. MS.ID.008025.

ABSTRACT

The purpose of this study was to introduce students to herbal chemistry, guide them in extracting, purifying, and testing phytochemicals in a college laboratory setting. One of the most economical and readily available herbs is garden cress seed, which is a small, pinhead-sized seed that has found nutritional value. The seed has been used as a traditional medicine around the world for centuries, and it is cost-effective seed to introduce in chemistry laboratories. Students have already been exposed to spices and herbs in cooked meals where they smelled and tasted herbs and may also have heard about the medicinal purpose of the herb. Students partake in labs involving planting the garden cress seed within the first day of experimentation and the remaining lab time can be used to extract and isolate the phytochemicals using organic solvents. The extraction method can be applied to the stem, leaves, and roots of the planted seed in the second laboratory period, and the third lab can be used to perform analytical methods to test the quality of the extraction and determine the antibacterial properties of the herb. Compared to the other labs, the innovative experiments on herbs provided exposure to lab techniques and increased collaboration among students with the support of the instructor.

Introduction

Naturally occurring herbal plants provide cost-effective, nontoxic chemicals that are available in most parts of the world. Experimenting with herbs provides multiple avenues of discovery that are engaging and will open the possibility of inquiry-based learning. The herbs can be used to teach all levels of college chemistry, and they can be performed with minimal equipment requirements. Furthermore, students that are experimenting with herbs can reduce the monotony of focusing only on answering pre- and post-lab questions as well as writing a lengthy lab report, which can be overwhelming for beginning students. Nontoxic herbal plants get the attention of students because most plants have an attractive smell and are familiar to those who have seen them used for cooking in the kitchen. The plant provides different compositions, and all parts of the plant—the roots, stem, leaves, fruits, and flowers— provide unique opportunities for experimentation. In this study, garden cress herb was used for several types of chemistry experiments for all levels. The availability of the garden cress plant, which is grown throughout the world, its rich source of essential nutrients, and its phytochemical properties have given it varied uses for experimentation to determine its properties as an alternative medicine. Previously, garden cress seed was used in folk medicine as a laxative, antimicrobial, antidiabetic, antioxidant, and anti-inflammatory agent. Presently, garden cress seed is found to consist of nutrient-rich protein (25%), and lipids (16%), while the powdered seed has a high fiber content [1]. The existence of anthocyanins in garden cress seed opened an opportunity for the herb to be tested by the valuable technique of acid-base titration. Chemistry students were challenged to develop their quantitative skills by following colors versus pH as an alternative to cookbook titration protocols.

Standard extraction procedures were followed to extract the phytochemicals found in garden cress plants. The samples were powdered seeds and the plants were grown in the laboratory. Several polar and nonpolar solvents were employed to extract the major phytochemical groups known as carotenoids and polyphenols. The accuracy of the method was tested by the percentage yield

of the results [2]. Antimicrobial properties of garden cress plants were tested using Kirby-Bauer method of diffusion and the results were conclusive. The diffusion discs as well as diffusion wells were employed, and the control antibiotics was penicillin as a control disc. Several modifications such as a variety of disc material, sample concentrations, disc paper, and temperature and humidity variables were applied (Figure 1).

