Metabolome-Based Analysis of Herbal Cough Preparations Via Headspace Solid-Phase Microextraction GC/MS and Multivariate Data Analyses: A Prospect for Its Essential Oil Equivalency

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ABSTRACT: Liquid cough preparations containing essential oils pose a challenge for isolating and quantifying their volatile components from such a complex matrix enriched with nonvolatile constituents and excipients. This study aims to develop a strategy integrating QC analysis of seven natural cough preparations in the Egyptian market and to assess volatile variation among the preparations using multivariate data analyses. Cough preparations were subjected to headspace solid-phase microextraction (HS-SPME) for determination of their essential oil composition mediating for their actions and to assess volatile differences among them. HS-SPME is a suitable technique for sample preparation that allows for extraction and enrichment of volatiles from complex nonvolatile matrices and their direct desorption into the gas chromatography analytical system. A total of 88 volatile components were identified belonging to seven classes, viz., aromatics, aliphatic hydrocarbons, mono/sesquiterpene hydrocarbons, and oxygenated mono/sesquiterpenes. Oxygenated monoterpenes, viz., menthol, cineole, thymol, and (E)-anethole, were the major volatiles identified in five cough preparations (79.5−98.6%), whereas aromatics, chiefly cinnamate derivatives, constituted the second class amounting for 50.5 and 27.4% in the other two cough preparations. Meaningful results regarding the products’ efficacy and safety were extrapolated from this analytical procedure, where artificial preservatives (parabens) were detected in five cough preparations. This study established an efficient strategy for exploring volatile profiling and defining different markers among the different cough preparations. Additionally, authenticity of listed herbal ingredients in the cough preparations was also confirmed in certain preparations, while other formulations failed to show representative volatile components. Volatile variation among preparations was assessed using multivariate data analyses in an attempt to prioritize cough preparations for usage, suggesting the preference of Bronchicum and Babetone among examined cough products.

INTRODUCTION

Acute cough is a troublesome manifestation of infectious or noninfectious causes, affecting the upper respiratory tract. The effect of persistent cough itself may be harmful and deleterious to patients, particularly infants, by interfering with breathing, and leads to further complications including syncopal episodes, urinary incontinence, muscle ache, insomnia, and fatigue if untreated. Thus, cough is a symptom with many facets: a protective mechanism for the lungs, a warning sign of disease, and a detrimental symptom when it is persistent. To mitigate against cough complications, early diagnosis and management of chronic productive cough are warranted for sustaining a healthy lung. Cough treatment primarily targets managing the underlying cause; hence, cough remedies are categorized as either antitussives or expectorants, viz., mucolytic, proteolytic enzymes, antihistamines, and bronchodilators. A large number of patients self-prescribe nonprescription over-the-counter cough medi-

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several biological activities such as antimicrobial, anti-inflammatory, antioxidant, expectorant, sedative, and bronchodilator effects, all of which support attenuating cough manifestations. Natural aromatic drugs mostly incorporated in herbal cough remedies include that of anise (Pimpinella anisum L. Apiaceae), eucalyptus (Eucalyptus globules, Myrtaceae), fennel (Foeniculum vulgare, Apiaceae), guava (Psidium guajava L., Myrtaceae), thyme (Thymus vulgaris L., Lamiaceae), and tilia (Tilia cordata Mill, Tiliaceae).6

Gas chromatography coupled to mass spectrometry (GC−MS) is the analytical platform of choice for the separation and identification of main essential oil constituents. Nevertheless,

Figure 1. SPME-GC−MS chromatogram of headspace volatiles collected from cough products; Alveolin-P, Sinawet, Alveolin, Babytone, guava, Bronchicum, and Pentamix. The corresponding compound names for volatile peaks follow those listed in Table 2. 33, cineole; 13, methylparaben; 55, anethole; 15, propylparaben; 47, menthol; 54, thymol; 8, methylcinnamate; 12, ethylcinnamate; 35, fenchone; and 82, β himachalene.

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when essential oils are present in complex mixtures containing nonvolatile components, *viz.* sugars, as typical in cough preparations or when present at low levels, a selective and sensitive extraction step for volatiles is warranted. Compared to steam distillation in which heat is applied for volatile extraction, headspace solid-phase microextraction (HS-SPME) is an alternative technique used for volatile extraction, found superior to steam distillation for being solvent-free and involving no heat application. Additionally, SPME allows for the isolation and enrichment of volatiles from gas or liquid samples, over a fused-silica fiber and then subsequent desorption of these analytes directly into the GC injection port. This operation allows for the assessment of the true aroma in investigated cough preparations without development of artifacts by extraction and meanwhile favors the detection of less-abundant volatiles.

