Quantification of allicin by high performance liquid chromatography-ultraviolet analysis with effect of post-ultrasonic sound and microwave radiation on fresh garlic cloves

Sankhadip Bose, Bibek Laha[1], Subhasis Banerjee[2]

Departments of Pharmacognosy, [1]Pharmaceutics, and [2]Pharmaceutical Chemistry, Gupta College of Technological Sciences, College of Pharmacy, Asansol, West Bengal, India

Submitted: 30-07-2013 Revised: 29-08-2013 Published: 28-05-2014

ABSTRACT

Background: Garlic (*Allium sativum* L.) has been accepted universally to be applied in food, spice and traditional medicine. The medicinal and other beneficial properties of garlic are attributed to organosulfur compounds. **Objective:** As of today no simultaneous analysis has been performed; hence the transformation of allicin to its degraded products during cultivation and storage is open into doubt. **Materials and Methods:** In our present work, we have tried to develop a sensitive and reproducible analytical method to measure allicin by high performance liquid chromatography-ultraviolet analysis with effect of post-acoustic waves and microwave radiation on fresh garlic cloves. **Results:** The process revealed the effect of different radiation techniques on fresh garlic retains the principle component, allicin in its pure form and generated higher yield than the conventional way of extraction. **Conclusion:** Therefore, materializing these techniques in the pharmaceutical industry will definitely be proved beneficial in term of time as well as money. Most interestingly, the methods ruled out possibilities of degradation of organosulfur compounds as well.

Key words: Acoustic waves, allicin, garlic, high performance liquid chromatography-ultraviolet analysis, microwave radiation

Access this article online

Website: www.phcog.com

DOI: 10.4103/0973-1296.133279

Quick Response Code:

INTRODUCTION

Garlic (*Allium sativum* Linné) is a widely distributed plant and is used in all parts of the world not only as a spice and food, but also as a popular remedy.[1] Numerous studies have previously demonstrated that garlic may be useful for the prevention of carcinogenesis, cardiovascular and age-related diseases.[2] Especially, it has been strongly suggested that its medicinal and beneficial properties are attributed to specific organosulfur compounds.[3-6] It is known that garlic contains three γ-glutamyl peptides, that is, γ-L-glutamyl-*S*-(2-propenyl)-L-cysteine, γ-L-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine and γ-L-glutamyl-*S*-methyl-L-cysteine; their corresponding sulfoxide derivatives, viz., (+)-*S*-(2-propenyl)-L-cysteine sulfoxide (alliin), (+)-*S*-(*trans*-1-propenyl)-L-cysteine sulfoxide (isoalliin), (+)-*S*-methyl-L-cysteine sulfoxide (methiin) and (1*S*,3*R*,5*S*)-5-methyl-1,4-thiazane-3-carboxylic acid 1-oxide (cycloalliin) [Figure 1].

Sulfoxides have been reported to have some medicinal properties; for example, alliin shows anticancer effects[4,5] and alliin and cycloalliin present lipid-lowering effects.[3,5] In contrast, γ-glutamyl peptides have been reported to lower blood pressure[4-6] and to have cholesterol-lowering effect.[4,6] However, once garlic is crushed, these compounds are transformed into other compounds such as allicin, ajoene, dithiins and diallylpolysulfides.[6,7] Therefore, it is important to control sample preparation to minimize artificial errors. The biosynthetic pathway of organosulfur compounds in garlic has been proposed to involve transformation of γ-glutamyl peptides into their corresponding sulfoxides by γ-glutamyl transpeptidase and oxidase.[8-10] Moreover, it has been reported that, the amount of organosulfur compounds do not remain same during cultivation and storage[10-13] [Figure 2].

Address for correspondence:
Dr. Sankhadip Bose, Department of Pharmacognosy, Gupta College of Technological Sciences, College of Pharmacy, Ashram More, G.T. Road, Asansol - 713 301, West Bengal, India.
E-mail: sankha.bose@gmail.com

Therefore, an analytical method for the simultaneous determination of all sulfoxides and their precursors, ɣ-glutamyl peptides, in a garlic sample is required to evaluate the quality of garlic even after different cooking or extraction methods where microwave radiation and acoustic waves are used. Our present study aims at determining the allicin content by high performance liquid chromatography-ultraviolet (HPLC-UV) analysis. Chromatograms obtained after normal extraction, microwave extraction, acoustic waves effect by the probe and bath sonication and quantitative result of the amount of allicin in a commercial garlic sample were reported. Microwave radiation and acoustic waves are of importance because many food materials and medical formulations are prepared by using them. Therefore, it is important to determine the stability and quantity of allicin after conducting various extraction processes.

