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Effect of hydroxylated solvents on the active constituents of Salvadora persica root "Siwak"

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ABSTRACT

Previously, the antimicrobial activity of *Salvadora persica* was traced to benzyl isothiocyanate. In the present study known inactive compounds were isolated from extracts obtained by different solvents including β-amyrin, β-sitosterol, stigmasterol glucoside, benzyl cyanide and sulphur. However, some inactive compounds were present only in the ethanol and methanol extracts. This observation indicated that these compounds most likely are artifacts resulted from interaction with the solvents used in extraction. Pure benzyl isothiocyanate was kept with different solvents for 72 h and after TLC study they were heated under reflux for 8 h to explore the possibility of interactions. Only solvents with OH groups reacted with benzyl isothiocyanate and gave products similar to those isolated from the alcohol extracts. In conclusion extraction of *S. persica* with hydroxylated solvents will alter the structure of the active compound benzyl isothiocyanate and leads to loss of antimicrobial activity.

1. Introduction

The roots of *Salvadora persica* (Miswak or Siwak) is used, traditionally, as natural tooth brush. The tradition inherited from Prophetic medicine backed the widespread of Siwak in Islamic countries (Al Lafi and Ababneh, 1995). The antimicrobial effect of Siwak was traced to benzyl isothiocyanate (Sofrata et al., 2011; Mennicke et al., 1988). While benzyl isothiocyanate is a major compound, two minor active derivatives were also reported from Siwak (Abdel-Kader et al., 2017a).

Other compounds including β-sitosterol, *m*-anisic acid, urea derivative salvadourea, oleic, linolic, stearic acids, glucosinolates; glucotropaelin and sinigrin were also isolated from Siwak (Ray et al., 1975; Abd El Rahman et al., 2003; Ezmirly and Seif El-Nasr, 1981). Kaempferol, quercetin, quercetrin, rutin and quercetin glucoside are examples of flavonoid derivatives reported from Siwak (Abdel Waheb et al., 1990).

Allyl isothiocyanate is reported to react with alkyl alcohols to form the corresponding *O*-alkyl allylthiocarbamate. The products of the interaction existed in two conformational isomers (Kanamori-Kataoka et al., 2011). Effect of aqueous medium with different pH on allyl isothiocyanate was also investigated (Pecháček et al., 1997).

Analysis of some marketed Siwak products recommended for dental care revealed that non of them contain benzyl isothiocyanate, the major active agent of Siwak (Abdel-Kader et al., 2017b,c; Abdel-Kader et al., 2018). That is most likely due to the decomposition of the active compound during extraction. These results encourage us to investigate the effect of extraction solvents on benzyl isothiocyanate contents and suggest the best way to protect from decomposition.

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2. Materials and methods

2.1. General

^{1}H, ^{13}C NMR spectra and 2D NMR experiments (COSY, HSQC and HMBC) were obtained using standard Bruker program on Ultra-Shield Plus 500 MHz (Bruker) (NMR Unite at the College of Pharmacy, Prince Sattam Bin Abdulaziz University) spectrometer operating at 500 MHz for protons and 125 MHz for carbon atoms, respectively. The chemical shift values are reported in δ (ppm) relative to the residual solvent peak. Coupling constants (J) are reported in Hertz (Hz). GC/MS analysis were performed 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 × 0.25 mm ID, 25 mm thickness column. GC injector and detector temperatures were set at 200–220 °C respectively. Column temperature was programmed from 80 °C (held for 5 min) to 200 °C at a rate of 10 °C/min and to 220 at a rate of 1 °C/min. Helium was used as carrier gas at flow rate 0.74 mL/min. Injected sample volume 1.0 mL with split ratio of 1:100. X-ray data were collected on a Bruker APEX-II D8 Venture area diffractometer using graphite monochromatic Mo Kα radiation (λ = 0.71073 Å) at 100(2) K. Silica gel 60/230–400 mesh (EM Science) was used for column chromatography and silica gel 60 F254 (Merck) was used for TLC. Benzyl isothiocyanate was obtained from Sigma-Aldrich Company, St. Louis, MO, USA.

