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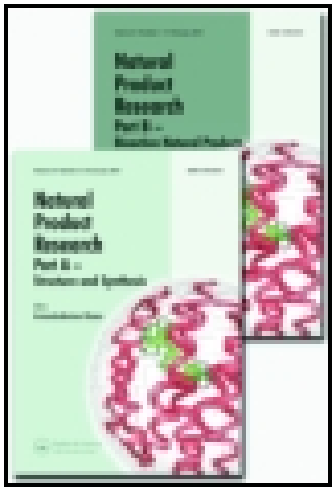

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Tamara Mengoni[a], Dolores Vargas Peregrina[a], Roberta Censi[a], Manuela Cortese[b], Massimo Ricciutelli[b], Filippo Maggi[a] & Piera Di Martino[a]

[a] School of Pharmacy, University of Camerino, Camerino, Italy
[b] HPLC-MS laboratory, University of Camerino, Camerino, Italy

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[a]*School of Pharmacy, University of Camerino, Camerino, Italy;* [b]*HPLC-MS laboratory, University of Camerino, Camerino, Italy*

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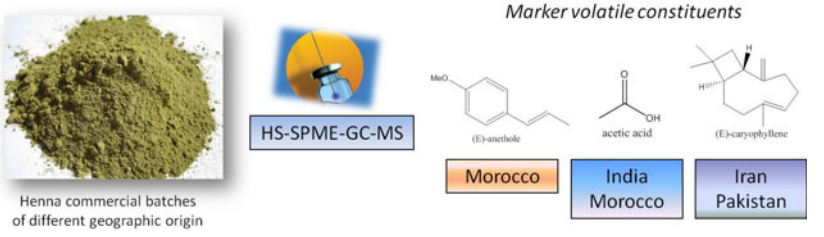

The aim of this work was to provide a characterisation of volatile constituents from different commercial batches of henna (*Lawsonia inermis*) leaves of different geographic origin. Headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC–MS) was used for the purpose. A total of 72 components were identified by GC–MS in the headspace of different henna samples which proved to differ considerably from each other, because they were characterised by different classes of components, mainly aliphatic compounds (9.0–64.7%), terpenoids (5.8–45.5%) and aromatics (7.9–45.2%), with alkanes (0.9–18.5%), aldehydes (2.1–18.8%) and carboxylic acids (3.1–29.3%), monoterpenes (3.4–30.0%) and sesquiterpenes (0.8–23.7%) and phenyl propanoids (0.6–43.1%), being the most abundant, respectively. Major representatives of these groups were *n*-hexadecane (0.5–4.7%), (2*E*)-hexenal (0.5–11.7%) and acetic acid (2.8–24.5%), limonene (0.8–14.7%), carvol (3.8–7.1%), geranyl acetone (1.4–7.9%) and (*E*)-caryophyllene (3.3–8.4%), and (*E*)-anethole (0.6–35.0%), respectively. We assume that factors such as the manufacturing process, the storage conditions and the different geographic origin of the samples may contribute to such variability.

Keywords: henna; *Lawsonia inermis*; HS-SPME; GC–MS

1. Introduction

Derived from cut and dried leaves of *Lawsonia inermis* L. (Lythraceae), henna has been used for centuries for body art, especially in Arabic and Hindu cultures (De Groot 2013). Today, henna is used as a hair dye and for temporary tattoos on the skin, frequently for self-use and easy domestic application, because of its strong colouring properties from the active component lawsone (2-hydroxy-1,4-naphthoquinone) and because people perceive henna as a natural product without the unfavourable effects of chemical colouring agents. Henna use has increased recently because of the fashion of temporary tattoos in both Europe and the USA, even if its use

*Corresponding author. Email: filippo.maggi@unicam.it

in the USA is not approved by the food and drug administration (FDA), which does not classify lawsone as a colourant permitted for direct application on the skin (FDA 2012). On the contrary, henna is authorised as a colouring agent for hair, and is widely available in India, the Middle East, Europe, Australia, Canada and the USA.