MATERIALS AND METHODS

Sources of reagents

Sodium chloride was purchased from Qualigens, Mumbai, Dichloromethane from Merck Specialties Pvt. Ltd., Mumbai, Methanol and water of HPLC grade were procured from Merck Specialties Pvt. Ltd., Mumbai.

Fresh garlic cloves (*A. sativum* L.) were obtained from the cultivated fields of Asansol area in West Bengal, India which was authenticated (Authentication no. CNH/1-1/(201)/2013/Tech. II/35) by head, Shibpur Botanical Garden, Shibpur, Howrah, West Bengal, India.

Standard allicin

The synthesis of allicin was performed following the method proposed by Bocchini *et al*.[10] According to this, an equimolar amount of perbenzoic acid in 20 ml of dichloromethane, was slowly added to a solution of allyl disulfide (1.46 g in100 ml of dichloromethane), under rapid magnetic agitation while cooling at −10°C. The reaction mixture was allowed to stand at room temperature for 1 h. The excess of acid was removed by washing the mixture with a sodium bicarbonate solution. The dichloromethane solution was rinsed with distilled water, dried over anhydrous sodium sulfate and the solvent was removed by rotary-evaporation. The dried product was weighed and standard solution was made by dissolving the pure allicin in methanol. Data obtained out of HPLC-UV analysis showed that allicin accounted for >99% of the standard.

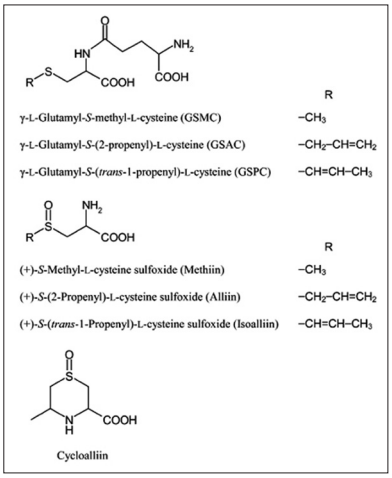

Figure 1: Chemical structure of organosulfur compounds in garlic

Garlic samples

A sample of 30 g of garlic cloves in 300 ml of distilled water were crushed for 1 min in a blender mixer.

Microwave extraction[14]

The above mixture was transformed to a specially designed round bottom flask with which a condenser was fitted. The entire assembly was finally kept inside the microwave extractor (Catalyst System, Pune, Model No. CATA 2R). It was refluxed for 10 min with the percentage power of 20 at 140 watt (41°C) and subsequently processed to get the pure allicin. The generalized purification process has been given just next to all extraction process.

Probe sonication

This was done in a frontline probe sonicator (Frontline, Ahmedabad, Model No. Sonicator FS 600) taking the same amount of mixture that of earlier in an Erlenmayer flask. The process was repeated twice for 20 min with the transducer frequency of 20 kHz at an interval of 10 min between each sonication.

Bath sonication

Bath ultrasonic assisted extraction was carried out in an ultrasonic bath (Enertech Electronics Pvt. Ltd., Mumbai, Model No. 2K 205035). Equal amount of the same mixture was transferred to an Erlenmayer flask and kept in an ultrasonic bath twice for 20 min with the transducer frequency of 20 kHz at an interval of 10 min between each sonication. The temperature was kept constant (25 ± 1°C) by periodical adding of ice in the bath.

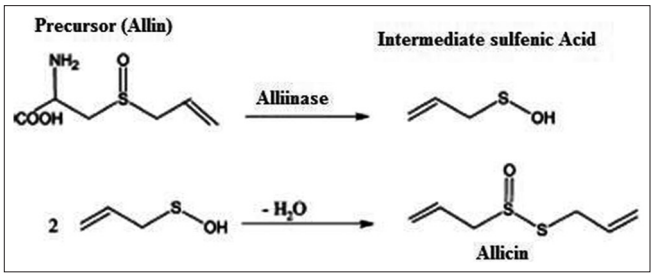

Figure 2: Simplified mechanism of allicin formation

Cold maceration

The earlier amount of the mixture was kept for cold maceration in a well closed container separately for 3 h with frequent mechanical stirring.