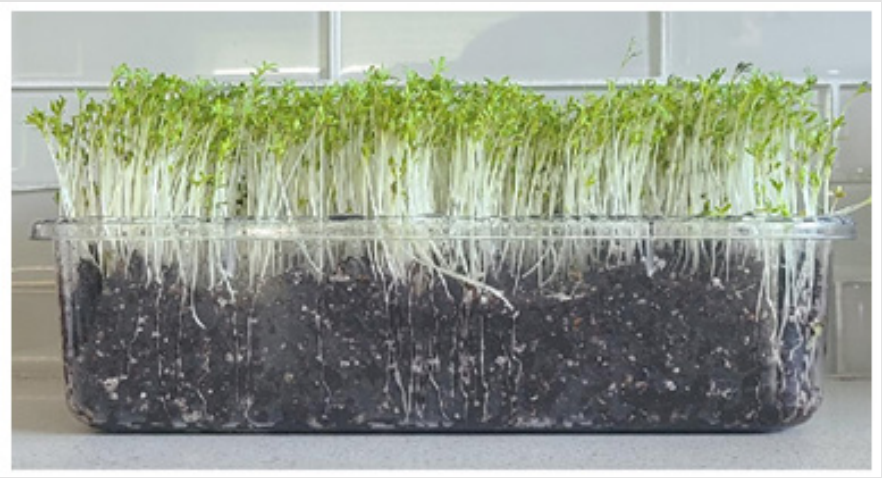

Figure 1: Fast growing garden cress seeds within six days from planting (Height = 7 cm).

Material and Methods

At the beginning of the first lab, students spread a small amount of the seed in moist soil and watered the plant, and they used the remaining lab period to extract the analyte from the garden cress seed. During the week, students had a chance to watch the seed germinate, sprout, and grow to a height of about 3 inches. Several variables, including the quantity of water, the temperature, and fertilizers, can be changed for experimentation. The plant, stem, leaves, and roots were cut, crushed, and blended with the traditional mortar and pestle, and the liquid extract was used for the extraction and for the antimicrobial test. Two grams of garden cress seed powder were dissolved in 20 mL of solvent at room temperature, and the solvents used for this experiment were chloroform, dichloromethane, water, ether, acetone, toluene, cyclohexane, ethanol, methanol, acetic anhydride, and acetonitrile. Except for water, the other solute-solvent mixture was stirred thoroughly for fifteen minutes before decanting and centrifuging. Water was removed from organic solvents by using $CaCl_2$ as the drying agent.

In the laboratory, nine students worked with several solvents to isolate the secondary metabolites from garden cress. They were all successful in extracting the analytes from the sample. The solvents were dried off on a hot plate, and other testing methods were performed to confirm the analyte. Thin-layer and column chromatography were achieved to further separate the analyte. Students carried out various pH exercises while identifying the colors of the garden cress extract. The garden cress was soaked in water, carefully decanted to separate the slimy solid from the liquid and centrifuged to get a clearer liquid. Six different masses of the garden cress were used by each student to determine the pH requirement to achieve the color change, and to calculate the pH from the amount of the 0.1 M NaOH used for the experiment. The colors displayed range from acidic red to green and purple as the pH approaches a basic solution due to the presence of anthocyanin pigments, which are the most readily available color indicator in many common plants. These results can be confirmed with an ultraviolet-visible spectrophotometer. The last experiment that was performed in the laboratory employed the Kirby-Bauer method of antimicrobial testing [3]. These experiments build students' advanced laboratory skills. Students learned to clean and sanitize their working area, melt and pour nutrient agar, smear bacteria on a plate, prepare a disk, place the disk on the smeared bacteria, and incubate the disk in a 37°C humid incubator. The antimicrobial disk diffused overnight, and the zone of inhibition was measured using a ruler [3] (Figure 2).