The polar fraction of *L. inermis* has been characterised in depth in several studies.

Polar components identified in henna aerial parts are aldehydes, organic acids, flavonoids, phenolic compounds (Siddiqui & Kardar 2001; Mikhaeil et al. 2004; Hema et al. 2010) and condensed tannins (Musa & Gasmelseed 2012). Also identified were 1-methyl-1-*H*-pyrrole, indole, aromatic compounds, such as toluene and styrene, and aliphatic compounds, many of them appearing to be unsaturated (Keheyan & Giulianelli 2006). Large amounts of carbohydrates, phenolic glycosides, gums and mucilages were also found (Jain et al. 2010; Hsouna et al. 2011).

Concerning non-polar components, a few studies have reported the occurrence of sterols (Siddiqui & Kardar 2001), aliphatic compounds, aromatic and heterocyclic compounds (Hema et al. 2010; Hsouna et al. 2011), and free and esterified fatty acids (Hsouna et al. 2011; Jacob & Saral 2013).

Studies focused on qualitative analysis of volatile components in henna as a finished product, that is, sold in the market as a hair dye, however, are lacking. Thus, the aim of our work was to provide a characterisation of volatile profiles of henna samples. Starting from different commercial henna hair, hand and tattoo batches found in the retail trade, we performed a headspace solid-phase microextraction (HS-SPME) coupled to gas chromatography–mass spectrometry (GC–MS) analysis to characterise the volatile constituents occurring in different commercial samples of different geographic origin. We used SPME technique because this is a non-destructive and non-invasive method to evaluate aroma compounds in different kinds of matrices (Demyttenaere et al. 2001; Hamm et al. 2003; Demyttenaere, Dagher, et al. 2003a, Demyttenaere, Sánchez Martinez, et al. 2003b; Demyttenaere, Moriña, et al. 2004a, Demyttenaere, Vanoverschelde, et al. 2004b; Van Lancker et al. 2008). It has been largely applied to aroma analysis in combination with GC and GC–MS, offering solvent-free and rapid sampling with low cost and ease of operation; moreover, it is sensitive, selective and also compatible with low detection limits (Pawliszyn 1997).

2. Results and discussion

The volatile components detected in the headspace of the six henna samples are reported in Table 1, while the relative chromatograms are depicted in Figure S1. A total of 72 components were identified in the headspace of the six henna samples, accounting for 69.0–91.4% of the total peak areas. The samples proved to differ considerably from each other, because they were characterised by different classes of components, mainly aliphatic compounds (9.0–64.7%), terpenoids (5.8–45.5%) and aromatics (7.9–45.2%), with alkanes (0.9–18.5%), aldehydes (2.1–18.8%) and carboxylic acids (3.1–29.3%), monoterpenes (3.4–30.0%) and sesquiterpenes (0.8–23.7%), and phenyl propanoids (0.6–43.1%), being the most abundant, respectively. Major representatives of these groups were *n*-hexadecane (0.5–4.7%), (2*E*)-hexenal (0.5–11.7%) and acetic acid (2.8–24.5%), limonene (0.8–14.7%), carvol (3.8–7.1%), geranyl acetone (1.4–7.9%) and (*E*)-caryophyllene (3.3–8.4%), and (*E*)-anethole (0.6–35.0%), respectively.

Interestingly, acetic acid is a marker of fermentation, which probably occurs during manufacturing of henna. Being a weak acid, acetic acid slightly lowers pH of henna solution (about 5.5), allowing to extend the duration of the colour (Leung & Foster 2003). Therefore, samples with high levels of this compound may be considered of higher stability.

Table 1. Headspace components of henna samples determined by GC–MS.

