



Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com



Original article

## GC quantitative analysis of benzyl isothiocyanate in *Salvadora persica* roots extract and dental care herbal products

Maged Saad Abdel-Kader<sup>a,b,\*</sup>, Elsadig Hassan Khamis<sup>c</sup>, Ahmed Ibrahim Foudah<sup>a</sup>, Mohammed Hamed Alqarni<sup>a</sup>

<sup>a</sup> Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, P.O. Box 173, Al-Kharj 11942, Saudi Arabia

<sup>b</sup> Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University, Alexandria 21215, Egypt

<sup>c</sup> Department of Pharmaceutical Chemistry, College of Pharmacy, Prince Sattam Bin Abdulaziz University, P.O. Box 173, Al-Kharj 11942, Saudi Arabia

### ARTICLE INFO

#### Article history:

Received 24 November 2017

Accepted 5 February 2018

Available online 11 February 2018

#### Keywords:

*S. persica*

Benzyl isothiocyanate

GC

ICH guidelines

Quantitative

### ABSTRACT

An accurate, sensitive, precise and simple method was developed utilizing Gas Chromatography for the quantitative analysis of benzyl isothiocyanate in Siwak extract and dental care herbal products claimed to contain Siwak. Rtx (30.0 m × 0.25 mm ID, 25 μm thickness) column was used and helium as carrier gas at a flow rate of 0.74 mL/min. The retention time of standard benzyl isothiocyanate was 13.470 min under the described conditions. Linear regression data analysis indicated a good linear relationship between peak height measurement and concentration of benzyl isothiocyanate in the range of 10–50 μg/ml ( $R^2 = 0.9971$ ). The regression equation was  $y = 11.471x$ . The developed GC method was subjected to validation requirements set by the ICH for precision, accuracy, and robustness. The entitled GC analyses expected to be valuable for the determination of benzyl isothiocyanate in Siwak extracts and other formulations containing Siwak extract. The amount of benzyl isothiocyanate reflects the efficacy of the products.

© 2018 The Authors. Production and hosting by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

### 1. Introduction

Miswak or Siwak (*Salvadora persica*) is known long back in history for use as natural tooth brush for oral hygiene. Based on the tradition inherited from Prophetic medicine the use of Siwak is widespread in Islamic countries. The aqueous extract of *S. persica* roots was reported to have antimicrobial activity (Al Lafi and Ababneh, 1995; Almas, 1999; Almas et al., 1997). Benzyl isothiocyanate was identified as the major antimicrobial component in Siwak (Sofrata et al., 2011; Mennicke et al., 1988). Our investigation for the organic extract of *S. persica* roots directed by antimicrobial activity resulted in the identification of two minor active derivatives: 3-methoxy benzyl isothiocyanate and 3-hydroxy benzyl isothiocyanate in addition to the major component benzyl isothio-

cyanate (Abdel-Kader et al., 2017a). Recently we reported on the quantification of benzyl isothiocyanate in Siwak as well as Siwak products using RP18 HPLC method (Abdel-Kader et al., 2017b). Benzyl isothiocyanate (Benzyl mustard oil) is a naturally-occurring constituent in many cruciferous vegetables. Benzyl isothiocyanate inhibits chemically induced cancer in animal models (Srivastava and Singh, 2004). Gas chromatography (GC) is the method of choice for the analysis of compounds that can be vaporized without decomposition (Pavia et al., 2006). Since members of isothiocyanate family are suitable for GC analyses several methods were developed for their estimation in Mustard seeds (Martoni and Lavric, 2013). Benzyl isothiocyanate was estimated in Papaya fruit using GC methods (Sheu and Shuy, 1996; Tang, 1971). In this work we report on the quantitative analysis of benzyl isothiocyanate in Siwak alcohol extract as well as some herbal products labeled to contain Siwak.

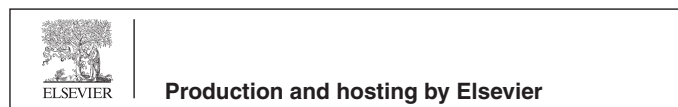
### 2. Material and methods

#### 2.1. Standard and chemicals

Standard benzyl isothiocyanate (Fig. 1) obtained from Sigma-Aldrich company, St. Louis, MO, USA. The solvents used for the analyses were of HPLC grade.

