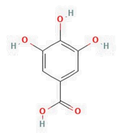
Analysis of Phytochemicals from Watercress leaves

Allyssa Fisher, James Villanueva, Bidisha Sengupta*
Department of Chemistry and Biochemistry, Stephen F. Austin State University, Nacogdoches, TX, 75965
*senguptab@sfasu.edu

Background

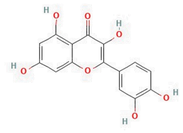
Watercress, or *Nestertium officianale*, is a member of the *Brassicaceae* family, along with other cruciferous vegetables including broccoli, cabbage, and kale. Literature shows that watercress is robust in nutritional value. Consumption of watercress has been linked to decreased likelihood of developing various diseases such as cancer, atherosclerosis, diabetes, neurological diseases, and cardiovascular diseases.[1] The therapeutic properties of watercress may be attributed to its rich phytochemical profile. Watercress contains classes of chemical compounds known as antioxidants, phenolic acids, glucosinolates, and flavonoids.[2] These phytochemicals are biologically important because they protect biomolecules from degradation by oxygen radicals.[3] In this study, the phytochemicals in watercress were extracted using different solvents. High performance liquid chromatography (HPLC) was utilized to separate the chemical constituents of watercress and gain an understanding of its chemical composition. In addition, the total antioxidant capacity (TAC), total phenolic content (TPC), and total flavonoid content (TFC) were determined using various methods.

Phenolic acids

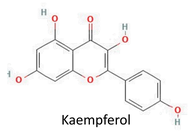


Gallic acid

Flavonoids

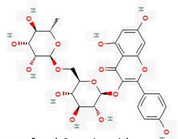


Quercetin

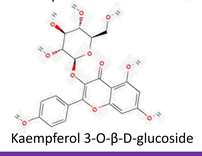


Kaempferol

Kaempferol derivatives



Kaempferol-3-rutinoside



Kaempferol 3-O-β-D-glucoside

Objectives and Approaches

Hypothesis: Watercress contains a high level of phytochemicals, and the alcohol extracts contain the highest concentration of antioxidants, flavonoids, and phenolic acids compared to other solvents.

Main Objective: Analyze the phytochemicals present in various watercress extracts

Approaches taken

- HPLC of watercress extracts
- Total phenolic acid content determination using Folin-Ciocalteau method
- Total Flavonoid content using aluminum chloride method
- Total antioxidant capacity using DPPH method
- ATR-FTIR of watercress extracts

Experimental Techniques

Instrumentation

HPLC (Jasco 4000)
- Flow Rate: 1 mL/min
- Stationary Phase: Kinetex 5 µm C18 100 Å, LC Column 250 x 4.6Mm
- Mobile Phase: 65% ACN / 35% DI H_2O

UV/Vis Absorption Spectroscopy
Shimadzu UV 2550 Spectrophotometer

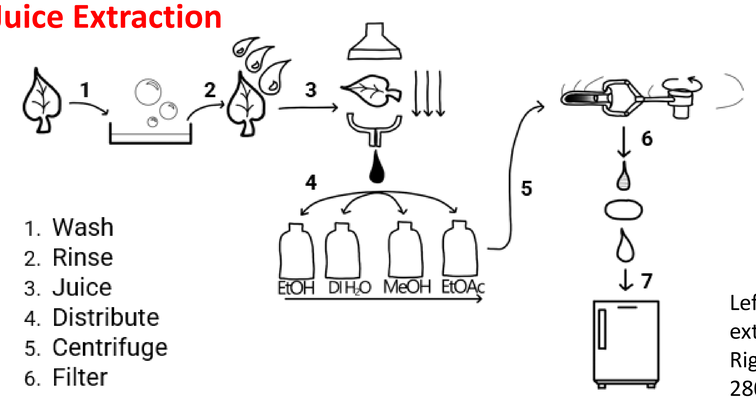
Fluorescence Spectroscopy
PerkinElmer 6500 fluorimeter
- Excitation and emission slit widths were 10/10 nm unless specified.

ATR-FTIR: Jasco FT/IR-4X Fourier transform infrared spectrometer, MCT detector

Acknowledgement:
We thank Welch Foundation Grant (AN-0008) at Stephen F. Austin State University. We also thank Stephen F. Austin State University SURE 2023 program for funding this project.