N.	Component[a]	RI calc.[b]	RI lit.[c]	Samples						ID[d]
				Mogano IRAN	Mohini INDIA	Minardi INDIA	Sahara MOROCCO	Zarqa PAKISTAN	Leaves MOROCCO	
1	Formic acid	510	512				3.3		0.3	Std
2	Acetic acid	644	645	11.9	24.5	6.8	26.0	13.8	2.8	Std
3	Hexanal	803	801	0.6		0.9		1.0		Std
4	Furfural	832	825	1.1	1.5	0.4		3.3	0.2	RI,MS
5	(2*E*)-Hexenal	858	857	1.4	0.6	1.4	11.7	0.5	1.9	RI,MS
6	(3*Z*)-Hexenol	863	850					0.4		RI,MS
7	2-Acetylfuran	916	900	2.4	0.5			5.3	1.2	RI,MS
8	1,2-Butanolide	924	914		0.4					RI,MS
9	Benzaldehyde	962	961	2.1	0.4	2.2			0.3	Std
10	1-Octen-3-ol	986	982	0.3						Std
11	6-Methyl-5-hepten-2-one	991	981	1.2			1.3	1.7		RI,MS
12	*n*-Decane	1001	1000			0.9				Std
13	(2*E*,4*E*)-Heptadienal	1014	1015			1.2				RI,MS
14	*p*-Cymene	1024	1023					3.0		Std
15	Limonene	1031	1026	14.7	1.0	3.0	2.7	0.8	0.4	Std
16	Benzyl alcohol	1042	1037		2.6				0.7	RI,MS
17	Fenylacetaldehyde	1046	1047	3.8	3.0	3.2	8.2		0.4	RI,MS
18	γ-Terpinene	1060	1065	0.8				1.2		Std
19	3,5-Octadien-2-one	1076	1076			2.1				RI,MS
20	*p*,α-Dimethylstyrene	1089	1089					5.0		RI,MS
21	(*E*,*E*)-3,5-Octadien-2-one	1096	1098			1.0				RI,MS
22	Linalool	1103	1100	1.2			1.0	4.7	1.5	Std
23	*n*-Nonanal	1105	1101		1.1		3.4			RI,MS
24	6-Methyl-3,5-heptadiene-2-one	1108	1110	2.0		3.5				RI,MS
25	2-Phenylethanol	1117	1124		1.1				0.8	RI,MS
26	Camphor	1141	1144	0.2		0.4			2.2	Std
27	Menthone	1153	1155	0.7		1.2			0.7	RI,MS

(*Continued*)

Table 1. (*Continued*).

N.	Component[a]	RI calc.[b]	RI lit.[c]	Samples						ID[d]
				Mogano IRAN	Mohini INDIA	Minardi INDIA	Sahara MOROCCO	Zarqa PAKISTAN	Leaves MOROCCO	
28	Isopropyl-1-methylcyclohexanol	1163	1156				2.1		2.9	RI,MS
29	Terpinen-4-ol	1177	1177	1.5				0.4		Std
30	α-Terpineol	1191	1183	0.6				1.5	1.3	Std
31	Butoxyethoxyethanol	1193	1192				1.3			RI,MS
32	Methyl chavicol	1196	1198	1.2		1.3			2.0	RI,MS
33	*n*-Dodecane	1201	1200			1.9				Std
34	*n*-Decanal	1206	1205		1.8		3.6			Std
35	β-Cyclocitral	1217	1218	0.4		0.6		0.7		Std
36	Pulegone	1236	1233						1.1	RI,MS
37	Carvol	1242	1253	4.2		3.8			7.1	RI,MS
38	Anisaldehyde	1251	1249	1.0		0.3			3.0	RI,MS
39	Linalyl acetate	1256	1254	1.6						RI,MS
40	(*E*)-Anethole	1281	1279	6.6	0.6	3.8		0.6	35.0	Std
41	*n*-Tridecane	1294	1300					0.5		Std
42	Carvacrol	1299	1297	0.4						Std
43	Eugenol	1355	1346				1.6	2.3	3.1	Std
44	3,4-Dimethylacetophenone	1358		2.9	2.0					MS
45	α-Copaene	1369	1370	1.4		5.2		0.4	2.7	RI,MS
46	Diphenyl ether	1393	1393		0.9					RI,MS
47	*n*-Tetradecane	1398	1400	0.9	1.1		0.7	1.2	0.9	Std
48	β-Cedrene	1403	1400	2.5					0.8	RI,MS
49	(*E*)-caryophyllene	1411	1414	3.8		8.4		5.0	3.3	Std
50	Octylcyclohexane	1440	1439	0.3						RI,MS
51	2-Methoxynaphthalene	1441	1433		1.5					RI,MS
52	α-Humulene	1446	1447	0.3				1.5	0.7	Std
53	Geranyl acetone	1453	1453	4.2	2.5	5.4	1.4	7.9	2.5	RI,MS
54	α-Isomethyl ionone	1474	1473		1.3					RI,MS