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).

2.3. Extraction and isolation

Fresh roots of S. persica "Siwak" (3 kg) were cut into small pieces. One kg was extracted with 95% ethanol, another 1 kg was extracted with methanol at room temperature until exhaustion. Both extracts were treated separately with the same procedure. The combined extracts were concentrated under reduced pressure using rotary vacuum evaporator and was partitioned in separating funnel by liquid-liquid extraction starting with $CHCl_3$ (3 × 500 mL) followed by EtOAc (3 × 300 mL). Third kg of the fresh roots was extracted with $CHCl_3$ at room temperature till exhaustion.

Part of the $CHCl_3$ fractions from each extraction process (4 gm) were separately chromatographed over silica gel column (150 g, 3 cm i.d.) using a gradient of pet. ether/EtOAc. Fractions, 150 mL each, were collected, screened by TLC and similar fractions were pooled.

2.4. Common isolates in all the $CHCl_3$ fractions

Early fractions eluted with 100% pet. ether found to contain sulphur. Fractions eluted with 5% EtOAc in pet. ether afforded different quantities of benzyl isothiocyanate. Fractions eluted with 10% EtOAc in pet. ether afforded traces of benzyl cyanide. Other compounds including β-Amyrin was eluted with 25% EtOAc in pet. ether, β-sitosterol was eluted with 50% EtOAc in pet. ether, while stigmasterol glucoside was eluted with 100% EtOAc.

2.5. $CHCl_3$ fractions from ethanol extract

Early fractions of this extract eluted with 5% EtOAc in pet. ether afforded 40 mg of compound **1** having lower polarity than benzyl isothiocyanate.

2.6. $CHCl_3$ fractions from methanol extract

Early fractions of this extract eluted with 5% EtOAc in pet. ether afforded 38 mg of compound **2** having lower polarity than benzyl isothiocyanate.

2.7. Interaction between benzyl isothiocyanate and different solvents

Standard benzyl isothiocyanate (100 mg aliquots each) were kept with $CHCl_3$, acetone, diethyl ether, EtOAc, ethanol, methanol, 2-propanol and *n*-butanol for 72 hrs at room temperature. Same solutions were heated under reflux for 8 h. Mixtures were checked by TLC before and after reflux. Methanol, ethanol, 2-propanol and *n*-butanol aliquots afforded **1–4** respectively.

2.8. Suphur

Colourless crystals insoluble in common organic solvents. M.p. 117 °C.

2.9. O-Ethyl benzyl thiocarbamate **1**

$C_{10}H_{13}NOS$, semisolid, ^{1}H and ^{13}C NMR ($CDCl_3$): Tables 1 and 2. GC–MS: t_R = 26.762, EIMS m/z (%): 195 (M^+, 20), 166 (M^+-CH_2CH_3, 28), 149 (M^+-H, OCH_2CH_3, 12), 106 (benzyl-NH, 20), 91 (benzyl, 100).

2.10. O-Methyl benzyl thiocarbamate **2**

$C_9H_{11}NOS$, semisolid, ^{1}H and ^{13}C NMR ($CDCl_3$): Tables 1 and 2. GC–MS: t_R = 24.847, EIMS m/z (%):181(M^+, 22), 166 (M^+-CH_3, 18), 149 (M^+-H, OCH_3, 16), 106 (benzyl-NH, 14), 91 (benzyl, 100).

2.11. O-Isopropyl benzyl thiocarbamate **3**

$C_{11}H_{15}NOS$, semisolid, ^{1}H and ^{13}C NMR ($CDCl_3$): Tables 1 and 2. GC–MS: t_R = 28.976, EIMS m/z (%):209 (M^+, 7), 106 (benzyl-NH, 10), 91 (benzyl, 100).