The main goal of this study was to analyze essential oil components present in cough preparations using metabolomics tools coupled to multivariate data analyses for their classification and or volatile marker identification. Another goal of this study was to establish the feasibility of the technique employed herein, that is, HS-SPME as a fast and efficient method for the quality control assessment of cough preparations, authenticating their volatile composition in accordance with the manufacturers’ labels. Multivariate data analysis provides powerful exploratory tools to untangle and visualize omics data by compression and de-meaningful results from such complexity, *viz.*, principal component analysis (PCA) and orthogonal projections to least squares-discriminant analysis (OPLS-DA). Seven cough preparations formulated as syrups (Alveolin-P, Sinawet, Alveolin, Bebetone, guava, Bronchicum and Pentamix) and of common usage in the Egyptian market were the working model of this study. For Pentamix, two different batches were obtained from the same supplier and included in the analysis.

## RESULTS AND DISCUSSION

### GC–MS Volatile Profiling in Cough Preparations

The main goal of this study was to assess volatile composition in cough preparations in an attempt to (a) provide a better quality control method for their volatile analysis in such a matrix containing nonvolatiles, (b) rationalize for their effective and safe usage for cough treatment, and (c) prioritize preparations based on its volatile profile. SPME volatile analysis led to the identification of 88 volatile components (Table 2, Figure 1) belonging to seven classes, *viz.*, oxygenated mono/sesquiterpenes, aromatic compounds, oxygenated aliphatic compounds, and mono/sesquiterpene hydrocarbons.

**Oxygenated Monoterpenes.** Oxygenated monoterpenes constituted the major components in five of the analyzed cough preparations amounting for 98.6, 93.5, 81.7, 80.7, and 79.4% of all volatiles identified in Pentamix, Sinawet, Alveolin-P, Bronchicum, and Alveolin, respectively. The cyclic monoterpeno alcohol menthol was the principal volatile detected in the two products Pentamix and Sinawet, detected at 94.2 and 45.2%, respectively. Interestingly, neither of these preparation labels indicated oil of Mentha in its formulation (Table 1), which is also evident by the detection of menthol-related peaks, that is, neomenthol and other minor constituents, *viz.*, menthone and isomenthone (0.5, 1.4, and 0.2%, respectively) in Pentamix. Although stated to contain fennel, eucalyptus, and thyme extracts (Table 1), Pentamix profile revealed only traces of the marker monoterpenes for these extracts, where (*E*)-anethole, cineole, thymol, and carvacrol were detected at trace levels of 0.03, 0.1, 0.7, and 0.9%, respectively. These results suggest that

| Table 1. Commercial Cough Preparations Included in This Study and Their Composition |
| --- |
| No. | Preparation name | Manufacturer | Composition |
| 1 | Alveolin-P | EGPI (Egyptian group for pharmaceutical industries) | 1. Each 5 mL contains: Grindelia herb liquid extract 16.65 mg, thyme herb liquid extract 16.65 mg, menthol, and menthone each 1.35 mg, (standardized to contain 0.6% grineolin) |
| 2 | Sinawet | Sigma pharmaceutical Industries | 2. Each 5 mL contains: inositol 0.10 mg, Grindelia root extract 3.3 ml (standardized to contain 0.06% grineolin), thyme herb liquid extract 16.65 mg (standardized to contain 0.01% *thymus vulgaris*, *E*-limonene, *E*-menthene, *E*-caryophyllene, and bergapten) |
| 3 | Alveolin | EGPI (Egyptian group for pharmaceutical industries) | 3. Each 5 mL contains: Pimpenella root liquid extract 0.005 ml (standardized to contain 0.03% geraniol), thyme herb liquid extract 16.65 mg, t-anethole, and Bee propolis extract 20 mg (standardized as: NLT 0.1% total flavonoids, rose hip flower and shell extract 0.015 g, (standardized as: NLT 0.01% total flavonoids, *E*-menthol, and menthone each 0.05 mg (standardized to contain 0.05%) |
| 4 | Bronchicum | Sano pharmaceutical industries | 4. Each 5 mL contains: fennel fruit extract (1:2−3) 5 g (phenolic compound calculated as thymol % not less than 0.03%), Primula fluid extract (1:2−2.5) 2.5 g (premulic acid = not less than 0.25%) |
| 5 | guava | Pharaonia, Egypt | 5. Each 100 mL contains: guava leaf extract 5 g (standardized as: NLT 0.1% total flavonoids, rose hip flower and shell extract 0.015 g, (standardized as: NLT 0.01% total flavonoids, *E*-menthol, and menthone each 0.05 mg (standardized to contain 0.03%) |
| 6 | Bronchicum | Sano pharmaceutical industries | 6. Each 5 mL contains aqueous guava leaf extract 0.125 g, potato powder extract 5 g, phenolic compound calculated as thymol % not less than 0.03%, Primula fluid extract (1:2−2.5) 2.5 g, pressed as: NLT 0.1% total flavonoids, rose hip flower and shell extract 0.015 g, (standardized as: NLT 0.01% total flavonoids, *E*-menthol, and menthone each 0.05 mg (standardized to contain 0.03%) |
| 7 | Pentamix | DBK for pharmaceutical industries, Egypt | 7. Each 10 mL contains: guava leaf extract 0.5 g, pressed as: NLT 0.1% total flavonoids, rose hip flower and shell extract 0.015 g, (standardized as: NLT 0.01% total flavonoids, *E*-menthol, and menthone each 0.05 mg (standardized to contain 0.03%) |
either the used herbal materials were not assayed for their essential oil content or that the extraction technique and/or processing procedures failed to extract or preserve the essential oils in the product.