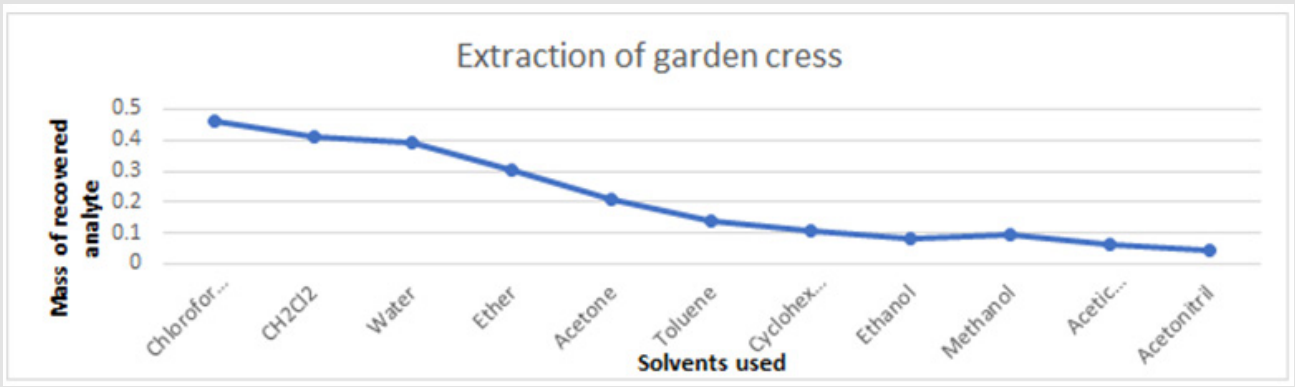

Figure 2: Extraction of garden cress seeds in different solvents.

Result and Discussion

Students performed the solid-liquid extraction method to separate the analyte in the garden cress powder, and the cold solvent was evaporated to determine the percentage yield. Students were already familiar with simple and fractional distillation, and they used different solvents to extract the sample. The following solvents were used to extract the secondary metabolites from garden cress seed. Water was used for the extraction of anthocyanins, starches, tannins, saponins, terpenoids, polypeptides, and lectins. Ethanol was used for the extraction of tannins, polyphenols, polyacetylenes, flavonols, terpenoids, sterols, and alkaloids. Methanol was used for the extraction of anthocyanins, terpenoids, saponins, tannins, xanthoxylene, totarol, quassinoids, lactones, flavones, phenones, and polyphenols. Chloroform was used for the extraction of terpenoids and flavonoids. Ether was used for the extraction of alkaloids, terpenoids, coumarins, and fatty acids. Acetone was used for the extraction of phenol and flavonols [4]. The primary metabolites of garden cress are carbohydrates, which are soluble in water and alcohol, along with proteins and lipids, which are soluble in organic solvents. Because of these nutrients, the mass of the extract showed slight variations. The results are tabulated in (Table 1). The extracted analyte is shown in the first row, while the precipitated solvent is listed in the second row. The total recovery rate is shown in the third row, with an excellent result of 98% recovery rate. The nonpolar solvent dichloromethane has 10% extraction, and polar solvents like acetone account for phenols and flavonols, which are the only extracts listed in the literature. Terpenoids and flavonoids are the extracts in chloroform, however 23% of the extract also includes the primary metabolites, such as lipids, and is included in the analyte. Methanol accounts for 4% of the sample's anthocyanins, flavones, lactones, phenones, polyphenols, quassinoids, saponins, tannins, terpenoids, totarol, and xanthoxyllins. Chemical analysis of the analyte using thin-layer and column chromatography were used to purify the product further.

Table 1: Nass of extracted phytochemicals using different solvent.

Solvent	$CHCl_3$	CH_2Cl_2	Water	Ether	Acetone	Toluene	Cyclohexane	Ethanol	Methanol	Acetic anhydride	Acetonitrile
Analyte (g)	0.46230	0.41020	0.39300	0.30300	0.20527	0.14080	0.10670	0.07867	0.09267	0.06000	0.04300
Precipitate (g)	1.90140	1.58980	1.71500	1.70650	1.44825	1.77360	1.84210	1.93143	1.91343	1.36120	2.21010
Total recovered (g)	2.36370	2.00000	2.10800	2.00950	1.65352	1.91440	1.94880	2.01010	2.00610	1.42120	2.25310