2.12. O-Butyl benzyl thiocarbamate **4**

$C_{12}H_{17}NOS$, semisolid, ^{1}H and ^{13}C NMR ($CDCl_3$): Tables 1 and 2. GC–MS: t_R = 30.631, EIMS m/z (%): 223 (M^+, 6), 190 (M^+-SH, 7), 168 (M^++ 2H-Butyl, 46), 106 (benzyl-NH, 15), 91 (benzyl, 100).

3. Results and discussion

Early fractions from the columns of all $CHCl_3$ extracts eluted with pet. ether afforded colourless crystals with characteristic odour insoluble in all common organic solvents. X-ray crystallography study enable the identification of the crystals as sulphur (Fig. 1). Benzyl isothiocyanate (Sigma Aldrich a), benzyl cyanide (Sigma Aldrich b) (Fig. 2), β-amyrin (Okoye et al., 2014), β-sitosterol (Bulama et al., 2015) and stigmasterol glucoside (Ridhay et al., 2012) were identified by comparison of their spectral data with the literature. Except benzyl isothiocyanate all these compounds were inactive when tested for antimicrobial activity.

Only the $CHCl_3$ fraction of the ethanol extract afforded **1** (Fig. 2) as a chromatographically homogenous compound in both TLC and GC (Fig. 3). The ^{1}H NMR of **1** showed three spine systems. The most down field system at δ_H 7.21–7.37 ppm assigned for 5 overlapped aromatic protons in a monosubstituted ring. Second spine system as indicated from COSY experiment composed of CH_2 doublet and NH proton. The third spine system includes O-CH_2-CH_3. All signals of ^{1}H and ^{13}C NMR are present as duplicate in approximate

Table 1
^{1}H NMR data of **1–4** in ppm (multiplicity, *J* in Hz).[a]

Pos.	**1**		**2**		**3**		**4**	
	Conf. A	Conf. B	Conf. A	Conf. B	Conf. A	Conf. B	Conf. A	Conf. B
NH	7.06 (bt)	7.96 (bt)	6.85 (bs)	7.56 (bs)	6.74 (bs)	7.77 (bs)	7.10 (t, *J* = 5.5)	8.10 (t, *J* = 5.5)
2–6	7.21–7.37 (m, aromatic)		7.17–7.30 (m, aromatic)		7.28–7.41 (m, aromatic)		7.16–7.26 (m, aromatic)	
CH_2-arom.	4.72 (d, *J* = 5.7)	4.40 (d, *J* = 5.9)	4.67 (d, *J* = 5.6)	4.34 (d, *J* = 6.1)	4.76 (d, *J* = 5.7)	4.37 (d, *J* = 5.7)	4.65 (d, *J* = 6.0)	4.28 (d, *J* = 6.1)
O-R	CH_2 4.47 (q, *J* = 7.1)	4.51 (q, *J* = 7.1)	CH_3 3.90 (s)	3.79 (s)	CH 5.68 (p, *J* = 6.2)	5.61 (p, *J* = 6.2)	CH_2 4.38 (t, *J* = 6.7)	4.38 (t, *J* = 6.7)
	CH_3 1.30 (t, *J* = 7.1)	1.31 (t, *J* = 7.1)			$(CH_3)_2$ 1.46 (d, *J* = 6.0)	1.38 (d, *J* = 6.0)	CH_2 1.59 (m)	1.59 (m)
							CH_2 1.35 (h, *J* = 7.5)	1.25 (h, *J* = 7.5)
							CH_3 0.90 (t, *J* = 7.9)	0.86 (t, *J* = 8.0)

[a] Assignments were done based on DEPT, COSY, HSQC and HMBC experiments.