1,8-cineole is a monoterpenoid oxide, well recognized for its antiseptic, mucolytic, and spasmytic actions on the respiratory tract in addition to its therapeutic benefits in inflammatory airway diseases, that is, asthma and chronic obstructive pulmonary disease. Marked levels of cineole were detected in Alveolin and Sinawet syrups at 77 and 47%, suggesting the presence of eucalyptus and thyme extracts in these products, respectively. However, only negligible amounts of cineole were perceived (less than 1% of total volatiles) in all other preparations’ volatile profile despite that they are all (except guava syrup) labeled to include either eucalyptus or thyme extracts. Such findings warrant more for developing marker assays for each essential oil in its finished cough product to be assayed for efficacy prediction.

The natural terpenoid thymol and its positional isomer carvacrol were the major constituents in thyme oil, which is frequently incorporated in cough preparations for its antimicrobial, antitussive, and expectorant effects. Although being stated in most cough preparations as thyme extract, highest thymol and carvacrol levels were detected in Bronchicum and Babetone volatile profiles at 48.6/27.3 and 7.8/4.3%, respectively, and with carvacrol detected exclusively in Alveolin-P at 5.7%. Much lower levels of both positional isomers were detected in Alveolin, Sinawet, and Pentamix at less than 1%, implying that herbal extracts should be standardized for their marker constituents. These markers and their relative levels need to be clearly stated in the preparations’ label to assist in dose examination and efficacy verification.

(E)-Anethole (anise camphor) is an aromatic ether that is widely used as a flavoring agent and contributes to the odor and flavor of anise and fennel. Both anise and fennel were listed in three preparations, viz. Pentamix, Babytone, and Alveolin-P, either as oil or fruit extract (Table 1). As expected, the highest level of (E)-anethole was found in the Alveolin-P profile (66.4%), which includes anise oil, anise extract, whereas Babytone and Pentamix preparations incorporating fennel extracts revealed much lower levels (1.5 and 0.03%, respectively). Interestingly, (E)-anethole was also detected at 21.5% in the volatile blend of guava syrup, although it was not stated as an active ingredient in its label. Apart from imparting an agreeable flavor to liquid formulations, (E)-anethole is likely to function as an expectorant, antimicrobial, anti-inflammatory, and a sweetening agent in cough products. Estragole (4-allylanisole, methyl chavicol, or 1-methoxy-4-prop-2-enylbenzene) is a naturally occurring volatile common in Apiaceae members, particularly fennel. Genotoxic and carcinogenic effects of estragole have been recognized, a matter that demands accurate and specific methods for the determination of estragole in herbal pharmaceutical products. Estragole was detected in three cough products at relatively low levels ca. 0.5, 2, and 2.7% in Babetone, Alveolin-P, and guava, respectively. Even though no safe exposure limit has been determined for estragole, its detection at such reduced levels guarantees the preparation safety.

Aromatic Compounds. Aromatic compounds were detected in most preparations ranging from 10–50%, with highest levels detected in Babetone exemplified in cinnamate methyl and ethyl esters at 22 and 24%, respectively. Cinnamate esters are important flavoring compounds additional to their potent biological effects, viz. antioxidant, antimicrobial, hepatoprotective, and anxiolytic activities. The abundance of cinnamates in Babetone volatile profile is likely attributed to its bee propolis content, aside from thyme and fennel (Table 1). Methyl-4-methoxysalicylate isomers collectively amounted for 14% of the aromatic volatile blend of Bronchicum, which are likely to be derived from Primula fluid extracts, accentuating their expectorant and antimicrobial medicinal benefits. Methyl and propyl parabens were both detected with appreciable amounts (2.3–13.5%) in all examined preparations, except for Babetone, which revealed 2.8% of methyl parabens, whereas Bronchicum and Pentamix were almost free of parabens. Parabens are usually added by manufacturers as an antimicrobial preservative in several liquid pharmaceutical products, although there are some health concerns about their safety for consumption by pregnant women and infants, although not stated in cough products’ label composition. Absence of parabens in Bronchicum cough preparation confirms the natural origin of all its ingredients as revealed by SPME and provides a wider safety margin for its usage. Two benzoic acid derivatives (4-isopropoxybenzoic acid and benzoic acid, 4-propyloxy-, propyl ester) were detected in guava syrup and collectively amounted for 15.1% of its total volatile profile. These metabolites are not likely to be derived from the listed plant ingredients used, that is, tilia or guava but are more prone to be artifacts during preparation and/or extraction.