\* Corresponding author at: Department of Pharmacognosy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, P.O. Box 173, Al-Kharj 11942, Saudi Arabia.  
E-mail address: [mpharm101@hotmail.com](mailto:mpharm101@hotmail.com) (M.S. Abdel-Kader).

Peer review under responsibility of King Saud University.



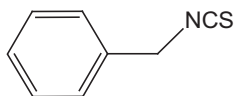


Fig. 1. Structure of benzyl isothiocyanate.

**Table 1**  
Test Results for calibration curve.

Concentration ( $\mu\text{g/ml}$ )	Peak area
10	130,047
20	232,873
30	349,368
40	449,821
50	573,173

## 2.2. Preparation of standard solutions

A stock solution of benzyl isothiocyanate 100  $\mu\text{g/ml}$  in methanol was prepared. Different concentrations were prepared from the stock solution starting from 10 to 50  $\mu\text{g}$ . The calibration curve of peak area versus benzyl isothiocyanate concentration was constructed using the prepared solutions (Table 1).

## 2.3. Plant material and dental care herbal formulations

The roots of *Salvadora persica*, family Salvadoraceae were purchased from the local market at Al-Kharj city in March 2016. The plant material was identified by the Taxonomist Dr. Mohammad Atiqur Rahman, Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC) at the College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. Voucher specimen (# 9011) was preserved at the herbarium of this center. Dental care herbal products labeled to contain Siwak extract were purchased from the local market at Al-Kharj city, Saudi Arabia. Other formulations were purchased from Hyderabad, India.

## 2.4. Extractions procedure for Siwak roots

The fresh roots of *S. persica* (20 g) were cut into small pieces and extracted with ethanol ( $3 \times 100 \text{ mL}$ ) by maceration. Extracts were combined, evaporated under reduced pressure using rotary vacuum evaporator, transferred to 20 mL volumetric flask, volume was completed using ethanol. Solutions were kept in refrigerator till the time of analyses.

## 2.5. Preparation of dental care herbal products for analysis of benzyl isothiocyanate

Accurately weighed 10 g from each product labeled to contain Siwak extract was separately extracted with ethanol ( $3 \times 70 \text{ mL}$ ) for 30 min. The ethanol extracts from each sample were combined and separately concentrated under reduced pressure using rotary vacuum evaporator. The concentrates of each sample were separately dissolved in 10 mL ethanol using volumetric flasks and stored in refrigerator till the time of GC analysis. Two hundred mL of mouth wash product labeled to contain Siwak extract were extracted with  $\text{CHCl}_3$  three times, 100 mL each. The combined  $\text{CHCl}_3$  soluble fractions were evaporated under reduced pressure and transferred to 10 mL volumetric flask using ethanol.

## 2.6. Chromatographic conditions

GC method was developed using SHIMAZU-GC/MS. The GC model 2010 plus equipped with Flame Ionization detector (FID)

and autosampler model AOC-Zoi was used. The GC connected with Mass Spectrometer model MS-2010-ultra equipped with electron multiplier detector and quadruple system analyzer. GC column used was Rtx 30.0 m  $\times$  0.25 mm ID, 25  $\mu\text{m}$  thickness column. GC injector and detector temperature were set at 200–220  $^\circ\text{C}$  respectively. Column temperature was programmed from 80  $^\circ\text{C}$  (held for 5 min) to 200  $^\circ\text{C}$  at rate of 10  $^\circ\text{C}/\text{min}$ , and to 220 at rate of 1  $^\circ\text{C}/\text{min}$ . Helium was used as carrier gas at a flow rate 0.74 mL/min. Injected sample volume 1.0 mL with split ratio of 1:100.

## 2.7. Method validation

The international conference on harmonization (ICH) guidelines were followed for the validation of the proposed GC method (ICH, 1996; ICH, 2005).

### 2.7.1. Linearity

The linearity of the method for estimation of benzyl isothiocyanate was checked between of 10–50  $\mu\text{g/ml}$  via plotting concentrations against peak areas (Fig. 2).

### 2.7.2. Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) as well as the limit of quantification (LOQ) were calculated using the standard deviation (SD) method. The two parameters were calculated from the slope of the calibration (S) plot and the SD of the blank sample applying the following equations:

$$\text{LOD} = 3.3\bar{\sigma}/S$$

$$\text{LOQ} = 10\bar{\sigma}/S$$

where,  $\bar{\sigma}$  is the standard deviation of the lowest standard level and S is the slope of the standard curve.