Table 2
^{13}C NMR data of **1–4** in ppm.[a]

Pos.	**1**		**2**		**3**		**4**	
	Conf. A	Conf. B	Conf. A	Conf. B	Conf. A	Conf. B	Conf. A	Conf. B
1	137.3	137.1	137.1	136.8	137.4	131.7	137.3	137.2
2–6	127.6–128.7		127.6–128.8		126.7–128.4		127.5–128.6	
CH_2-arom.	48.8	46.8	49.2	47.0	48.8	46.9	48.8	46.8
C = S	190.7	189.6	191.5	190.3	190.0	188.8	190.8	189.5
O-R	CH_2 66.4	67.7	CH_3 57.2	58.3	CH 74.0	75.8	CH_2 70.4	71.5
	CH_3 14.4	14.3			$(CH_3)_2$ 22.0	21.9	CH_2 30.8	30.6
							CH_2 19.1	19.1
							CH_3 13.9	13.8

[a] Assignments were done based on DEPT, COSY, HSQC and HMBC experiments.

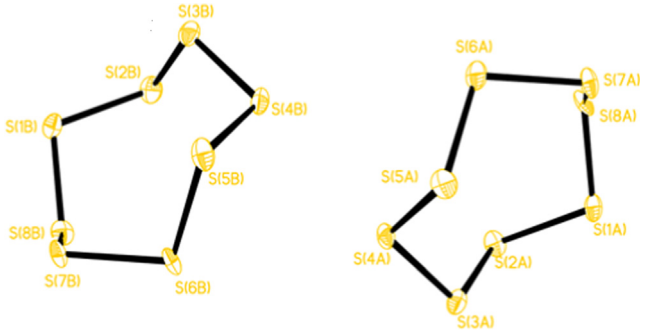

Fig. 1. X-ray crystal structure of sulphur.

ratio 3:2 as indicated from the integration of the ^{1}H NMR spectrum. ^{13}C NMR showed quaternary carbon signal at δ_C 189.63 and 190.65 ppm assigned for C=S. Comparing the data of **1** with benzyl isothiocyanate enable the assumption that **1** resulted from the addition of ethanol on the C=N of benzyl isothiocyanate. This addition result in the formation of two conformers (Fig. 4) (Kanamori-Kataoka et al., 2011). Although N—C single bond classically allows free rotation, in case of **1** the formation of hydrogen bonding between the N—H and either the oxygen or sulphur atoms led to the formation of two distinct conformational isomers. Conformer **A** has energy of 121.3763 K Cal/mol while Conformer **B** has energy of 583.4052 K Cal/mol as calculated by Chem3D Pro 12.0. Consequently, conformer **A** is more stable than conformer **B** and it was assigned to the higher ratio conformer. This fact was further supported by ^{1}H NMR data where the NH of conformer **B** appears at δ_H 7.96 as it form hydrogen bonding with the more electronegative oxygen atom. The same NH proton in conformer **A** form hydrogen bonding with the less electronegative sulphur atom with a chemical shift of δ_H 7.06. Integration of the ^{1}H NMR gave higher ration to the NH at δ_H 7.06. MS data showed an M^+ at m/e 195 fully supporting the structure of **1**. Compound **1** (*O*-Ethyl benzylthiocarbamate) is only present in the $CHCl_3$ fraction of the ethanol extract. This observation proved that **1** is an artifact produced from the reaction of benzyl isothiocyanate with ethanol.

Similarly, two conformational isomers of **2** are formed from the reaction of benzyl isothiocyanate with methanol in the methanol extract $CHCl_3$ fraction. The O-CH_2-CH_3 spine system in **1** was replaced by O-CH_3 at δ_H 3.79 and 3.90 for conformer **B** and **A**, respectively, in the ^{1}H NMR of **2**. The presence of compound **2** in

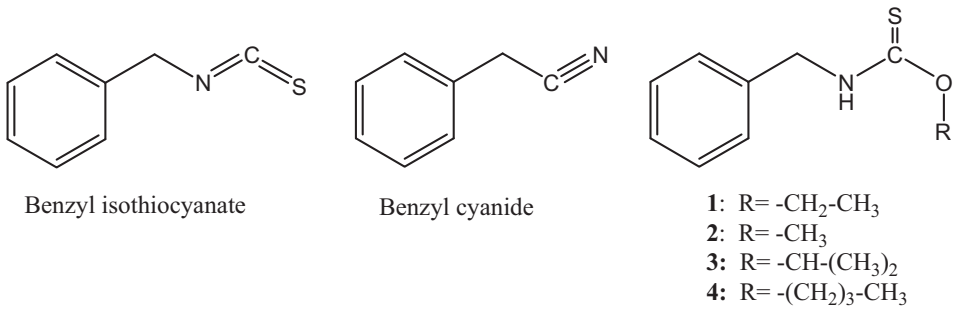

Fig. 2. Isolated benzyl derivatives from different $CHCl_3$ extracts of *S. persica*.