Oxygenated Hydrocarbons (Oxylipids). Insignificant amounts of oxygenated hydrocarbons were present in the volatile blend of all examined cough preparations, except for Babetone, which contributed 2.8% of the total identified volatiles. Vaccenol and sorbic acid (1.6 and 0.5%, respectively), in addition to traces of other fatty acids, mark a positive identity for rose hip flower extract, indicated in Babetone syrup (Table 1). In that respect, Alveolin and Alveolin-P labels are stated to include flora rosa liquid extract (Table 1), yet its volatile profile revealed for negligible amounts of these marker compounds. Anti-inflammatory and immunomodulatory effects of short chain fatty acids, viz. sorbic acid, has been recently reported, which augments its usage in relieving cough manifestations.

Sesquiterpene Hydrocarbons. A total of 22 sesquiterpene hydrocarbons were identified in the volatile profiles of four of the examined cough preparations that are guava, Babetone, Alveolin, and Bronchicum at 35.9, 23.6, 8.1, and 2.8%, respectively. Guava profile was the richest in sesquiterpene hydrocarbons, supporting P. guajava identity in the preparation (Table 1) as guava encompasses a sesquiterpene-rich volatile profile. Analysis of rose hip flower volatiles present in Babetone indicated its enrichment in sesquiterpenes in addition to organic and fatty acids. The chemical composition of bee propolis volatiles is very dependent on floral origin and may justify for the enrichment of sesquiterpenes in Babetone. β-Himachalene, longifolene, α-murolene, and γ-himachalene were the major sesquiterpene hydrocarbons characterized in guava, Babetone, and Alveolin. Sesquiterpene hydrocarbons are known to exhibit anti-inflammatory, antimicrobial, and antinociceptive activities, aiding in treatment of such ailments. Farnesene and β-caryophyllene are two characteristic key odors of fennel that were detected in the Babetone volatile profile. In that regard, it should be noted that the Pentamix aroma profile did not reveal any sesquiterpene hydrocarbons (less than 1%), despite being indicated to contain fennel and guava on its label (Table 1).
identifiable. Babetone ranks the second as a cough preparation indicated label composition and moreover is devoid of any encompass genuine herbal ingredients matching the most to the can be concluded that Bronchicum syrup is the product to volatile pro-
cadinol. Higher levels of these compounds were expected in the preparations to include Babetone and guava at were also detected at trace levels in two of the analyzed cough veri-
ty the authenticity of the used herbal material and demands further assessment of their permissive limits is thus needed. The volatile composition as compared to the other five cough preparations, clustering in a separate group 1a. By examining the volatile profile of these two products (Table 2), it can be observed that aromatic and oxygenated monoterpenes amounted for more than 93% of their volatile profiles. Within groups, segregation was noted between Alveolin present on one side and Bronchicum, Babytone, guava, and Alveolin-P on the other side (Figure 3a). Interestingly, the close clustering of Pentamix products of different batch numbers confirms that at least for this product, no obvious difference exists in its volatile composition among the different batches.

In summary and in context of the identiﬁed volatile pool, it can be concluded that Bronchicum syrup is the product to encompass genuine herbal ingredients matching the most to the indicated label composition and moreover is devoid of any parabens. Babetone ranks the second as a cough preparation having consistency between formulation composition and identiﬁed volatiles, although entailing trace amounts of methyl parabens. All other preparations included parabens, and further assessment of their permissive limits is thus needed. The volatile proﬁle of Pentamix syrup did not reveal any of the marker volatiles characteristic of its ﬁve herbal ingredients and instead was suggested to contain Mentha oil. Both Alveolin and Alveolin-P share same composition, except for substituting eucalyptus in Alveolin with anise oil in Alveolin-P. Markers conﬁrming the presence of thyme, eucalyptus, primula, and anise oil were readily revealed from SPME volatile proﬁles of both preparations, whereas markers for proving rose ﬂowers’ and Grindelia’s presence are still missing. Sinawet volatile proﬁle only conﬁrmed thyme presence aside from including menthol, probably added as a ﬂavoring agent, although it was not reported later in its label. In contrast, guava preparation revealed markers of P. guajava, yet those of tilia were poorly represented.