(*Continued*)

Table 1. (*Continued*).

N.	Component[a]	RI calc.[b]	RI lit.[c]	Samples						ID[d]
				Mogano IRAN	Mohini INDIA	Minardi INDIA	Sahara MOROCCO	Zarqa PAKISTAN	Leaves MOROCCO	
55	*ar*-Curcumene	1478	1477	0.7		0.7		7.9		RI,MS
56	(*E*)-β-Ionone	1482	1486	1.9	1.0	2.6	1.4		2.3	Std
57	α-Zingiberene	1491	1492					1.0		RI,MS
58	*n*-Pentadecane	1496	1500	0.6	4.1		1.4			Std
59	Dihydroactinidiolide	1517	1513	5.4	2.3	6.8	4.7			RI,MS
60	β-Sesquiphellandrene	1519	1518					7.3	1.9	RI,MS
61	Caryophyllenyl alcohol	1561	1569	0.4		1.7				RI,MS
62	2-Methylpentadecane	1560	1562		1.2					RI,MS
63	Caryophyllene oxide	1570	1571	2.7		5.6				Std
64	*n*-Hexadecane	1598	1600	0.5	4.7	0.9	2.5	0.6		Std
65	Isopropyl dodecanoate	1622	1618				5.4			RI,MS
66	*ar*-Turmerone	1656	1660					0.5		RI,MS
67	*n*-Heptadecane	1699	1700		4.4		2.0			Std
68	*n*-Octadecane	1800	1800		3.0					Std
69	Hexahydrofarnesyl acetone	1836	1838	1.3		1.8	0.8		0.6	RI,MS
70	Farnesyl acetone	1900	1895			0.3			0.2	RI,MS
71	Methyl palmitate	1911	1909			0.1		0.5		RI,MS
72	Methyl oleate	2094	2095			0.2		0.3		RI,MS
	Total identified (%)			91.4	69.6	79.3	86.6	80.6	84.7	
	Grouped compounds (%)									
	Aliphatics			20.7	48.0	21.2	64.7	23.7	9.0	
	Aldehydes			3.1	5.0	3.9	18.8	4.8	2.1	
	Ketones			3.1		6.6	1.3	1.7		
	Esters					0.3	5.4	0.8		
	Alkanes			2.3	11.1	3.6	6.5	2.3	0.9	
	Carboxylic acids			11.9	24.5	6.8	29.3	13.8	3.1	
	Aromatic compounds			17.5	12.0	10.7	9.9	7.9	45.2	
	Terpenoids			50.9	8.1	47.4	12.03	43.7	29.3	
	Monoterpene hydrocarbons			15.4	1.0	3.0	2.7	5.0	0.4	

(*Continued*)

Table 1. (*Continued*).

N.	Component[a]	RI calc.[b]	RI lit.[c]	Samples						ID[d]
				Mogano IRAN	Mohini INDIA	Minardi INDIA	Sahara MOROCCO	Zarqa PAKISTAN	Leaves MOROCCO	
	Oxygenated monoterpenes			14.5	2.5	10.8	2.4	14.5	16.4	
	Sesquiterpene hydrocarbons			8.7		14.3		23.0	9.4	
	Oxygenated sesquiterpenes			4.5		9.4	0.8	0.5	0.8	
	Norisoprenoids			2.3	2.4	3.1	1.4	0.7	2.3	
	Other terpenoids			5.4	2.3	6.8	4.7			
	Others			2.4	0.9			5.3	1.2	

[a] Compounds are listed in order of their elution from a HP-5MS column.
[b] Linear retention index on HP-5MS column, experimentally determined using homologous series of C_8–C_{30} alkanes.
[c] Relative retention index taken from Adams and NIST 08 libraries for apolar capillary column.
[d] Identification methods: MS, by comparison of the mass spectrum with those of the computer mass libraries Wiley, Adams, FFNSC2 and NIST 08; RI, by comparison of RI with those reported in the literature (Adams 2007; NIST 08 2008; FFNSC2 2012); STD, by comparison of the retention time and mass spectrum of available authentic standard.