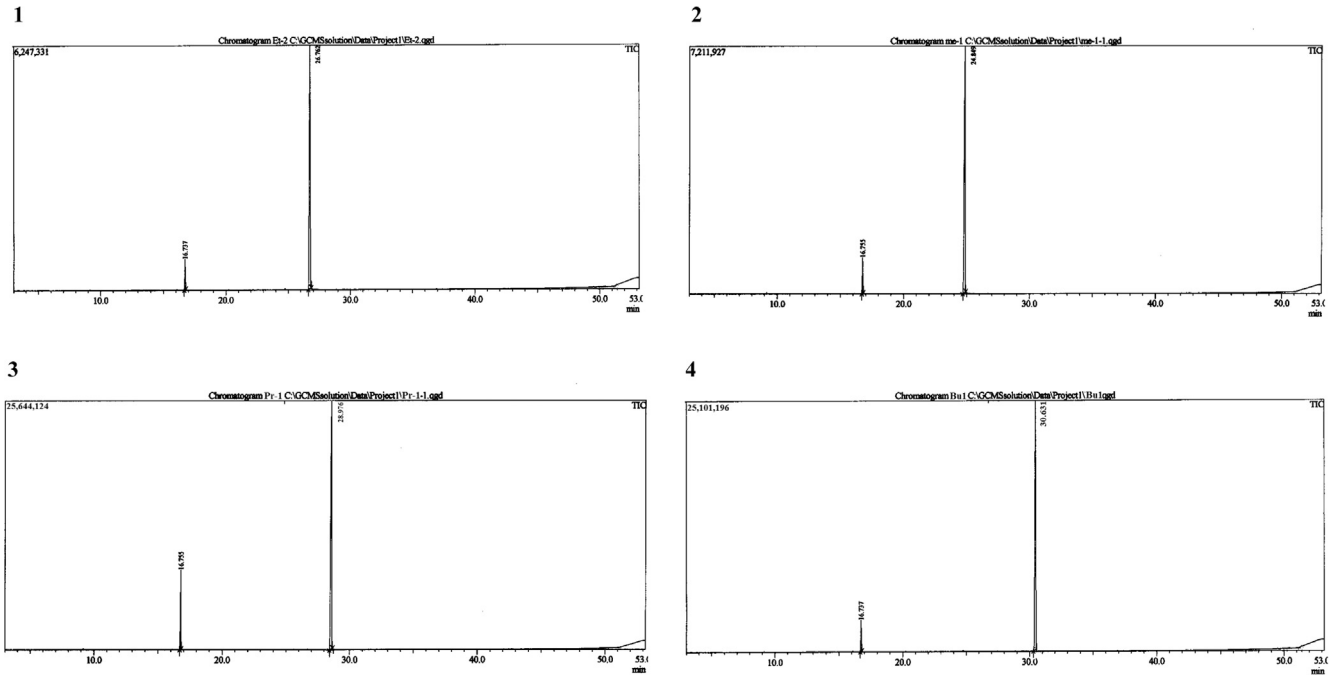

Fig. 3. GC chromatograms resulted from reflux of Benzyl isothiocyanate with ethanol (**1**), methanol (**2**), 2-propanol (**3**) and *n*-butanol (**4**).