Absolute quantiﬁcation of the major volatiles detected in preparations of known effect on cough was attempted for cineole, thymol, and carvacrol (Figure 2). Results revealed enrichment of cineole in Alveolin and Sinawet at 10 and 2.5 mg/mL. Cineole levels ranged in other preparations from 0.1 to 0.4 mg/mL. In contrast, Bronchicum followed by Babetone were the most abundant in thymol at 3.3 and 0.3 mg/mL, respectively, and found at ca. 0.1 mg/mL in guava and Alveolin, see Figure 2.

### Multivariate Data Analyses of Cough Preparations. Unsupervised Multivariate Data Analysis

Multivariate data analysis tools were employed to assess the differences between cough preparations regarding their volatile composition. Hierarchical cluster analysis (HCA) dendrogram examination of group 1a showed that Sinawet and Pentamix are closer in composition as compared to the other five cough preparations, clustering in a separate group 1a. By examining the volatile proﬁle of these two products (Table 2), it can be observed that aromatic and oxygenated monoterpenes amounted for more than 93% of their volatile proﬁles. Within groups, segregation was noted between Alveolin present on one side and Bronchicum, Babytone, guava, and Alveolin-P on the other side (Figure 3a). Interestingly, the close clustering of Pentamix products of different batch numbers conﬁrms that at least for this product, no obvious difference exists in its volatile composition among the different batches.

The PCA score plot revealed that cough preparations could be differentiated to some extent (Figure 3b), being segregated on both sides of PC1. In agreement with HCA, Pentamix, and Sinawet segregated on the positive side (positive PC1 values) versus negative score values for the other ﬁve cough products. The metabolite loading plot for PC1 (Figure 3c) revealed the most variant volatile components, mediating for such a scattering pattern. Menthol and cineole contributed most positively along PC1, while (E)-anethole was more abundant on the negative side, contributing for preparation segregation in PCA along PC1.

### Supervised Multivariate Data Analysis

OPLS-DA-derived S-plot provided a better overview of the classiﬁcation model metabolite marker outcome (Figure 4b). Markers revealed from the OPLS model included menthol, in agreement with PCA loading results (Figure 3c). Menthol comprised the major volatile detected in Pentamix and Sinawet, being absent from all other cough preparations (Table 2).

### CONCLUSIONS

To the best of our knowledge, this study presents the ﬁrst application of headspace SPME for the quality control analysis of cough preparations analyzed using chemometric tools. HS-SPME–GC–MS was employed for volatile proﬁling of seven cough preparations, with the analytical technique ﬁnding a
Table 2. Relative Percentage of Aroma Compounds Detected in Cough Products Analyzed Using SPME-GC-MS (n = 3)\textsuperscript{a}

