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Optimization of the extraction condition for benzyl isothiocyanate contents in *Salvadora persica* roots "Siwak"

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ABSTRACT

Benzyl isothocyanate is the major active antibacterial metabolite in *Salvadora persica* roots "Siwak" beside two minor isothiocyanate derivatives namely; 3-methoxy benzyl isothiocyanate and 3-hydroxy benzyl isothiocyanate. The extraction condition effect on the amount of benzyl isothiocyanate was explored in detailed study. Both cold and hot extraction with different solvents was applied. The amount of benzyl isothiocyanate was estimated using HPLC and HPTLC. The results indicated that cold extraction of the fresh samples with chloroform offers the maximum amount of benzyl isothiocyanate. Drying process leads to great loss of the active component of Siwak.

1. Introduction

The use of *Salvadora persica* (Siwak) as natural tooth brush is an old tradition inherited from Prophetic medicine (Al Lafi and Ababneh, 1995). The antimicrobial effect of Siwak is mainly due to benzyl isothiocyanate (Sofrata et al., 2011; Mennicke et al., 1988). Two other minor active isothiocyanate derivatives 3-methoxy benzyl isothiocyanate and 3-hydroxy benzyl isothiocyanate were also identified (Abdel-Kader et al., 2017a).

Our analysis of some marketed Siwak containing products revealed that none of them contain benzyl isothiocyanate (Abdel-Kader et al, 2017b; Abdel-Kader et al, 2017c; Abdel-Kader et al, 2018). In a previous study the effect of extraction solvents indicated that hydroxylated solvents such as methanol and ethanol react with benzyl isothiocyanate to form the corresponding inactive thiocarbamate derivatives (Abdel-Kader et al, 2019). Other solvents used in the experiments were of analytical grades.

In the current study different extraction conditions and samples preparation were applied to optimize the condition that offers maximum concentration of the antimicrobial agent benzyl isothiocyanate.

2. Materials and methods

2.1. Standards and chemicals

Benzyl isothiocyanate was obtained from Sigma-Aldrich, St. Louis, MO, USA. HPLC grade hexane, acetonitrile, ethyl acetate and methanol (E. Merck, Darmstadt, Germany) were used for the analysis. Deionized water was obtained from Milli-Q system (Millipore, Bedford, MA, USA). Ortho-phosphoric acid (H_3PO_4) 88% was purchased from Fisher Scientific Company (UK).

2.2. Plant material

The roots S. *persica*, family Salvadoraceae were purchased from the local market at Al-Kharj city in March 2016 and was identified as described earlier (Abdel-Kader et al, 2017a).

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2.3. Extraction

Fresh Siwak samples 20 gm each were extracted with chloroform, acetone and ethanol. The extraction was performed with each solvent using two different techniques. Aliquots were extracted with the stated solvents by maceration at room temperature. The process was repeated three times using fresh solvents. The second applied technique was the continuous hot extraction using soxhlet apparatus.

All extraction procedures were repeated using 20 gm of air dried Siwak samples.

2.4. HPTLC analysis

The analysis was performed using 10×20 cm glass plates coated with 0.2 mm layers of silica gel 60 F_{254} (E-Merck, Germany). Camag Automatic TLC Sampler 4 (ATS4) sample applicator (Switzerland) fitted with a Camag microlitre syringe in the form of spot by manual contact method without using the nitrogen gas was used for samples application. Application rate was constant at 150 nl/s was used. The plates were developed in a Camag Automatic Developing Chamber 2 (ADC2) after saturated for 30 min at 22 °C with mobile phase to a distance of 8 cm with n-Hexane-ethyl acetate 9:1 as mobile phase. Plates were left to dry for 10 min and the Silica gel layers were covered tightly with 10×20 glass plates. Spots were detected under UV light at 191 nm (Abdel-Kader et al, 2017b).

2.5. HPLC analysis

Waters Alliance e2695 separating module (Waters Co., MA, USA) using UV detector (Waters 2998) with autosampler and column oven was used for the analysis. The instrument was run under the control of "EMPOWER" software installed with equipment for data collection and acquisition. C_{18} reverse phase column (250×4.6 mm, particle size 5 μm) maintained at room temperature using acetonitrile and water in the ratio of 1:1 as mobile phase were used for the quantification. The flow rate was adjusted to fixed rate of 1 mL/min. The detection wavelength was 190 nm and the injection volume was 10 μL (Abdel-Kader et al, 2017c).

2.6. Methods validation

The HPTLC and HPLC methods were validated for linearity, precession, accuracy, robustness, specificity, limit of detection (LOD) and limit of quantification (LOQ) following the international conference on harmonization (ICH) guide lines (ICH, 2005) as described earlier (Abdel-Kader et al, 2017b; Abdel-Kader et al, 2017c).

