

Different Approaches for Quantification of the Bioactive *Metastable Thiosulfinates* in Garlic Using a Validated High Performance Liquid Chromatography Method

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ABSTRACT

Aims:

In this study *Allium sativum* extracts were evaluated for antibacterial activity, High Performance Liquid Chromatography method was developed and validated for direct quantification of alliin and indirect quantification of metastable bioactive thiosulfinates. Study Design: The experimental research design composed of bioassay using Disc-diffusion method and chemical assay which involved differential quantification of bioactive metastable thiosulfinates in *Allium sativum* using a validated HPLC method. Original Research Article 2 Place and Duration of Study: Chemical Engineering Laboratory, School of Engineering, Moi University, Eldoret, Kenya. The study duration was from June 2015 to August 2016.

Methodology:

Antibacterial activity conducted for gram-positive *Staphylococcus aureus* and *Pseudomonas aeruginosa* while *Escherichia coli* was bioassayed as the gram-negative bacteria.

The bioactive metastable thiosulfinates were differentially quantified using HPLC method through direct quantification of alliin.

Results:

The highest percent yield of 0.32% was realized from a garlic cloves blend marc of 25 g. The activity of the extracts was noted to be dependent on the duration of time after reconstitution but before assaying. This was revealed from evaluation of antibacterial activity constancy which indicated that the garlic extract exhibited a pattern of decreasing zone of inhibition from above 14 mm at 3 hours reducing to 6 mm after 24 hours. The HPLC method developed enabled elution of alliin at 2.5 minutes illustrating high levels of accuracy from the calculated mean percent recovery \pm SD that ranged from 99.06 ± 0.08 to 99.56 ± 0.11 and 99.08 ± 0.12 to 99.34 ± 0.03 for the inter-day and intra-day respectively which is regarded optimum for the method application. The data obtained for quantification is in agreement with the results of bioactivity constancy evaluation in that as the bioactivity of the garlic extracts diminishes with time after extraction, so the % bioactive thiosulfinates falls along the time intervals from 22.9% at 0 hour to 10.0% after 24 hours.

Conclusion:

The devised method for differential quantification of bioactive thiosulfinates proved to be valid and accurate hence applicable for evaluating the metastable bioactive constituents of garlic extracts

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