| no | RT min | RI   | compound                          | Alveolin-P | Sinawet | Alveolin | Babetone | guava | Bronchicum | Pentamix |
|----|--------|------|-----------------------------------|------------|---------|----------|----------|-------|------------|----------|
|    |        |      | average (standard deviation)      |            |         |          |          |       |            |          |
|    |        |      | aliphatic hydrocarbon             |            |         |          |          |       |            |          |
| 1  | 12.558 | 1264 | tridecane                         | 0.1        | (0.04)  | 0.8      | (0.31)   | (0.19) |          | 0.43     |
| 2  | 14.75  | 1441 | pentadecane                        | 0.2        | (0.21)  | 0.02     | (0)      | (0)   | 0.03       | (0)      |
| 3  | 17.783 | 1647 | hexadecane                         | 0.1        | (0.05)  | 0.03     | (0.01)   | (0.02) |            | (0.03)   |
|    |        |      | total aliphatic hydrocarbons      | 0.4        | 0.8     | 0.5      | 0        |       |            |          |
|    |        |      | aromatic                           |            |         |          |          |       |            |          |
| 4  | 11.408 | 1179 | benzoic acid                       | 0.2        | (0.19)  | 0.9      | (0.08)   | (0.16) | (0.59)     | 0.2      |
| 5  | 12.45  | 1256 | ethyl salicylate                   | 0.1        | (0.02)  | 0.2      | (0.11)   | (0.4) |            | (0)      |
| 6  | 12.508 | 1261 | p-anisaldehyde                     | 0.6        | (0.46)  | 0.2      | (0.01)   | (0.01) | 0.02      | (0)      |
| 7  | 13.835 | 1366 | methyl-p-anisate                   | 0.04       | (0.01)  | 0.03     | (0.01)   | (0)   |            | (0)      |
| 8  | 13.851 | 1367 | methyl cinnamate                   | 22         | (2.9)   | 3        | (0.06)   |       |            |          |
| 9  | 14.117 | 1389 | 2’-hydroxy-5’-methoxy-acetophenone  | 0.03       | (0.06)  | 0.2      | (0.01)   | (0)   |            | (0.4)    |
| 10 | 14.492 | 1420 | methyl 4-methoxyisalicylate        | 0.01       | (0.02)  | 0.01     | (0.01)   | (0)   |            | (0.14)   |
| 11 | 14.783 | 1444 | ethyl cinnamate                    | 24         | (2.54)  | 0.2      | (0.01)   |       | 8          | (0.01)   |
| 12 | 14.825 | 1447 | methyl 4-methoxyisalicylate isomer | 0.1        | (0.01)  |          | 8        |       |            | (0)      |
| 13 | 14.975 | 1459 | methylparaben                      | 13.5       | (1.18)  | 4.3      | (0.9)    | (6.8) | 2.8        | 5.7      |
|    |        |      | total aromatics                    | 18.4       | 6.1     | 9.9      | 50.5     | 27.4  | 15.5       | 1.4      |
|    |        |      | monoterpene hydrocarbon            |            |         |          |          |       |            |          |
| 18 | 7.14   | 910  | α-thujene                          | 0.2        | (0.03)  | 0.01     | (0.01)   |       | 0.3       | (0.42)   |
| 19 | 8.117  | 966  | β-myrcene                          | 0.1        | (0.02)  | 0.01     | (0.01)   |       | 0.1       | (0)      |
| 20 | 8.743  | 1002 | O-cymene                           | 0.2        | (0.19)  | 0.4      | (0.02)   | (0)   | 0.01      | (0.04)   |
| 21 | 8.808  | 1006 | limonene                           | 0.4        | (0.02)  | 0.03     | (0.02)   | (0)   | 0.04      | (0.03)   |
| 22 | 9.725  | 1065 | terpinolene                        | 0.02       | (0.02)  | 0.01     | (0.01)   |       | 0.02      | (0)      |
|    |        |      | total monoterpene hydrocarbons     | 0.2        | 0.1     | 0.2      | 1.1      | 0.1   | 0.1       | 0.02     |
|    |        |      | oxylipids                          |            |         |          |          |       |            |          |
| 23 | 10.15  | 1092 | sorbic acid                        | 0.3        | (0.26)  | 0.5      | (0.48)   | (0.17) | 0.1       | (0.11)   |
| 24 | 13.592 | 1346 | capric acid                        | 0.01       | (0.01)  | 0.2      | (0.22)   | (0)   | 0.01      | (0)      |
| 25 | 13.742 | 1358 | β-damascenone                      | 0.01       | (0.01)  | 0.2      | (0.08)   | (0.04) |            | 0.01     |
| 26 | 14.317 | 1406 | linoeleic acid                     | 0.04       | (0)     | 0.01     | (0.01)   |       |            | (0)      |
| 27 | 15.87  | 1528 | dodecanoic acid                    | 0.01       | (0.01)  |          | 0.1      |       |            |          |
Table 2. continued

