A RAPID METHOD BASED ON UV SPECTROPHOTOMETRY FOR QUANTITATIVE DETERMINATION OF ALLICIN IN AQUEOUS GARLIC EXTRACTS

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ABSTRACT
Garlic *(Allium sativum)* is known for its medicinal and health food use. However, garlic extracts are also used in bio-pesticides. This study reports research on the optimisation of a fast and cheap method for assaying allicin, the active constituent of garlic extract, based on UV spectrophotometry. Garlic cloves were extracted using water. The allicin content of the garlic extracts was analysed after passing the extract through SPE cartridge and eluted with solvents of various polarities. The most polar solvent used was water which eluted the allicin in 4 ml, while methanol and ethanol did not. The absorbance of UV radiation at 240 nm and 254 nm wavelengths by the garlic fraction eluted with water gave a ratio of A240 nm/ A254 nm H" 1.4 – 1.5, which is typical for allicin.The garlic available in the Kenyan market was assayed for allicin using the optimised method. Some pesticides in the market containing garlic were also assayed for allicin. The results obtained compared well with documented values.

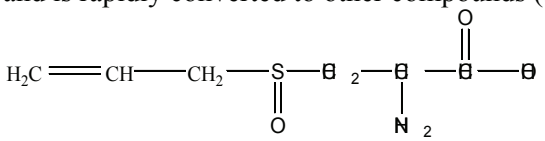
Key words: UV spectrophotometry, high performance liquid chromatography, allicin, garlic cloves

1.0 INTRODUCTION

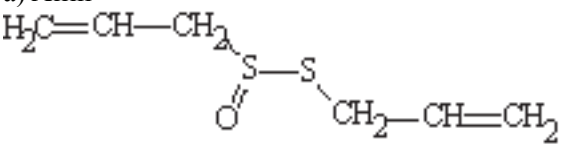
Garlic *(Allium sativum)* is a bulb crop found in the family of onions, chives and shallots. It is believed to have originated from central Asia and its use as a condiment and for medical purposes predates written history.

Garlic has long been known to have uses in pest control for its repellent effects. its primary use is in flavouring foods. Garlic is stated to possess diaphoretic, expectorant, antispasmodic, antiseptic, bacteriostatic, antiviral, hypotensive and anthelmintic properties, and to be a promoter of leukocytosis. Traditionally, it has been used to treat chronic bronchitis, respiratory catarrh, recurrent colds, whooping cough, bronchitic asthma and influenza (Astal, 2003; Abdulkadir *et al*., 2005). Modern use of garlic and garlic preparations is focused on their antihypertensive, anti-atherogenic, antithrombotic, antimicrobial, fibrinolytic, cancer preventive and lipid-lowering effects (Mathew and Biju, 2008).

Most of its therapeutic value, as well as its flavour and odour are attributed to suphur compounds contained within the garlic clove. One such compound is the amino acid, alliin (Figure 1a). Once a clove is cut or crushed, alliin is converted through an enzymatic reaction with aliinase to allicin (Figure 1b), the main contributor to garlic's widespread medicinal and anti-microbial benefits. Allicin is a very unstable compound and is rapidly converted to other compounds (Sendl, 1995; Jiben *et al*., 2006).



a) Aliin



b) Allicin

Figure 1: Structures of garlic extract active constituents

Extracts and oils from garlic bulbs have been formulated into a range of pest control products marketed as crop protection products for use against many pest organisms. However, these products are not without their limitations, with a lack of consistency of effect being the most serious. For example, Flint *et al*. (1995) compared the effects of two commercial products, Garlic Barrier Ag_ and ENVIRepel with those of chopped garlic extract and steam-distilled garlic oil, on the silver leaf whitefly *Bemisia argentifolii*. Garlic oil sprays and ENVIRepel gave no protection, whereas a 10% solution of Garlic Barrier Ag_ reduced insect numbers and a 10% solution of chopped garlic extract provided the best protection. Other studies provide conflicting results (Liu and Stansly, 1995). It is possible that between batches variation in the efficacy of these products is responsible for such results.