The standard deviation value was calculated based on the standard deviation of y-intercepts of regression lines.

### 2.7.3. Accuracy

Recovery was used to verify the method accuracy. Standard addition method was used as indication of recovery. Extra amounts of benzyl isothiocyanate standard (50, 100, and 150%) were added to pre-analyzed samples of benzyl isothiocyanate (10, 15, 35  $\mu\text{g}$ ) and the mixtures were reanalyzed. The percentage of concentration recovery and the corresponding relative standard deviation (RSD, %) were obtained at each concentration level.

### 2.7.4. Precision

Intra-day variation was measured as an indication of repeatability while intermediate precision was determined by assessment of inter-day variation for analysis of benzyl isothiocyanate at the five

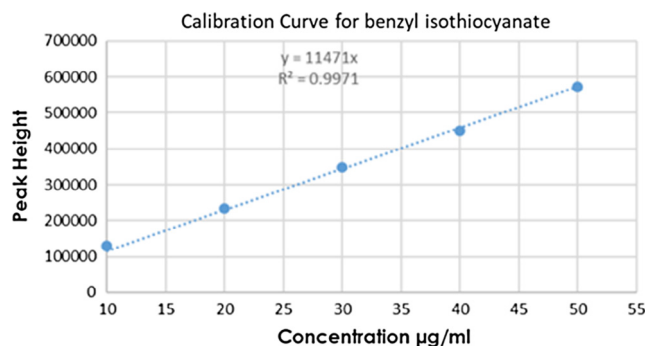


Fig. 2. GC calibration plot of standard benzyl isothiocyanate.

selected concentrations (12, 17, 23, 32, and 43  $\mu\text{g}$ ) in six replicate. Repeatability and intermediate precision are reflection of the method precision.

#### 2.7.5. Specificity

Specificity of the developed GC method was verified by analyzing of benzyl isothiocyanate include other volatile compound as menthol and comparing the GC chromatogram obtained for a mixture of the studied for benzyl isothiocyanate in the samples with that obtained from the mixture to ensure the identity of an analyte; results obtained show that developed method are unaffected by the present of impurities and there is no interference between the two compound.

#### 2.7.6. Robustness

Robustness of the newly developed method was explored to prove that small deliberate changes in the chromatographic conditions like programmed temperatures  $\pm 10^\circ\text{C}$  and carrier gas flow rate  $0.74 \pm 0.2$  mg/min will not affect the quantification.

#### 2.8. Quantification of benzyl isothiocyanate in *S. Persica* extract and dental care herbal products containing Siwak extract

The ethanol extract of *S. persica* and dental care herbal products were analyzed and their corresponding chromatograms were obtained using same conditions described for the analysis of standard benzyl isothiocyanate. The peak areas corresponding to the Rt