Purification of each extract

Each aqueous extract was filtered and diluted to 500 ml with water. The diluted extract (10 ml) was saturated with sodium chloride and subsequently extracted with 10 ml of dichloromethane (3 times). All the fractions were pooled, dried over anhydrous sodium sulfate and dessicated by means of rotary-evaporation.

UV spectroscopic analysis of allicin

A preliminary analysis of standard allicin using a UV-visible detector (Thermo Scientific, UK, Model: Evaluation 201, Sl. No. SA30240002) with spectral range 200-600 nm was made under the chromatographic conditions. The UV spectrum was recorded for each purified extract in order to ascertain the presence of pure allicin.

HPLC analysis of allicin

The HPLC system consisted of the following components: A pump (Lachrom Hitachi, Mumbai, Model no. L-7400, pump no. L-7110), a rheodyne injector (Cotati, CA, USA, Model 7725i), a kromasil C18 column (250 × 4.6 mm i.d., 5 μm particle size). The column was operated in isocratic mode (50:50 MeOH: H_2O) at a flow rate of 0.5 ml/min. Unless otherwise specified, the UV detector was set at 254 nm for all operations.

RESULTS AND DISCUSSION

The λ_{max} obtained of all the extracts including the standard allicin was marginal, i.e. 220 nm. Therefore, the inference can be drawn from the above fact that irrespective of the extraction method, one of the principle component allicin remains unchanged despite of microwave or acoustic waves exposure [Figure 3].

The chromatogram obtained by analyzing a solution of standard allicin (10 μg/ml) was shown [Figure 4]. The response was linear in the range 1-20 μg/ml. The linear regression equation was: y = 98173x, (y = area in arbitrary units, x = concentration in μg/ml), with $R^2 = 1$.

The UV detector response (254 nm), with acoustic waves and microwave radiation effect of allicin, resulted higher than that obtained when standard allicin was used [Figure 5]. The higher response was ascribed to changes in the absorption spectrum due to the presence of more amount of allicin in original state. Figures 6 and 7 clearly demonstrated the newer techniques not only generated a high amount of allicin but also unaltered allicin chemically. Three replicate injections of a standard solution of allicin (10 μg/ml) yielded a relative standard deviation of 1.3%.

The HPLC data given in Table 1 clearly suggested that the allicin was not at all degraded by the effect of ultra-sonic and microwave radiation [Figures 5-7]. Even, from the different area and peak height, it was easily visible that the amount of allicin was more if the probe, bath ultra-sonication and microwave radiation were applied for a specific time as described above.

According to the manufacturer, it may require sonication at the time of preparation of different garlic formulations. However, it was not confirmed earlier that whether that ultra-sonic radiation will create degradation of different organo-sulphur compounds especially allicin or not. Our present study clearly indicates, despite of different extraction methods the

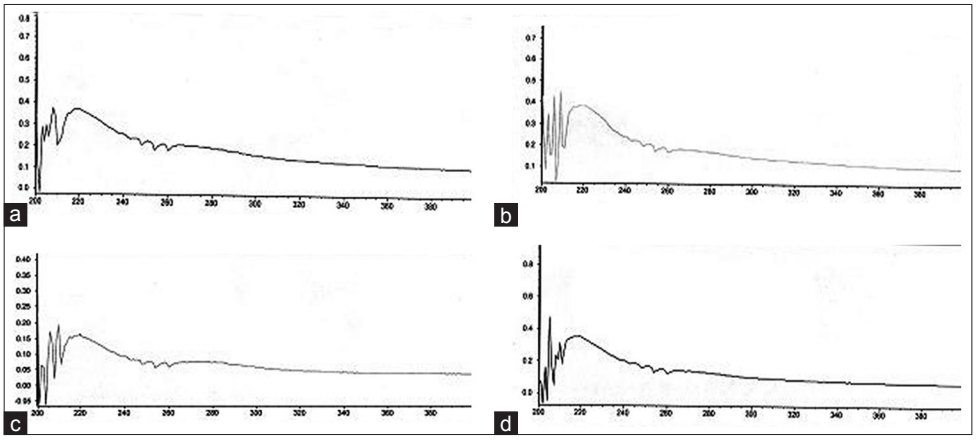

Figure 3: Ultraviolet spectra of allicin from (a) normal maceration extract, (b) probe sonication extract, (c) bath sonication extract and (d) microwave extract. Wave length in X axis and absorbance in Y axis

allicin content was unaltered, the yield was much higher in compare with the conventional way of extraction as well. The above fact in turn supports microwave cooking system too.