Various components of garlic and aged garlic extract, including allicin, S-allylcysteine (SAC) and volatile metabolites of allicin have been determined in various matrices by high performance liquid chromatography (HPLC), gas chromatography (GC), HPLC–mass spectroscopy (HPLC-MS) and GC–MS (Robert *et al*., 2001). Lawson *et al*. (1991) employed a HPLC method to quantify allicin and other thiosulfinates in garlic clove homogenates. The methods reported in literature are tedious and require expensive instruments. For quick quantification of allicin in a garlic extract in an industrial environment, fast and cheap methods would be easily adopted. It was therefore the objective of this study to provide a fast and cheap method based on UV spectrophotometry for determination of allicin in aqueous garlic extracts.

2.0 MATERIALS AND METHODS

2.1 Sample Preparation

Garlic, originating from South Africa, Malaysia and parts of Kenya were purchased from different market outlets. The outer skins of garlic cloves were peeled and the cloves crushed in a mortar using a pestle. 1.0 g of the fine garlic mash was soaked in 10 ml of cold (refrigerated) distilled water and put in a refrigerator (< 5^{o}C). After 24 h, the garlic solution was filtered through Whatman No. 42 filter papers under vacuum. The filtrate was preserved in a refrigerator (< 5^{o}C).

2.2 Solid Phase Extraction (SPE) Optimisation and UV Absorbance Measurements

C_{18} Sep pak cartridges were conditioned by rinsing with 5 ml methanol and then equilibrated with 10 ml of water. Approximately 1 ml of the garlic extract solution was introduced to the top of a cartridge and eluted with varying volumes of cold distilled water, ethanol, methanol, methanol/ water (2:3 v/v), methanol/ water (1:1 v/v) and methanol/ water (3:2 v/v) after adjusting the flow rate to about 1-2 ml min^{-1}. The fractions were collected in test tubes placed in an ice bath and their absorbances against eluting solvent was measured at 240 nm and 254 nm in a 1cm quartz cuvette using a UV–VIS Spectrophotometer (Shimadzu model UV – 1601 PC).

2.3 HPLC Fingerprinting of Crude Garlic Extract and SPE Fraction

A HPLC instrument Shimadzu liquid chromatograph with an LC–10 AD VP Shimadzu pump, SPD - 10 A VP Shimadzu UV/ VIS detector was used. The peaks were recorded in a Shimadzu chromatopac C-R8A integrator. An analytical column Shim pack VP-ODS (250 x 4.6 mm i.d, 5 µm) was used for the analysis. Aliquots of crude garlic extract and the SPE fractions were separately filtered through 0.45 µm syringe filters before injection into the HPLC column. The HPLC mobile phase was prepared by combining equal volumes of HPLC grade methanol and distilled water; the mobile phase was degassed in ultrasonic bath (Shimadzu) for 30 minutes. The HPLC conditions were as follows: column temperature, 28^{0}C; λ , 240 nm; flow rate, 0.8 ml min^{-1}; sample, 10 µl; run time, 15 minutes; attenuation, 2; chart speed, 2 mm min^{-1}.

2.4 Determination of Allicin Concentration in Garlic Cloves and Some Garlic Based Botanical PesticideProducts

Aqueous garlic extracts were prepared from the cloves as described in the sample preparation section. Allicin concentration of the extracts and the diluted (10 times with cold distilled water) garlic based pesticide products was analysed using the optimised SPE method and UV spectrophotometry. The eluting solvent for SPE was 4 ml of cold distilled water. The procedure was followed as described in the SPE optimisation method.