| no  | RT min | RI    | compound          | Alveolin-P | Sinawet | Alveolin | Babetone | guava | Bronchicum | Pentamix |
|-----|--------|-------|-------------------|------------|---------|----------|----------|-------|------------|----------|
| 28  | 16.158 | 1548  | ethyl nonanoate    | (0.01)     | (0.01)  | 0.1      | (0.01)   | 0.1   | (0.01)     |          |
| 29  | 20.242 | 1776  | ethyl caprate      | 0.12       | (0.02)  | 0.12     | 0.12     | (0.02) | 0.12       |          |
| 30  | 21.392 | 1836  | (Z)-vaccenic acid  | 0.4        | (0.25)  | 1.6      | (1.64)   | 0.01  | (0.01)     |          |
| 31  | 23.283 | 1935  | ethyl stearate     | 0.03       |         |          |          |       |            |          |
|     |        |       | total oxylipids    |            |         |          |          |       |            |          |
|     |        |       | oxygenated monoterpene |        |         |          |          |       |            |          |
| 32  | 8.6    | 993   | isocineole (1,4-cineole) | 1          | (0.45)  | 76.8     | (17.9)   | 0.1   | (0.01)     |          |
| 33  | 8.809  | 1012  | 1,8-cineole        | 1          | (0.45)  | 47       | (9.1)    | 0.1   | (0.01)     |          |
| 34  | 9.592  | 1056  | artemiseole        | 0.03       |         | 0.6      | 0.6      | 0.03  | (0.01)     |          |
| 35  | 9.85   | 1072  | fenchone           | 0.2        |         | 0.6      | 1.4      | 0.04  | 0.01       |          |
| 36  | 9.958  | 1079  | linalool           | 1.4        | (0.23)  | 0.2      | 0.2      | 0.4   | 0.03       |          |
| 37  | 9.967  | 1080  | isoamyl valerianate| 0.2        |         | 0.1      | 0.01     |       |            |          |
| 38  | 10     | 1082  | isodihydrolavandulyl aldehyde | 4.1        | (2.29)  | 0.8      | 0.8      | 0.1   | 0.01       |          |
| 39  | 10.317 | 1103  | fenchyl acetate    | 0.01       | (0.01)  | 0.01     | 0.01     | 0.1   | (0.01)     |          |
| 40  | 10.325 | 1105  | β-fenchol          |            |         | 0.1      | 0.01     | 0.1   | (0.01)     |          |
| 41  | 10.667 | 1127  | pinocarveol        | 0.3        | (0.3)   | 0.2      | 0.02     | 0.1   | 0.01       |          |
| 42  | 10.767 | 1134  | camphor            | 0.13       | (0.02)  | 0.1      | 0.1      | 0.2   | (0.01)     |          |
| 43  | 10.842 | 1139  | menthone           | 0.3        | (0.24)  | 0.1      | 0.1      | 0.1   | 1.4        | (2.7)    |
| 44  | 10.983 | 1149  | isomenthone        | 0.01       | (0.01)  | 0.01     | 0.1      | 0.01  | (0.01)     | (0.01)   |
| 45  | 11.033 | 1153  | neomenthol         | 0.2        | (0.03)  | 0.01     | 0.01     | 0.5   | 0.01       | (0.07)   |
| 46  | 11.125 | 1159  | borneol            | 0.3        | (0.25)  | 0.01     | 0.3      | 0.5   | (0.03)     |          |
| 47  | 11.183 | 1163  | menthol            | 0.4        | (0.16)  | 0.01     | 0.01     | 0.5   | 94.2       | (6.02)   |
| 48  | 11.475 | 1184  | estragole          | 2          | (0.14)  | 0.5      | 2.7      | (0.32) | (0.01)     |          |
| 49  | 11.475 | 1184  | methyl salicylate  | 0.04       |         | 0.04     | 0.2      | 0.3   |             |          |
| 50  | 12.042 | 1225  | pulegone           | 0.01       | (0.01)  | 0.01     | 0.02     | 0.04  | (0.07)     |          |
| 51  | 12.127 | 1232  | carvone           | 0.3        | (0.01)  | 0.3      | 3.1      | 0.01  | 0.02       | (0.03)   |
| 52  | 12.15  | 1234  | thymoquinone       | 0.03       | (0.02)  | 0.02     | 0.03     | 0.1   | 0.01       | (0.03)   |
| 53  | 12.533 | 1263  | bornyl acetate     | 0.01       | (0.01)  | 0.01     | 0.2      | 0.07  | 0.03       | (0.05)   |
| 54  | 12.663 | 1270  | (E)-anethole       | 66.4       | (3.33)  | 0.01     | 1.5      | 21.5  | 1.7        | 0.03     |
| 55  | 12.758 | 1272  | thymol            | 0.1        | (0.1)   | 0.1      | 7.8      | 0.3   | 48.6       | 0.7      |
| 56  | 12.883 | 1285  | carvacrol          | 0.7        | (0.36)  | 4.5      | 1.24     | 0.05  | 27.3       | 0.9      |
| 57  | 13.282 | 1321  | α-terpineol acetate| 0.4        |         | 0.01     | 0.4      | 0.02  |            |          |
| no | RT min | RI     | compound           | Alveolin-P | Sinsawet | Alveolin Babetone | guava | Bronchicum | Pentamix |
|----|--------|--------|--------------------|------------|----------|-------------------|-------|------------|----------|
| 58 | 13.492 | 1338   | eugenol            | (0.22)     | (0.01)   | (0.01)            | 0.7   | 0.1        |          |
| 59 | 13.967 | 1377   | methyleugenol      | (0.01)     | (0.02)   | (0.02)            | 0.02  | 0.5        |          |
