

Quantification of Kaempferol Conjugates in Watercress Juice and Methanol Extract: A Study Using HPLC and Protein Binding

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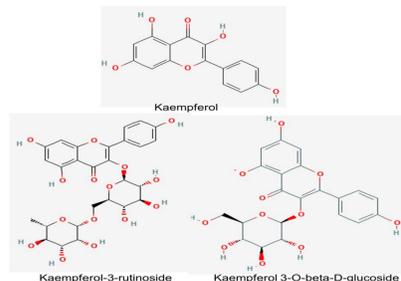
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Background

Flavonoids are a large group of biologically active polyphenolic compounds found in plants, that have gained importance in recent years due to their potential medicinal and therapeutic properties of high potency and low systemic toxicity. Kaempferol (3,4',5,7-tetrahydroxyflavone) is a polyphenol antioxidant dietary flavonoid found in a variety of fruits and leafy vegetables, specifically vegetables of the *Brassica* family, which includes our subject vegetable, watercress (WC) which is usually grown in marshy lands. Kaempferol (KMP) has been depicted to have revolutionary attribute in overall human health ranging from anti-cancerous to anti-inflammatory properties. Here we show a novel approach for quantifying kaempferol and its derivatives in watercress juice (WC2) and methanol extract (WCM) using HPLC and protein binding studies. Human serum albumin (HSA) is chosen as the model protein.



CHEMICAL STRUCTURE OF KAEMPFEROL AND IT'S DERIVATIVES

Objectives and Approaches

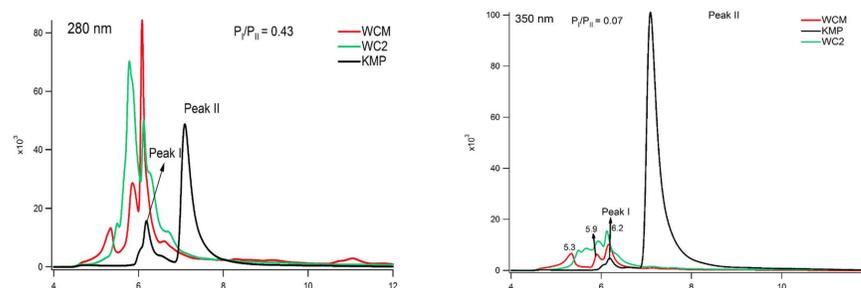
Hypothesis: Watercress has a high concentration of KMP, and the methanol extract contains more KMP than watercress juice.

- Our main objective is to quantify the amount of Kaempferol and its conjugates in Watercress Juice and extract.
- **The approaches taken were:**
 - HPLC of Juice and extract with KMP
 - Study of binding of juice and extract with Human Serum Albumin using optical spectroscopy and computational chemistry.

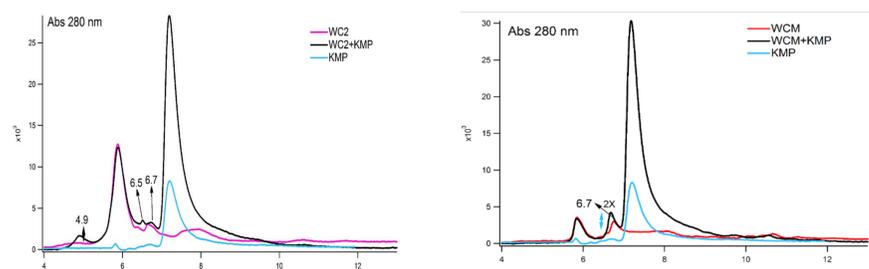
Experimental Techniques

- **HPLC (Jasco 4000) Flow Rate:** 0.5mL/min **Mobile Phase:** (50%ACN/30%MeOH/20%H₂O w/1% acetic acid).
- **UV/Vis Absorption Spectroscopy:** Shimadzu UV 2550 spectrophotometer.
- **Fluorescence Spectroscopy** PerkinElmer 6500 fluorimeter. Excitation and emission slit widths were 10/10 nm unless specified.
- **AutoDocktools4.0** was used for computational docking studies.