Quantitative data on the amount of allicin in garlic were obtained using the standard calibration curve and are summarized in Table 2 as micrograms of allicin per milliliter purified extracts obtained from different sonication and microwave extraction ± standard deviation.

Microscopic studies of normal garlic clove and after treatment of acoustic wave and microwave radiation for 20 min were also performed to observe the fracture of cell wall, which may responsible for getting more amounts of allicin and other organosulfur compounds. The transverse sections was done by using microtome instrument (Model no. SP-1120 from Sipcon Optical Industries, Haryana, India) and was observed under ×40 eyepiece of a projection compound microscope (Model no. 521328 from Magnus, New Delhi, India) and photographs were taken through a microscope attached camera (Model no. SIN S009906M from Moticam of 1.3 megapixel live resolution). It was clearly visible that the cell wall of fresh garlic cloves were dissolved properly in case of ultrasonic and microwave radiations [Figures 8 and 9] than the normal transverse section of garlic [Figure 10].

With different types of radiations so far employed on the extraction of allicin garlic cloves clearly indicated the presence of pure allicin with a higher yield, therefore, materializing these techniques in the pharmaceutical industry definitely prove beneficial in terms of time as well as money. Most interestingly, the methods ruled out possibilities of degradation of organosulfur compounds as well.

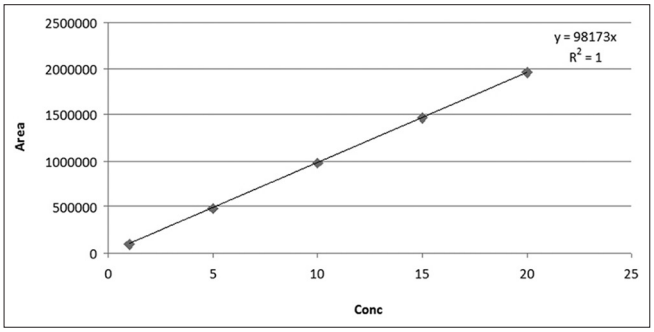

Figure 4: Calibration curve of standard allicin as obtained by high performance liquid chromatography-ultraviolet analysis

ACKNOWLEDGMENT

It's a great pleasure to the authors to express their gratitude to Dr. Kalyan Kumar Sen, Principal, Gupta College of Technological Sciences, Asansol, West Bengal, India for his valuable suggestions and constant support throughout the course of study. The authors are also indebted to Prof. Debesh Chandra Majumdar, Chairman, Trinity Trust, Asansol, West Bengal, India for providing necessary facilities to carry out this work. The grant from All India Council of Technical Education as MODROBS (Ref. No-8024/RIFD/MOD-32 (Pvt.) dated 20.07.2013) for the "Microwave Extractor" is immensely acknowledged. A sense of gratitude goes to the West Bengal University of Technology, Salt Lake, Kolkata for affiliating our Institution.

Table 1: HPLC output of allicin (standard, purified extracts obtained from different sonication and microwave extraction±standard deviation, triplicate analysis)

Component	R_t value (min)	Peak area	RSD (%)	Peak height
Standard allicin	5.07	979821	0.42089	956
Normal maceration extract	5.07	87068	0.40887	252
Probe sonication extract	5.09	816799	0.50704	1953
Bath sonication extract	5.09	935588	0.69736	1872
Micro-wave extract	5.06	837369	0.75486	436

HPLC: High performance liquid chromatography; RSD: Relative standard deviation

Table 2: Quantitative data of allicin (µg/ml purified extracts obtained from different sonication and microwave extraction±standard deviation, triplicate analysis)

Allicin extracts	Quantitative data of allicin (µg/ml)
Normal maceration extract	3.33±0.1
Bath sonication extract	8.32±0.01
Probe sonication extract	9.53±0.02
Micro-wave extract	8.5±0.1

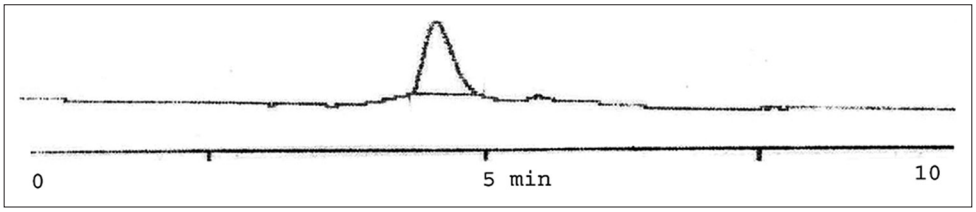

Figure 5: Chromatogram of standard solution of allicin (10 µg/ml) as obtained by high performance liquid chromatography and ultraviolet analyzer