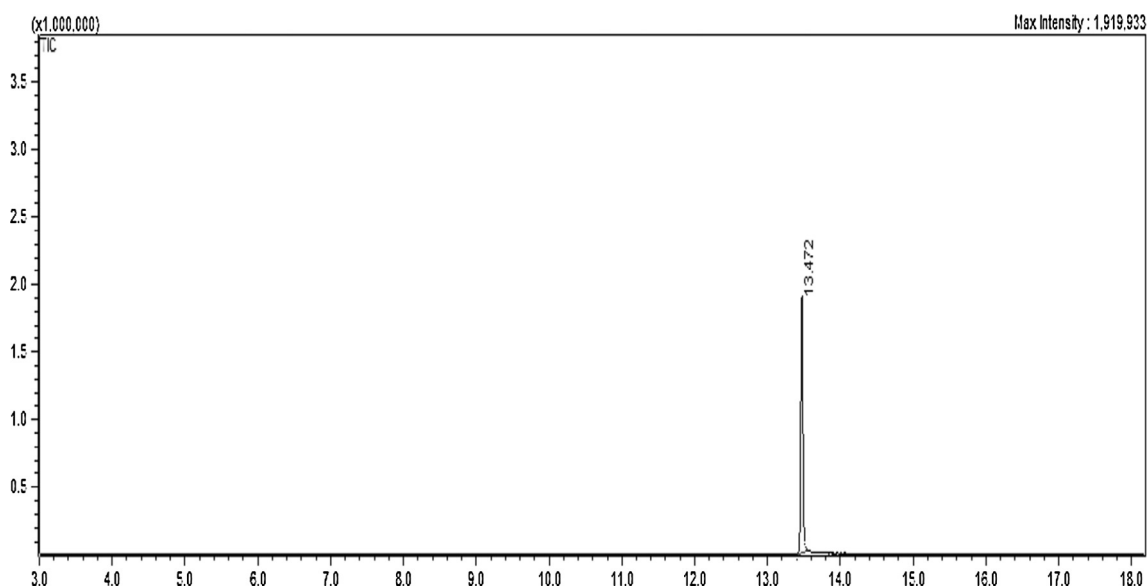


Fig. 3. GC chromatogram of standard benzyl isothiocyanate.

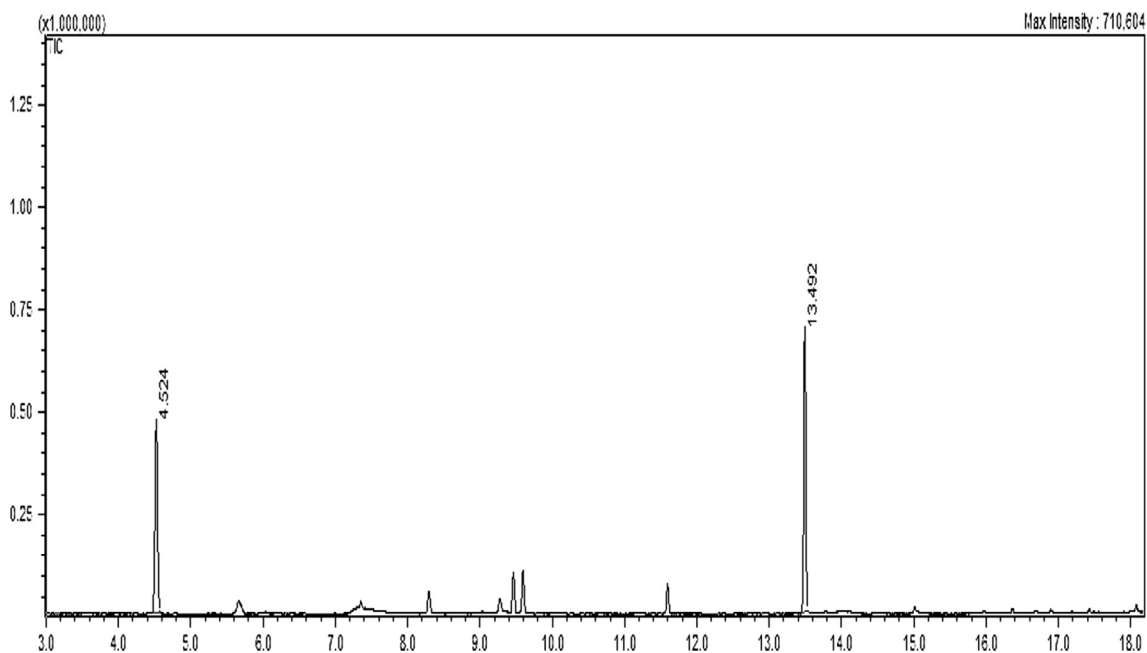


Fig. 4. GC chromatogram of *S. persica* roots ethanol extract.

**Table 2**

Linear regression data for the calibration curve of benzyl isothiocyanate (n = 6).

Linearity range ( $\mu\text{g}/\text{spot}$ )	10–50 $\mu\text{g}/\text{ml}$ Rang
Regression equation	$Y = 11,472x$
Correlation coefficient	0.9983
Slope $\pm$ SD	$11,472 \pm 455.53$
Intercept $\pm$ SD	$0 \pm 455.53$
LOQ <sup>*</sup>	2.03 $\mu\text{g}/\text{ml}$
LOD <sup>**</sup>	0.67 $\mu\text{g}/\text{ml}$

<sup>\*</sup> LOQ = 10  $\sigma/S$ .<sup>\*\*</sup> LOD = 3.3  $\sigma/S$ .

of benzyl isothiocyanate standard, if any, was recorded and the concentrations were obtained using the regression equation generated from the calibration plot.