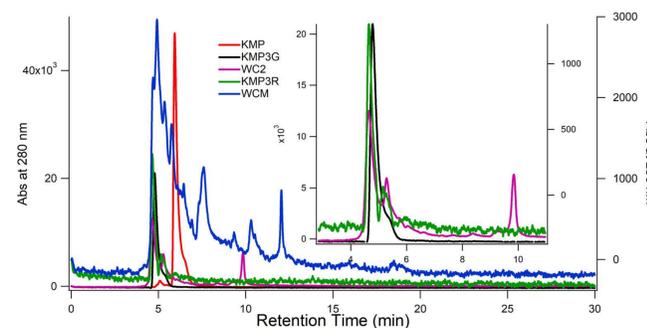
HPLC Analysis of Watercress Juice and Methanol Extract



Observations: The absorption of Peak II (PII) increases at 350 nm compared to 280 nm which indicated that (PII) is the KMP flavone which absorbs at ~350-370 nm

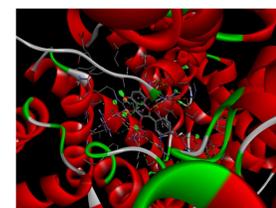
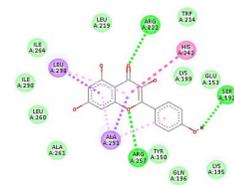


Observations: In both chromatograms WCM+KMP and WC2+KMP have a higher intensity in comparison to WCM and WC2.



Observations: 1. WC juice has more KMP3R compared to KMP3G. 2. WCM has more phytochemicals than WC2.

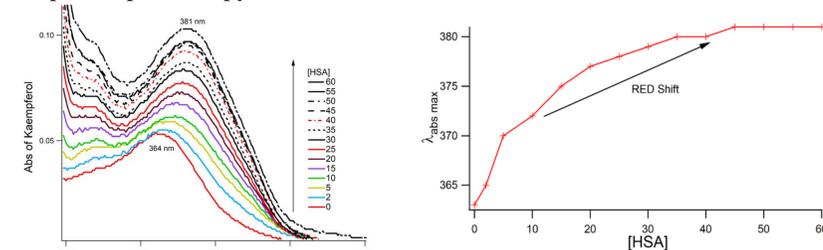
Docking Of Kaempferol and HSA



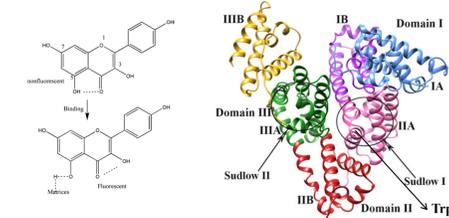
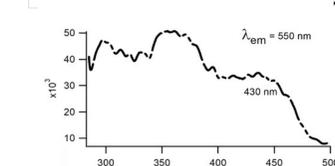
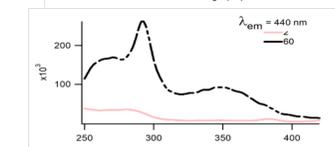
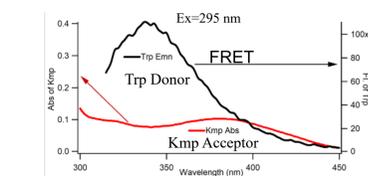
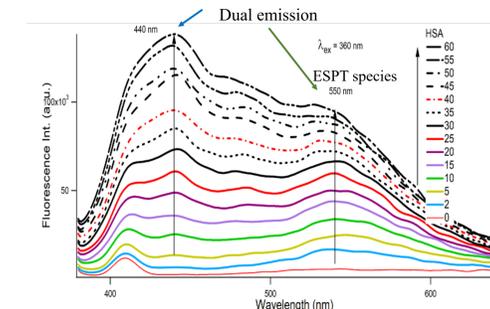
Observations: Kaempferol makes 3 H-bonds with 3 polar amino acids which corroborates with our absorption studies. Trp 214 is in close proximity of Kaempferol, which supports the fluorescence results

Protein binding studies

Optical Spectroscopy



With increasing [HSA], Kaempferol absorption increases, along with the absorption max wavelength shifts towards the right.



With the increase of [HSA], the fluorescence KMP also increased alongside displaying two distinct emission band regions.

Summary and Conclusions

1. HPLC studies suggest the presence of Kaempferol derivatives in Watercress juice and methanol extract.
2. The Watercress water extract seems to contain more of these derivatives compared to the methanol extract.
3. Fluorescence spectroscopic studies suggest the binding of Kaempferol with HSA at region close to Trp 214.

Future Studies

- Carry out CD spectroscopy study on HSA protein with Kaempferol, Watercress juice and extract and study the structural difference in the protein if any.
- Study the binding of Watercress Juice and Extract with HSA protein and compare with Kaempferol and its derivatives.
- Quantify Kaempferol derivative in Watercress Juice and Extract.

Acknowledgement:

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References

1. Wang, L.; Mei, Q.; Wan, D. Simultaneous Determination by HPLC of Quercetin and Kaempferol in Three *Sedum* Medicinal Plants Harvested in Different Seasons. *Journal of Chromatographic Science* **2014**, *52*(4), 334–338.
2. Mizzi, L.; Chatzitzika, C.; Gatt, R.; Valdramidis, V. HPLC Analysis of Phenolic Compounds and Flavonoids with Overlapping Peaks. *Food technology and biotechnology* **2020**, *58*(1), 12–19
3. <https://www.britannica.com/plant/watercress>