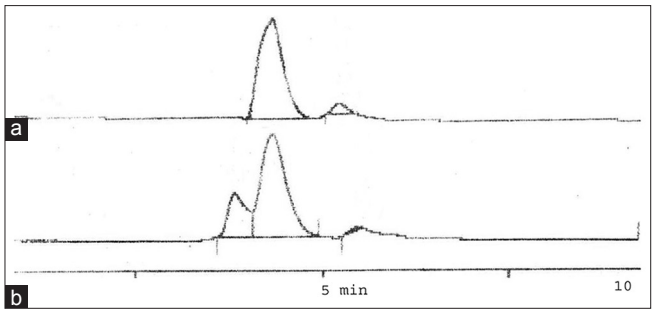

Figure 6: Chromatogram of purified extracts of garlic (10 µg/ml) as obtained by high performance liquid chromatography and ultraviolet detection where (a) bath sonication and (b) probe sonication were applied

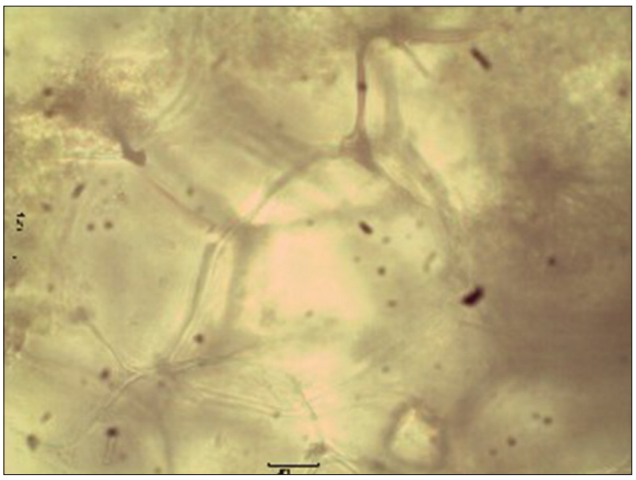

Figure 8: Transverse section of garlic clove after effect of ultrasonic radiation

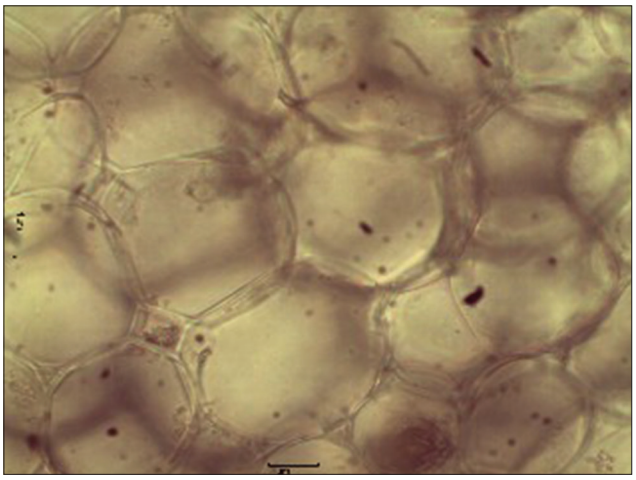

Figure 10: Transverse section of garlic clove in normal condition

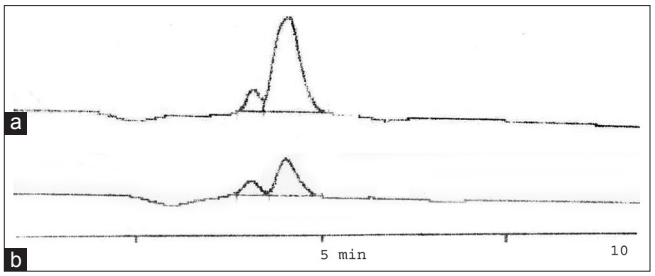

Figure 7: Chromatogram of purified extracts of garlic (10 µg/ml) as obtained by high performance liquid chromatography and ultraviolet detection where (a) microwave radiation was applied and (b) normal maceration was performed