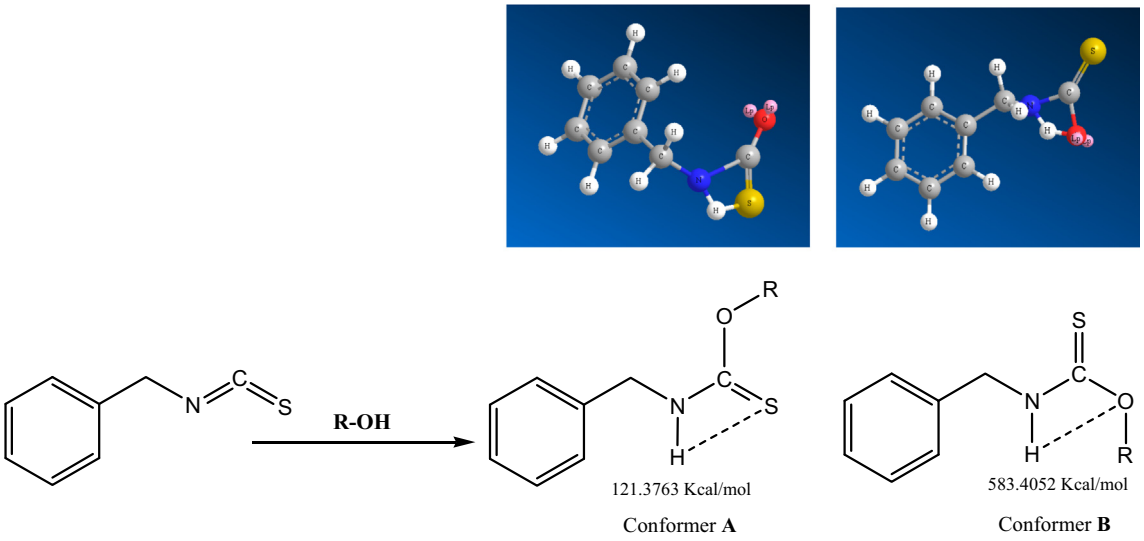

Fig. 4. Addition of alcohols on benzyl isothiocyanate to form two conformers.

the $CHCl_3$ fraction of the methanol extract enables the conclusion that it is formed during extraction with the solvent.

To explore the possible effect of extraction solvent on benzyl isothiocyanate, aliquots of 100 mg each were kept with $CHCl_3$, acetone, diethyl ether, ethyl acetate, ethanol, methanol, 2-propanol and *n*-butanol at room temperature for 72 h. Partial conversion of benzyl isothiocyanate to **1–4** was observed in ethanol, methanol, 2-propanol and *n*-butanol solutions respectively (Fig. 2). All solutions were heated under reflux for 8 h. Benzyl isothiocyanate was almost completely converted to **1**, **2, 4** in ethanol, methanol and *n*-butanol solutions respectively. However, only 40% conversion of benzyl isothiocyanate was observed with the secondary alcohol 2-propanol to give **3**. Other solvent showed no interaction with benzyl isothiocyanate. Compounds **1–4** showed no antimicrobial activity.

4. Conclusion

Extraction of Siwak with ethanol and methanol resulted in the conversion of the active antimicrobial compound benzyl isothiocyanate into the inactive *O*-ethyl benzyl thiocarbamate **1** and *O*-methyl benzyl thiocarbamate **2**, respectively. Reflux of benzyl isothiocyanate with 2-propanol and *n*-butanol resulted in the formation of *O*-isopropyl benzyl thiocarbamate **3** and *O*-butyl benzyl thiocarbamate **4**. Each compound (**1–4**) present in two conformational isomers as indicated from the NMR data. $CHCl_3$, acetone, diethyl ether and ethyl acetate didn't affect benzyl isothiocyanate even after heating under reflux. As analyses of marketed Siwak products indicated that they are all free from benzyl isothiocyanate. This fact is most likely due to the improper extraction or preservation of Siwak as the commonly used solvent for

extraction is ethanol. It is recommended that extraction with alcohols should be avoided with Siwak to keep its antimicrobial properties.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jsps.2018.11.001.

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