Students titrated the 50/50 alcohol-water extract of garden cress seeds to determine the presence of phytochemicals in the samples. Providing simple investigative instructions about following the color of the solution while recording the volume of the NaOH titrant and measuring the pH of the solution, helped students get fully engaged as they helped each other identify colors and learn to use pH meters. Students prepared a 0.1 molar standard solution of NaOH and titrated garden cress extract using a handheld pH meter, to develop manipulative skills while acquiring laboratory equipment handling skills, titration skills, and theoretical calculations in acid-base chemistry. The cost of commercially available garden cress seeds were $9.99/lb., and their availability in herbal stores will complement working with monetary constraints. The instructor avoided providing a recipe-style approach so that students could be creative and apply

an active learning model. The small teacher-to-instructor ratio allowed each student to work individually, and their results were accumulated at the end of the 2 hours and 50 minutes laboratory sessions. The titration result is displayed in (Figure 3). The pH range varied from 2.9 to 11.4, and the color changed from red to purple and brown. Two strains of bacterial pathogens that are found in the mouth were employed to the extraction of garden cress seed. The analyte from garden cress seed powder was extracted by dissolving it in eleven different solvents and evaporated to a high concentration. The nutrient agar, the control antibacterial penicillin disk, and the bacteria pathogens were bought from Carolina Biological Supply Company. The Myer-Bower method was carefully followed, and a thin layer of bacteria was spread on the agar surface, and four different disks were placed in the disc. The bacteria were left overnight to grow, and the diameter of the diffusion of the plant extract, which is the zone of inhibition, was measured with a ruler. A penicillin disc was used as a control, and the zone of inhibition was measured in mm.

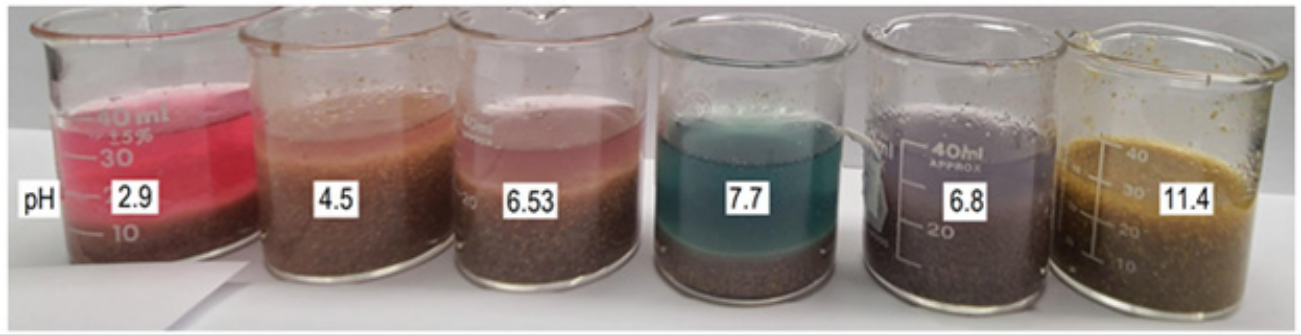

Figure 3: pH and color change of garden cress seed.

Conclusion

Worldwide, teaching institutions equipped with a chemistry lab have limited budgets to buy the necessary equipment and chemicals to train students. Implementing herbal extracts would reduce lab costs and get the training institutions closer to phytochemical extraction and their determinate testing. The extraction of phytochemicals was productive, and 100% of the students obtained a reasonable percentage yield. The available solvents made it possible to extract natural herbs as an alternative to toxic chemicals. To test the presence of anthocyanin, students followed a creative method of titration compared to the standard acid-base titration of a strong acid with a strong base, a weak acid with a weak base, and many variations of these combinations. Following color changes to determine the pH gave the student an innovative perspective and was found effective in learning the theory as well as increasing interaction between students. The process of extraction, isolation, and testing of phytochemicals also opened the opportunity for testing the products with titration and antimicrobial effectiveness, and the result was convincing. In conclusion, the quest for novel methods of extraction, isolation, and testing that was described in this study has avoided some of the more toxic chemicals and provided students with alternative methods of learning the principles of practical, hands-on, higher-level undergraduate chemistry experiments at minimal cost.

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ISSN: 2574-1241

DOI: 10.26717/BJSTR.2023.50.008025

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