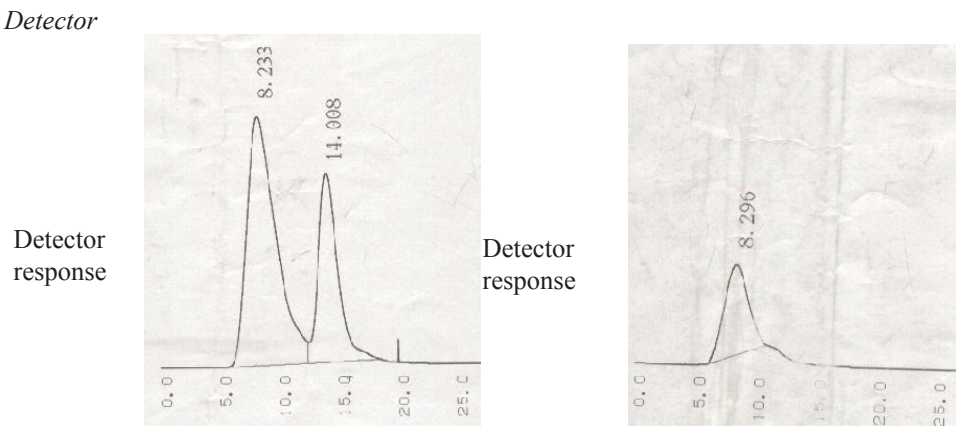
3.0 RESULTS

The SPE optimisation data for garlic extracts prepared as described previously and subjected to sample cleanup using SPE–C_{18} cartridges is presented in Table 1 and Figure 1.

Table 1: UV absorbance (A240 nm/ A254 nm) ratio of SPE eluants

	UV absorbance (A240 nm/ A254 nm) ratio				
	Eluting volume (ml)				
Mobile Phase	2.0	3.0	4.0	5.0	6.0
Water	1.43 ± 0.02	1.43 ± 0.02	1.46 ± 0.01	1.56 ± 0.01	1.65 ±0.006
Ethanol	1.55 ± 0.006	1.59 ± 0.006	1.70 ± 0.01	1.73 ± 0.01	1.80 ± 0.02
Methanol	2.03 ± 0.01	2.20 ± 0.01	2.34 ± 0	2.35 ± 0.006	2.50 ± 0.006
MeOH/ H_2O (2:3 v/v)	1.93 ± 0.006	1.93 ± 0.006	1.95 ± 0.01	2.01 ± 0.01	2.33 ± 0.02
MeOH/ H_2O (1:1 v/v)	1.66 ± 0.02	1.67 ± 0.01	1.69 ± 0.02	1.70 ± 0.01	1.78 ± 0.01
MeOH/ H_2O (3:2 v/v)	1.55 ± 0.01	1.58 ± 0.01	1.60 ± 0.006	1.63 ± 0.006	1.65 ± 0.01

Each value is a mean ± s.d. (n = 3).



a) Elution time (min) b) Elution time (min)

Figure 1: HPLC chromatograms of a) Aqueous garlic extract and b) SPE fraction of the aqueous garlic extract

The amounts of allicin in the garlic cloves originating from various geographical zones in Kenya and in the world are as given in Table 2. The allicin content was found in all the cases to be within the range reported in literature.

Table 2: Allicin concentration of aqueous garlic extracts

Origin of garlic bulbs	Concentration (µg/ ml)	% allicin in garlic bulb
~~Kenya – (Unknown source)~~	~~1609.4 ± 27.6a~~	~~0.40~~
Kenya – Mt. Kenya region	1650.6 ± 13.8a	0.41
Kenya – Isiolo region	1570.4 ± 19.8a	0.39
South Africa (source A)	1804.2 ± 4.0b	0.45
South Africa (source B)	1742.3 ± 10.5c	0.44
Malaysia (source A)	1756.1 ± 8.0c	0.44
Malaysia (source B)	1815.8 ± 6.9b	0.45

- ~~Source A and source B represents garlic from different vendors.~~
- Each value is a mean ± s.d. (n = 4).
- Means followed by the same letters are not significantly different at p= 0.05.