Methodology

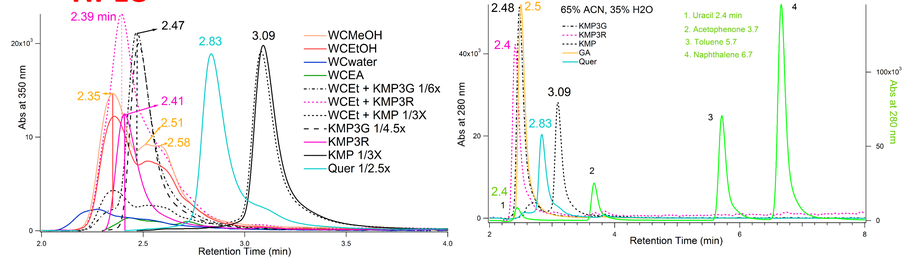
Juice Extraction



1. Wash
2. Rinse
3. Juice
4. Distribute
5. Centrifuge
6. Filter

Exploring the Constituent Phytochemicals

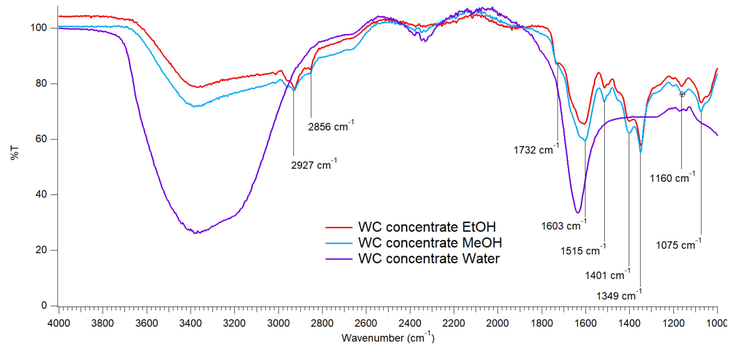
HPLC



Left: HPLC chromatograms with abs at 350 nm of watercress (WC) juice (water) and solvent extracts. The ethanol extract (WCEt) was spiked with the standards KMP, KMP3G and KMP3R. Right: Typical HPLC chromatograms of the phytochemical standards (used in this study) at abs 280 nm. The green solid line represents the standards for the C18 column.

Spectroscopic Measurements

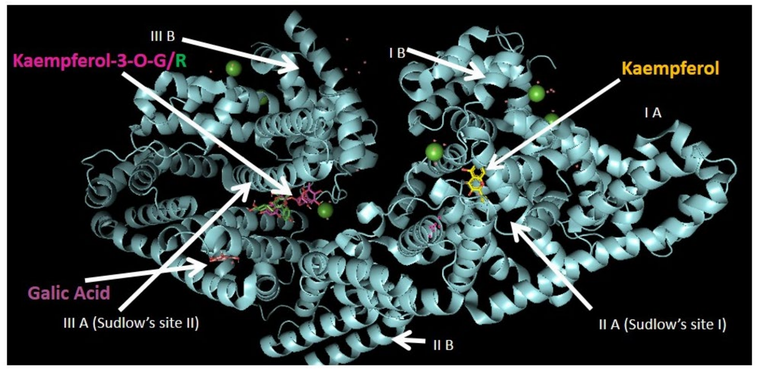
ATR-FTIR



Overlay of FTIR spectra of the three concentrated WC extracts

Computational Docking Studies using AutoDock vina

Binding Studies of WC with Protein using the standards



Biochemical Assays

Total Antioxidant Capacity (TAC)

$$TAC_{DPPH}\,(\%) = \frac{(A_{blank} - A_{sample})}{A_{blank}} \times 100\%$$

	AVG Absorbance	TAC_{DPPH}%
DPPH in EtOH	0.340 ± 0.017	
WC MeOH extract with DPPH in EtOH	0.131 ± 0.002	61.5
WC EtOH extract with DPPH in EtOH	0.149 ± 0.009	56.2
WC juice extract with DPPH in EtOH	0.206 ± 0.001	39.4

Total Phenolic Acid Content

The total phenolic acid content of each extract expressed as mg GA/g extract:

MeOH extract $\frac{12.0\frac{mg\ GA}{mL\ solution}}{0.105\frac{g\ extract}{mL\ solution}} = 114\ \frac{mg\ GA}{g\ extract}$

EtOH extract $\frac{10.0\frac{mg\ GA}{mL\ solution}}{0.105\frac{g\ extract}{mL\ solution}} = 95.2\ \frac{mg\ GA}{g\ extract}$

DI H_2O extract $\frac{6.50\frac{mg\ GA}{mL\ solution}}{0.105\frac{g\ extract}{mL\ solution}} = 1.9\ \frac{mg\ GA}{g\ extract}$

Total flavonoid content

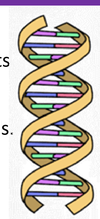
	x value (mg quercetin / mL)	Concentration of TFC in stock extracts (mg quercetin /mL)
MeOH extract	0.0179 ± 0.0004	0.179 ± 0.004
EtOH extract	0.0138 ± 0.0003	0.138 ± 0.003
DI H_2O extract	0.00012 ± 0.00006	0.0012 ± 0.0006

Observations

1. Mobile phase with more acetonitrile seems to elute more chemicals in the extracts.
2. HPLC and spectrophotometric studies suggest the presence of flavonoids, antioxidants, and phenolic acids in Watercress juice and solvent extracts.
3. The Watercress alcohol extracts seems to show more peaks compared to other extracts.

Future Studies

- Identify more individual phytochemicals in Watercress extracts.
- Run phytochemical standards for FTIR and compare to watercress extracts
- Determine if DNA binds to watercress extracts
- Explore the effect of the extract on healthy and diseased mammalian cells.



References
1. Chaudhary, S.; Hisham, H.; Mohamed, D. A Review on Phytochemical and Pharmacological Potential of Watercress Plant. *Asian J. Pharm. Clin. Res.* **2018**, *11* (12), 102. https://doi.org/10.22159/ajpcr.2018.v11i12.29422.
2. *Phytochemical Characterization and Antioxidant Properties of Baby-Leaf Watercress Produced under Organic Production System Alfredo Aires*; Carvalho, R., Rosa, E. A. S., Saavedra, M. J., Eds
3. Ştef, D. S. Total Antioxidant and Radical Scavenging Capacities for Different Medicinal Herbs. *Romanian Biotechnological Letters* **2009**, *14*, 4705–4710.