### 3. Results and discussion

The literature lacks any attempts to estimate benzyl isothiocyanate in *S. persica* and products labeled to contain its extract using GC. In Islamic countries, many products claimed to contain Siwak extract are available in the market for dental hygiene. The medicinal value of Siwak is dependent on the amount of benzyl isothiocyanates as previous research indicated that it is the main antimicrobial agent in the extract with distinct characteristic odour (Sofrata et al., 2011; Abdel-Kader et al., 2017a). GC method was developed using Rtx column at temperature starting with 80 °C (held for 5 min) to 200 °C at rate of 10 °C/min, and to 220 °C at rate of 1 °C/min. The flow rate of Helium was 0.74 mL/min. The peak corresponding to benzyl isothiocyanate was observed at retention time of 13.470 min. *S. persica* roots extract showed well

resolved peak for benzyl isothiocyanate under the described conditions (Fig. 3 and 4).

A valid linear relationship between peak area and concentration of benzyl isothiocyanate in the range of 10–50  $\mu\text{g}/\text{ml}$  was obtained from linear regression analysis (Fig. 2). The obtained regression equation was  $Y = 11,471x$ , where Y represents the FID response to the eluted peaks while X is the concentration of benzyl isothiocyanate. Table 2 represents the linear regression data for the plot of concentration against spot areas. The correlation coefficient ( $R^2$ ) was 0.9971 which is highly significant ( $P < 0.05$ ). LOD and LOQ were calculated and found to be 0.67 and 2.03  $\mu\text{g}/\text{mL}$ , for benzyl isothiocyanate (Table 2). These values reflect the wide range and high sensitivity of the method for detection and quantification of benzyl isothiocyanate effectively.

The accuracy of the method, as recovery, was 99.35–100.15%, with RSD values in the range 0.01–0.28. These results of percentage recovery indicated that the method is accurate (Table 3). Percentage coefficient of variation (% CV) of measured concentrations for each calibration level was taken as indication for precision. Table 4 represents the obtained results from determination of repeatability and intermediate precision, expressed as SD (%). RSD was in the range 0.17–2.14 for repeatability and 0.27–2.20 for intermediate precision indicating that the developed method is precise. Robustness of the method after introducing minor changes in the method such as pressure, initial temperature and flow rate are presented in Table 5. The low values obtained for % RSD (0.06–0.34) indicated that the propose GC method is robust.

Benzyl isothiocyanate peaks obtained from the analysis of ethanol extract of *S. persica* and dental care herbal formulations were examined by comparing their peak at  $R_t = 13.470$  min with those obtained from the standard benzyl isothiocyanate under the same conditions. The benzyl isothiocyanate content in ethanol extract of *S. persica* was estimated to be 0.073% w/w by the use of the linear regression equation. None of the examined dental care herbal

**Table 3**

Accuracy of the proposed method (n = 6).

Excess drug added to analyte (%)	Theoretical content ( $\mu\text{g}$ )	Conc. found ( $\mu\text{g}$ ) $\pm$ SD	% Recovery	% RSD
50	15	$14.47 \pm 0.17$	99.35	0.28
100	20	$19.13 \pm 0.12$	99.43	0.10
150	35	$35.53 \pm 0.05$	100.15	0.01

Accuracy = Mean overall recovery =  $100.23 \pm 0.13$ .**Table 4**

Precision of the proposed method.

Conc. ( $\mu\text{g}$ )	Repeatability (Intraday precision)		Intermediate precision (Interday)	
	Benzyl isothiocyanate found (n = 6)	% RSD	Benzyl isothiocyanate found (n = 6)	% RSD
12	11.82	2.14	12.46	2.20
17	18.08	1.19	17.79	1.14
23	22.81	0.85	24.08	0.65
32	32.98	0.17	31.89	0.27
43	43.11	0.32	43.34	0.31
		Av = 0.93%		Av = 0.89%

**Table 5**

Robustness of the proposed GC method.