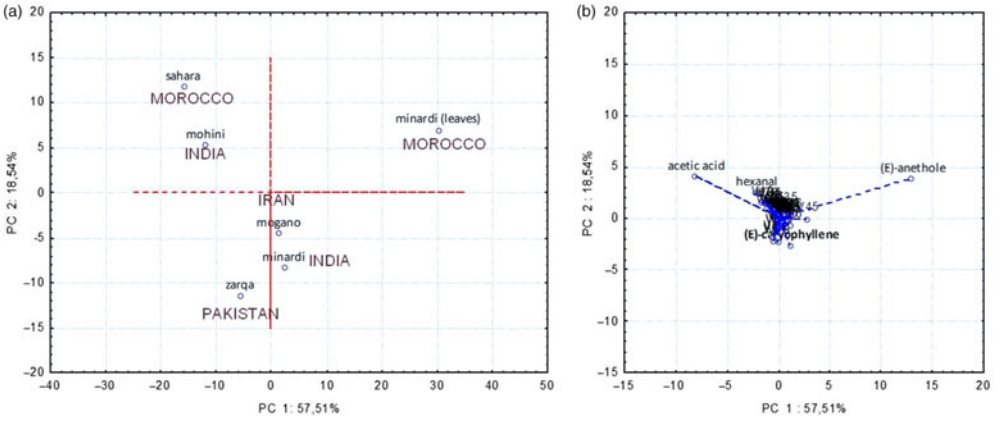

Figure 1. (a) Score plot (PCA) for main variation of volatile components among henna samples. (b) The PCA loading plot for henna volatiles extracted by HS-SPME.

Other components present in noteworthy levels were the sesquiterpenes *ar*-curcumene (7.9%) and β-sesquiphellandrene (7.3%) in the Zarqa sample (Pakistan) and the lactone dihydroactinidiolide (2.3–6.8%) in the Mogano (Iran), Mohini (India), Minaridi (India) and Sahara (Morocco) samples.

Multivariate analysis showed that the variability of data was generated mostly by the content of acetic acid, (*E*)-anethole and (*E*)-caryophyllene, as shown in the loading plot (Figure 1(b)). They were correlated with henna samples taking place in the same position of the score plot (Figure 1(a)). Sample 6 from Morocco was correlated with (*E*)-anethole, while samples 2 and 5 from India and Morocco, respectively, were mainly characterised by high levels of acetic acid. Finally, samples 1, 3 and 4, from Iran and Pakistan, respectively, were mostly correlated with sesquiterpenes such as (*E*)-caryophyllene.

3. Materials and methods

See Supplementary materials.

4. Conclusions

Our study found significant variability in the headspace volatile components of the different henna products analysed, confirmed by the principal component analysis. We assume that factors such as the manufacturing process, the storage conditions and the different geographic origin of the samples may contribute to such variability. Results obtained by SPME analysis showed different chromatographic profiles for the six henna samples, leading to the conclusion that the volatile components in the samples of henna are heterogeneous, depending on the different origin and manufacturing process of the samples. Since there are no studies in the literature that offer qualitative and semi-quantitative analysis and comparison of data between different samples of commercial henna to highlight their variability, the results of this project fill a gap in knowledge about this plant derivative that is widely used for cosmetic purposes, but still poorly understood.

Supplementary material

Supplementary material relating to this paper are available online at http://dx.doi.org/10.1080/14786419.2015.1055491, alongside Table S1 and Figure S1.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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