The concentration of allicin in selected commercially available garlic pesticide formulations is given in Table 3.

Table 3: Allicin concentration of some garlic based botanical pesticide products

Product	Allicin concentration (μg/ml)
Batch 1 – original protocol	258.6 ± 9.3a
Batch 3	645.7 ± 0.6b
Pyegar (A)	844.2 ± 3.6c
Pyegar (B)	953.1 ± 2.3d
Pyegar (C)	842.6 ± 1.3c
GC – 3	325.5 ± 2.7e

Each value is a mean ± s.d. (n = 4).
Means followed by the same letters are not significantly different at p= 0.05.

4.0 DISCUSSION

Reported research work shows that the absorbance ratio of allicin measured against water at wavelengths 240 nm and 254 nm respectively gives typical values in the range 1.4 to 1.5 (Han *et al*., 1995). A HPLC chromatogram of an aqueous extract of garlic bulbs is shown in Figure 1 (a). The absorbance ratio measured separately for each peaks gave values of 1.4 – 1.5 for peak with Rt ≈ 8.30 ± 0.25 min and 1.4 - 1.5 for peak with Rt ≈14.01 min. This shows that the peak eluting after 8.35 min was allicin.

≠ SPE clean-up provides a quick and cheap method for preconcentration and clean up of samples before analysis. SPE has been used in clean-up of samples for pesticide analysis, in pharmaceuticals, environmental analysis etc. SPE may be used either to retain the compound (s) of interest eluting the undesirable compound or by changing the solvent to prefentially elute the compounds of interest or vice versa.

The SPE optimisation data is presented in Table 1. From thenTable, the absorbance ratio of 1.4 to 1.5 was achieved with water as the eluent. Absorbance ratios that were not within the required range indicate presence of other compounds different from allicin. The maximum eluting volume which gave the correct absorbance ratio was found to be 4 ml.

4.1 Calculation of Alicin Concentration

The concentration (C) of allicin for SPE chromatography elutes that gave an absorbance ratio (A240 nm/ A 254 nm) of 1.4 – 1.5 was calculated as given below. The extinction coefficient (E) for allicin in water is 145.4 (1 cm cell @ 240 nm).

E_{1cm} = (Absorbance) (10000) / C (μg/ml)
Thus,
C (μg/ml) = Absorbance @ 240 nm (10000) / 145.4
(Han *et al*., 1995).

4.2 Allicin Concentration in Garlic Cloves

The amounts of allicin in the garlic cloves originating from various geographical

zones in Kenya and in the world are as given in Table 2. From Table 2, the allicin concentration of South Africa and Malaysia garlic were found to be significantly different at 95% confidence interval from the Kenyan garlic. Garlic from various regions of Kenya was found to be of comparable quality. Garlic cloves from South Africa and Malaysia were also found to be of comparable quality. The higher levels of allicin concentration in South Africa and Malaysia garlic as compared to Kenyan garlic imply that the former is of higher quality. The differences in allicin concentrations may be due to the different climatic conditions under which garlic is grown in the different regions and/ or the different varieties of garlic that exist. Iber *et al*. (1990) found significant variation in the amount of allicin depending on the variety and/or preparation of garlic; they found the allicin content of fresh bulbs to vary between 0.12 % and 0.53 %. Commercial supplement tablets varied from 0.006% to 0.26% allicin and garlic powder ranged from 0.06% to 0.69% allicin.

4.3 Allicin Concentration in Some selected Garlic-based Pesticide Formulations

Allicin was assayed in selected commercially available garlic pesticide formulations. Table 3 gives the concentration of allicin in some garlic based pesticide formulations; batch 1–original protocol and GC–3. Unformulated garlic extracts; batch 3, Pyegar (A), Pyegar (B) and Pyegar (C) from a local bio-pesticides manufacturer were also assayed for allicin and the results presented in Table 3. From Table 3 allicin values for the different extracts and products varied. However, the allicin concentration of Pyegar (A) and Pyegar (C) were not significantly different at 95% confidence interval. This implied reproducibility in garlic extract preparation. The difference in allicin levels in the unformulated garlic extracts may be attributed to difference in garlic quality and/ or possibly due to application of different extraction parameters. The difference in allicin concentration between the two garlic based products; batch 1–original protocol and GC–3 is not necessarily as a result of the difference in garlic quality used in each case but may be due to the difference in initial concentration of allicin in the extracts used in the formulations plus the effect of adjuvants.