Conc. ( $\mu\text{g}$ )	Variations			Results	
	Pressure (kpa) $\pm 5$	Initial T (°C) $\pm 10$	Flow rate (ml/min) $\pm 0.2$	Resolution	RSD (%)
40	41.5	80	0.74	13.47	0.06
	46.5	90	0.76	11.55	0.21
	36.5	70	0.72	15.44	0.34

product showed peak corresponding to benzyl isothiocyanate. The absence of benzyl isothiocyanate in the products may be due to improper treatment of the original plant materials or extraction method.

#### 4. Conclusion

In this work we developed and validated a GC method for the estimation of benzyl isothiocyanate in Siwak extract and selected dental care products. The developed GC method is simple, accurate, reproducible, sensitive, and is applicable for the analysis of any products containing benzyl isothiocyanate. Statistical data prove that the method is reproducible and highly selective for benzyl isothiocyanate analysis. The proposed method was applied for the quantification of benzyl isothiocyanate in the ethanol extract of *S. persica* and some dental care herbal products. The calculated amount of benzyl isothiocyanate was 0.073% w/w in the ethanol extract of *S. persica*. However, none of the examined dental care herbal product showed peak corresponding to benzyl isothiocyanate. The process of manufacturing of such products must be investigated to locate the step responsible for the loss of the active ingredients.

#### References

- Abdel-Kader, M.S., Muharram, M.M., Foudah, A.I., Alqarni, M.H., Salkini, M.A., 2017a. Antimicrobial isothiocyanate derivatives from *salvadora persica* root "Siwak" extract. *Indo Am. J. P. Sci.* 4 (05).
- Abdel-Kader, M.S., Kamal, Y.T., Alam, P., Alqarni, M.H., Foudah, A.I., 2017b. Quantitative analysis of benzyl isothiocyanate in *Salvadora persica* extract and dental care herbal formulations using reversed phase C18 high-performance liquid chromatography method. *Pharmacogn. Mag.*, S412–S416
- Al Lafi, T., Ababneh, H., 1995. The effect of the extract of the miswak (chewing sticks) used in Jordan and the Middle East on oral bacteria. *Int. Dent. J.* 45 (3), 218–222.
- Almas, K., 1999. The antimicrobial effects of extracts of *Azadirachta indica* (Neem) and *Salvadora persica* (Arak) chewing sticks. *Indian J. Dent. Res.* 10 (1), 23–26.
- Almas, K., Al-Bagieh, N., Akpata, E., 1997. In vitro antimicrobial effects of extracts of freshly cut and 1-month-old miswak (chewing stick). *Biomed. Lett.* 56, 145–149.
- International Conference on Harmonization (ICH) Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (B), 2005.
- International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use: Harmonized Tripartite Guideline on Validation of Analytical Procedures: Methodology, recommended for Adoption at Step 4 of the ICH Process on November 1996 by the ICH Steering Committee, IFPMA, Switzerland.
- Martoni, M.-R., Lavric, V., 2013. A simple method for the quantification of isothiocyanates from Mustard. *U.P.B. Sci. Bull. Series B* 75 (1), 63–72.
- Mennicke, W.H., Görler, K., Krumbiegel, G., Lorenz, D., Rittmann, N., 1988. Studies on the metabolism and excretion of benzyl isothiocyanate in man. *Xenobiotica* 18 (4), 441–447.
- Pavia, D.L., Gary, M.L., George, S.K., Randall, G.E., 2006. Introduction to Organic Laboratory Techniques. Thomson Brooks/Cole, pp. 797–817.
- Sheu, F., Shuy, Y.-T., 1996. Determination of benzyl isothiocyanate in papaya fruit by solid phase extraction and gas chromatography. *J. Food Drug Anal.* 4 (4), 327–334.
- Sofrata, A., Santangelo, E.M., Azeem, M., Borg-Karlson, A.K., Gustafsson, A., Pütsep, K., 2011. Benzyl isothiocyanate, a major component from the roots of *Salvadora persica* is highly active against Gram-negative bacteria. *PLoS One* 6 (8), e23045.
- Srivastava, S.K., Singh, S.V., 2004. Cell cycle arrest, apoptosis induction and inhibition of nuclear factor kappa B activation in anti-proliferative activity of benzyl isothiocyanate against human pancreatic cancer cells. *Carcinogenesis* 25 (9), 1701–1709.
- Tang, C.-S., 1971. Benzyl isothiocyanate of papaya fruit. *Phytochemistry* 10 (10), 117–121.