3. Results and discussion

The fact that all the analyzed S. *persica* "Siwak" containing products were free from benzyl isothiocyanate attracted our attention to discover the reasons behind this fact. Our previous study revealed that hydroxylated solvents react in various rates with benzyl isothiocyanate to produce two inseparable conformers of the inactive corresponding thiocarbamate. These reactions proceed at room temperature and accelerated by heating. The study also indicated that chloroform and acetone did not express any effect on benzyl isothiocyanate (Abdel-Kader et al, 2019). In the current study we focused on the effect of extraction conditions and samples preparation. Analysis of benzyl isothiocyanate, the major antibacterial agent in *S. persica* Roots, were performed on the obtained extracts using both HPTLC and HPLC validated methods as previously reported (Abdel-Kader et al, 2017b; Abdel-Kader et al, 2017c). Three solvent were used for extraction representing different classed of solvents. Chloroform is an oxygen free halogenated solvent, acetone containing carbonyl function while ethanol represents the category of hydroxylated solvent. Both fresh and air dried samples were used in the current study. With each solvent the fresh and dry samples were extracted by maceration at room temperature for three times as well as by continuous hot extraction technique using soxhlet apparatus. Consequently, twelve extracts were obtained and the amount of benzyl isothiocyanate was quantified by HPTLC and HPLC (Table 1).

The numbers obtained from both HPTLC and HPLC quantification indicated a great variation in the benzyl isothiocyanate contents. Three main factors including the extraction solvents, samples preparation conditions and the method of extraction critically affected the benzyl isothiocyanate contents. The effect of solvents revealed that chloroform gave the highest yield of benzyl isothiocyanate followed by acetone and finally ethanol. As a hydroxylated solvent ethanol expected to react with benzyl isothiocyanate leading to very low yield (Abdel-Kader et al, 2019). The loss due to the use of ethanol is great when compared with chloroform under all the used condition. For example fresh Siwak sample extracted with chloroform by maceration (FCM) estimated to contain 2.111–2.410% while the ethanol extracted samples (FEM) contain 0.071–0.063% as obtained from HPTLC and HPLC analysis respectively. Acetone on the other hand do not react directly with benzyl isothicyanate, however, the yield was also very low. Fresh Siwak sample extracted with acetone by maceration (FAM) found to contain 0.142–0.120% as estimated by HPTLC and HPLC respectively. The low yield could be explained by the water miscibility of acetone that facilitate contact between benzyl isothicyanate and polar components in the plant with hydroxyl groups that most likely react with the N=C=S function of benzyl isothiocyanate.

The effect of extraction methods also has a great impact on benzyl isothicyanate concentration. While FCM contains 2.111–2.410%, the fresh Siwak sample extracted with chloroform by soxhlet (FCS) contains 0.920–0.88% as indicated from the HPTLC and HPLC analysis respectively. This excessive lost upon hot extraction could be referred to either the volatile nature of benzyl isothicyanate or the decomposition of benzyl isothiocynate by elevated temperature as reported for other isothiocynate species (Ohta et al, 1995). Higher temperature will initiate faster chemical interaction with benzyl isothiocynate.

More than 90% of the benzyl isothiocyanate contents were lost from the sample subjected to drying at room temperature. FCM contains 2.111–2.410% while the dry samples extracted under same conditions (DCM) proved to contain 0.194–0.210% of benzyl

Table 1
Concentration of benzyl isothiocyanate (w/w%) in different Siwak samples as determined by HPTLC and HPLC.

Sample	Concentration of benzyl isothiocyanate (w/w %)	
	HPTLC	HPLC
FCM	2.111	2.410
FCS	0.920	0.880
DCM	0.194	0.210
DCS	0.210	0.170
FAM	0.142	0.120
FAS	0.122	0.110
DAM	0.032	0.037
DAS	0.056	0.061
FEM	0.071	0.063
FES	0.054	0.059
DEM	0.036	0.040
DES	0.015	0.021

F: Fresh samples; **D:** Dry samples; **M:** Maceration at room temperature; **S:** Soxhlet extraction; **C:** Chloroform; **A:** Acetone; **E:** Ethanol.

isothiocyanate by HPTLC and HPLC respectively. This loss occurs as a result of the volatile nature of benzyl isothiocyanate (Abdel-Kader et al, 2017b).

4. Conclusion

The effect of solvents, methods of extraction and samples preparation conditions on the yield of benzyl isothiocyanate in S. *persica* was explored. Fresh and dried samples of S. *persica* roots were extracted with chloroform, acetone or ethanol. Extraction performed by maceration at room temperature and by continuous hot extraction using sexhlet apparatus. The estimation of benzyl isothiocyanate was conducted by HPTLC and HPLC validated methods. Extraction with ethanol and acetone resulted in much less amounts of benzyl isothiocyanate compared to chloroform extracts. Hot extraction markedly led to great loss of benzyl isothiocyanate. More than 90% of the benzyl isothiocyanate contents were lost after drying of the samples. In conclusion the best yield of benzyl isothiocyanate can be obtained when fresh S. *persica* root samples are extracted with chloroform at room temperature.

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