Analysis and Characterization of Bioactive Compounds Isolated from *Humulus lupulus*

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Abstract

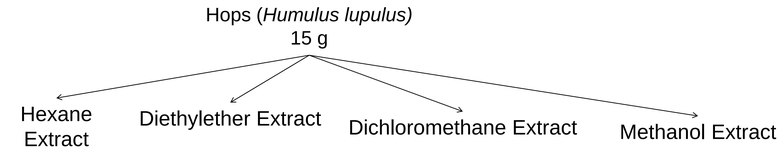
Bioassay guided fractionation of plant materials is a powerful technique that has lead to the discovery of many powerful contemporary medicines including anti-inflammatory medicines, and anti-angiogenics. The promise of cheap, powerful drugs has fueled this broad area of research. In this experiment, fractions prepared from the model plant, *Humulus lupulus*, were studied in depth using two separate anti-oxidant assays (DPPH and ABTS). Also included in the following study were four unknown pure compounds. Initial testing showed that, in general, the most active fractions from *H. lupulus* were those prepared in extremely polar or extremely non-polar solvents (PTT-9A = 0-60% free-radical compared to control, PTT-14A = 17-80% free-radical compared to control) . Furthermore, some fractions showed activity across a wide range of concentrations (500, 100, 50, and 10 ug/ml). These data suggest that some of the compounds isolated via sequential fractionation may be potent anti-oxidants. Investigation of the selected pure compounds showed varied activity at low concentrations (50 and 10 uM). This implies that the compounds tested were not potent radical scavengers. Furthermore, many of the pure compounds tested actually enhanced the production of free-radicals (A11i = 110% free-radical compared to control, A11j = 120% free-radical compared to control).

Introduction

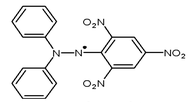
- Bioassay guided fractionation of plant materials has led to the discovery of a broad range of contemporary medicines:
  - Antibiotics
  - Cytotxic anti-tumor agents
  - Anti-angiogenics
- The promise of powerful drugs from common plants has fueled a strong new area of research
- Furthermore, the promise of cheap compounds isolated from common plants has gotten the interest of huge pharmaceutical companies which usually spend millions of dollars researching and synthesizing drugs
- The main body of the research presented in this study will be testing compounds fractioned from *Humulus lupulus*, or hops, used in the brewing of beer
- Xanthohumol was the most effective anti-proliferative agent in human breast cancer cells (MCF-7), colon cancer cells (HT-29) and ovarian cancer cells (A-2780)
- This study will focus on the anti-oxidatative capabilities of hops by applying common, straightforward colorimetric assays to sequentially extracted compounds from hops; unknown pure compounds will also be tested with the ABTS assay
- The next step of this study will extend our testing to anti-inflammatory activity through celluar assays which study the inhibition of inflammatory mediators such as:
  - COX-2
  - iNOS
- This may be a promising study as other anti-inflammatory mediators have already been isolated from hops, such as Xanthahumol
- Ethnopharmacological studies have become popular in recent years in an attempt to build thorough libraries of compounds in a variety of plants that have been used as medical treatments for thousands of years
- However, few of these studies are accompanied by bioassays especially assays testing for common and powerful medicinal effects such as antioxidative and anti-inflammatory activity

Methods

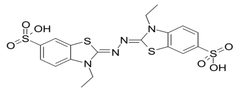
- Sequential fractionation of hops was performed according to this reaction scheme (from left to right with increasing polarity)



- 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) was used to quantify anti-oxidant capability of higher concentrations of crude compounds; it’s color change scheme and chemical structure are seen below:

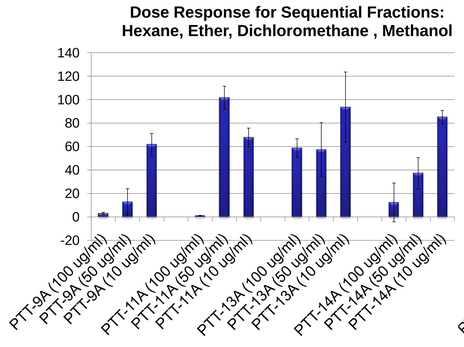


- 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) was employed to quantify the anti-oxidant capabilities of low concentrations of crude compounds as well as unknown pure compounds: it’s color change scheme and chemical structure are seen below:

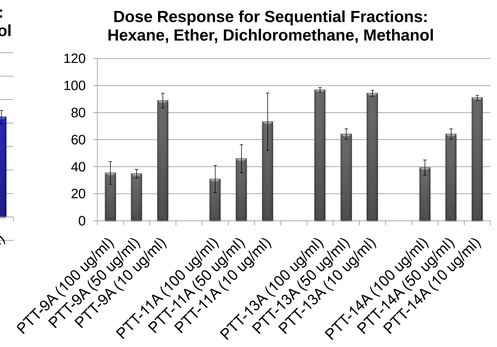


Results

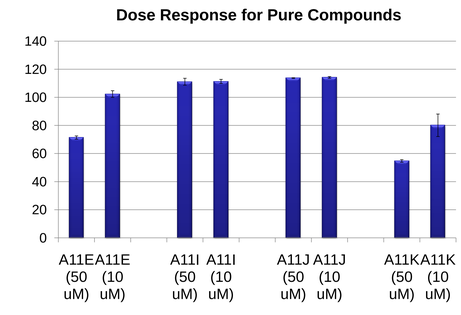
ABTS

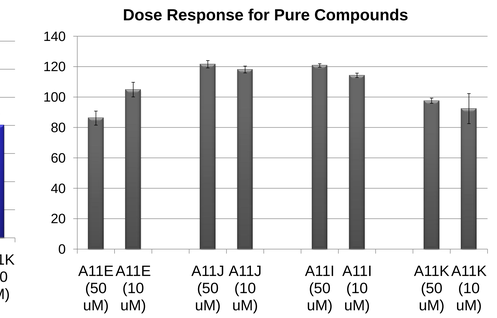


DPPH



*9A = Hexane, 11A = Ether, 13A = Dichloromethane, 14A = Methanol





Discussion

- Most of the crude fractions tested showed some measure of anti-oxidant activity in the DPPH tests at high concentrations
- ABTS testing of low concentrations of the crude fractions was a mixture of strong activity and no activity
- There was, however, a clear pattern among the crude fractions tested: the most active compounds were contained in the most polar and least polar fractions
- For the pure compounds low concentrations were selected because, in theory, there were no other compounds to obscure the anti-oxidant activity
- None of the pure compounds were as active as select crude fractions, however, relatively strong activity was seen in A11e
- A11k may seem to be an active-radical scavenger, however it was inconsistent between ABTS and DPPH
- Furthermore, most of the pure compounds actually enhanced the production of free radicals
- It is clear, even in these initial trials, that there is anti-oxidant potential in hops
- Further purification of the crude compounds mentioned in this poster will allow for a better understanding of the anti-oxidative potential of hops

Acknowledgments

- Summer Science Student Research Program – funding, housing, and research opportunity
- Dreyfuss Grant – funding for reagents, tools and instrumentation
- Kenyon College Department of Chemsitry

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