5.0 CONCLUSION

A fast and cheap method for the determination of allicin in garlic bulbs using UV spectrophotometry was optimised. The method could be used to carry out quality control of garlic bulbs; monitor batch consistencies in garlic based formulations and generally on standardisation of the garlic based formulations.

A study to improve the effectiveness of garlic based products will benefit agricultural sectors of developing countries, as these substance are not only of low cost, but also have less environmental impact in term of pesticidal hazard.

ACKNOWLEDGEMENTS

The authors acknowledge the funding of their research by Juanco SPS Ltd. Special

thanks goes to Paul Karanja, Food Biochemistry Laboratory, JKUAT for his technical assistance.

REFERENCES

Abdulkadir A., Chindo I. E., Azuka T. H. M.,Binta I. and AminuY. B. (2005). Effect of Different Aqueous Extracts of Garlic on Some Electrolytes and Urea Levels in Rats.

Journal of Pharmacy and Bioresources, **2**(1), pp 1-4.

Astal A. (2003). The Inhibitory Action of Aqueous Garlic Extract on the Growth of Certain Pathogenic Bacteria. *East and Central African Journal of Pharmaceutical Sciences,* **6**(1), pp 9-14.

Flint H. M.,Parks N. J., Holmes J. E., Jones J. A. and Higuera C. M. (1995). Tests of Garlic oil for the Control of the Silverleaf Whitefly, Bemisia Argentifolia Bellows and Perring (Homoptera:Aleyrodidae) in Cotton. *Southwestern Entomologist,* **20**, pp 137–150.

Han J., Lawson L., Hand G. and Han P. A. (1995). A Spectrophotometric Method for Quantitative Determination of Allicin and Total Garlic Thiosulfinates. *Biochemistry* **225**, pp 157–160.

Iber I., Winkler G., Muller B. and Knobloch K. (1990). Quantitative Determination of Allicin and Alliin from Garlic by HPLC. *Planta Medica,* **56**, pp 320-326.

Jiben R., Diaa, M. S., Patrick S. A.and John G. T. (2006). Chemical Constituents and Antimicrobial Activity of a Traditional Herbal Medicine Containing Garlic and Black cumin. *African Journal of Traditional, Complementary and Alternative Medicines,* **3** (2) pp 1–7.

Lawson L. D.; Wood S. G. and Hughes B. (1991). HPLC Analysis of Allicin and other Thiosulfinates in Garlic Clove Homogenates. *Planta medica,* **57**,pp 263-270.

Liu T. X. and Stansly P. A. (1995). Toxicity and Repellency of some Biorational Insecticides to Bemisia Argentifolia on Tomato Plants. *Entomologia Experimentalis et Applicata,* **74**, pp 137-143.

Mathew, B. C. and Biju, R. S. (2008). Neuroprotective Effects of Garlic. *Libyan Journal of Medicine,* **3**(1), pp 16–20.

Robert T. R., Richard D. H., Elaine K. F., Reginald J. R., Zhengy, Z., Joseph L., Sharon L. R.and Thomas G. H. (2001). Determination of Allicin, S – Allylcysteine and Volatile Metabolites ofGarlic in Breath, Plasma or Simulated Gastric Fluids. *Journal of nutrition,* **131**, pp 968S-971S.

Sendl A. (1995). *Allium Sativum* and *Allium Ursinum*: Part 1. Chemistry, Analysis, History, Botany. *Phyto Medicine,* **4,** pp 323-339