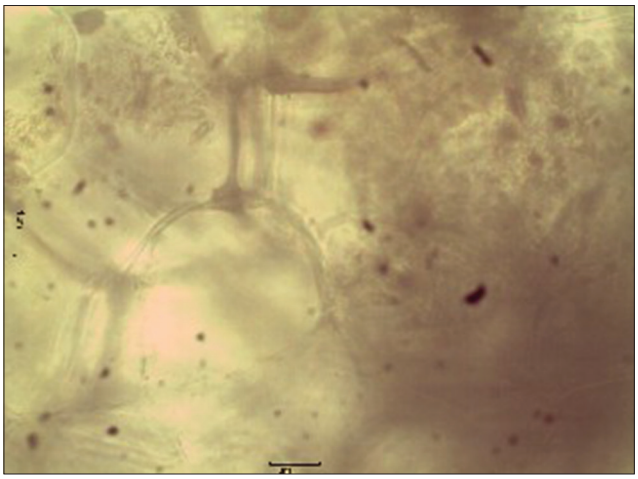

Figure 9: Transverse section of garlic clove after effect of microwave radiation

REFERRENCES

1. Young, NJ, Yong-Hoon C, Chul-Hak Y. Identification of an essential tryptophan residue in alliinase from garlic (*Allium sativum*) by chemical modification. Bull Korean Chem Soc 2001;22:68-76.
2. Hans-Peter K. Interaction of *Allium sativum* L. and *Leontopodium alpinum* cass. with mediators of inflammation involved in the molecular mechanisms of atherosclerosis. Dissertation zur Erlangung des Doktorgrades der Fakultät für Chemie und Pharmazie der Ludwig-Maximilians-Universität München; 2003.
3. Mukherjee M, Das AS, Das D, Mukherjee S, Mitra S, Mitra C. Role of oil extract of garlic (*Allium sativum* Linn.) on intestinal transference of calcium and its possible correlation with preservation of skeletal health in an ovariectomized rat model of osteoporosis. Phytother Res 2006;20:408-15.
4. Raghuveer C. Benificial effect of *Allium sativum* and *Allium tuberosum* on experimental hyperlipidemia and atherosclerosis. Pak J Physiol 2008;4:7-9.
5. Dkhil MA, Abdel-Baki AS, Wunderlich F, Sies H, Al-Quraishy S. Anticoccidial and antiinflammatory activity of garlic in murine *Eimeria papillata* infections. Vet Parasitol 2011;175:66-72.
6. Cheng-Tzu L, Tien-Wei H, Ke-Ming C, Ya-Ping T, Chong-Kuei L, Lee-Yan S. The antidiabetic effect of garlic oil is associated with ameliorated oxidative stress but not ameliorated level of pro-inflammatory cytokines in skeletal muscle of streptozotocin-induced diabetic rats. J Tradit Complement Med 2012;2:135-44.
7. Rahman K. Garlic and aging: New insights into an old remedy. Ageing Res Rev 2003;2:39-56.
8. Lawson LD. Bioactive organosulfur compounds of garlic and garlic products. Role in reducing blood lipids. In: Kinghorn AD, Balandrin MF, editors. Human Medical Agents from Plants, ACS Symposium Series 534. Washington; 1993. p. 34-55.
9. Agarwal KC. Therapeutic actions of garlic constituents. Med Res Rev 1996;16:111-24.

10. Bocchini P, Andalò C, Pozzi R, Galletti GC, Antonelli A. Determination of diallyl thiosulfinate (allicin) in garlic (*Allium sativum* L.) by high-performance liquid chromatography with a post-column photochemical reactor. Anal Chim Acta 2001;441:37-43.
11. Yeh YY, Liu L. Cholesterol-lowering effect of garlic extracts and organosulfur compounds: Human and animal studies. J Nutr 2001;131:989S-93.
12. Yanagita T, Han SY, Wang YM, Tsuruta Y, Anno T. Cycloalliin, a cyclic sulfur imino acid, reduces serum triacylglycerol in rats. Nutrition 2003;19:140-3.
13. Sang EB, Seung YC, Yong DW, Seon HL, Hyun JP. A comparative study of the different analytical methods for analysis of S-allyl cysteine in black garlic by HPLC. LWT Food Sci Technol 2012;46:532-5.
14. Mandal V, Mohan Y, Hemalatha S. Microwave assisted extraction – An innovating and promising extraction tool for medicinal plant research. Pharmacogn Rev 2007;1:7-18.

Cite this article as: Bose S, Laha B, Banerjee S. Quantification of allicin by high performance liquid chromatography-ultraviolet analysis with effect of post-ultrasonic sound and microwave radiation on fresh garlic cloves. Phcog Mag 2014;10:288-93.

Source of Support: Nil, **Conflict of Interest:** None declared.