|    |        |        | total oxygenated   |            |          |                   | 81.7  | 13.492     | 1338     |
|    |        |        | monoterpenes       |            |          |                   | 19.7  | 32.5       | 80.7     |
|    |        |        | sesquiterpenes     |            |          |                   | 0.8   | 0.9        | 0.1      |
| 60 | 16.272 | 1556   | spathulenol        | (0.01)     | (0.16)   | (0.1)             | 0.01  | 0.2        |          |
| 61 | 16.708 | 1586   | β-eudesmol         | (0.02)     | (0.02)   | (0.02)            | 0.02  | 0.1        |          |
| 62 | 16.817 | 1594   | cubenol            | (0.01)     | (0.01)   | (0.01)            | 0.01  | 0.1        |          |
| 63 | 16.983 | 1604   | guaiol             | (0.01)     | (0.02)   | (0.02)            | 0.2   | 0.02       |          |
| 64 | 17.017 | 1606   | spathulenol isomer | (0.01)     | (0.04)   | (0.04)            | 0.04  | 0.2        |          |
| 65 | 17.225 | 1617   | tau-cadinol        | (0.01)     | (0.04)   | (0.04)            | 0.2   | 0.04       |          |
| 66 | 20.608 | 1795   | hexahydrofarnesyl acetone | (0.01)     | (0.06)   | (0.06)            | 0.01  | 0.1        |          |
| 67 | 13.133 | 1309   | δ-calamene         | (0.01)     | (0.05)   | (0.05)            | 0.01  | 0.2        |          |
| 68 | 13.408 | 1331   | bergamotene        | (0.01)     | (0.01)   | (0.01)            | 0.01  | 0.04       | 0.02     |
| 69 | 13.6   | 1347   | ylangene           | (0.01)     | (0.01)   | (0.01)            | 0.01  | 0.2        |          |
| 70 | 13.808 | 1364   | β-elemene          | (0.01)     | (0.02)   | (0.02)            | 0.04  | 0.2        |          |
| 71 | 14.083 | 1386   | α-gurjunene        | (0.01)     | (0.05)   | (0.05)            | 0.04  | 0.2        |          |
| 72 | 14.242 | 1399   | farnesene          | (0.01)     | (0.01)   | (0.01)            | 0.01  | 0.2        |          |
| 73 | 14.25  | 1400   | caryophyllene      | (0.01)     | (0.12)   | (0.12)            | 0.1   | 0.2        | 0.04     |
| 74 | 14.3   | 1404   | α-bergamotene      | (0.01)     | (0.06)   | (0.06)            | 0.01  | 0.5        |          |
| 75 | 14.442 | 1416   | β-farnesene        | (0.01)     | (0.03)   | (0.03)            | 0.01  | 0.5        |          |
| 76 | 14.467 | 1418   | aromandendrene     | (0.01)     | (0.02)   | (0.02)            | 0.02  | 0.1        |          |
| 77 | 14.658 | 1434   | humulene           | (0.01)     | (0.02)   | (0.02)            | 0.02  | 0.1        |          |
| 78 | 14.85  | 1449   | α-curcumene        | (0.01)     | (0.13)   | (0.13)            | 0.02  | 2.4        |          |
| 79 | 14.9   | 1454   | α-muurolene        | (0.01)     | (0.09)   | (0.09)            | 0.01  | 4.4        |          |
| 80 | 14.975 | 1459   | longifolene-(V4)   | (0.01)     | (0.76)   | (0.76)            | 0.2   | 0.7        |          |
| 81 | 15.158 | 1475   | β-bisabolene       | (0.01)     | (0.15)   | (0.15)            | 0.01  | 2.3        |          |
| 82 | 15.208 | 1479   | β-himachalene      | (0.01)     | (1.38)   | (1.38)            | 0.01  | 13.9       |          |
| 83 | 15.358 | 1491   | δ-cadinene         | (0.02)     | (0.05)   | (0.05)            | 0.1   | 0.4        |          |
| 84 | 15.442 | 1498   | calamenene         | (0.01)     | (0.02)   | (0.02)            | 0.1   | 0.5        |          |
| 85 | 15.55  | 1506   | γ-himachalene      | (0.01)     | (0.01)   | (0.01)            | 0.17  | 0.5        | 0.01     |
powerful tool for the selective isolation and identification of volatiles in such a complex viscous matrix containing other drugs, diluents, or excipients. This study revealed that volatile constituents, even as adjuvants, can serve as determinants for verifying ingredient composition in the marketed cough preparations and to assess their suitability for quality control issues. Based on this headspace volatile result, efficacy of Bronchicum and Babetone cough products can be predicted.

Multivariate data analysis modeling was further used to elucidate the resemblance and disparity between preparations mostly

Table 2. continued

| no | RT min | RI  | compound                      | Alveolin-P | Sinawet | Alveolin | Babetone | guava | Bronchicum | Pentamix |
|----|--------|-----|-------------------------------|------------|---------|----------|----------|-------|------------|----------|
| 86 | 15.725 | 1518| α-calacorene                  | (0.17)     | (0.05)  | (0.01)   | (0.41)   |
| 87 | 17.242 | 1618| epi-bicyclesesquiphellandrene | 0.01       | 0.4     | 0.1      | (0.01)   |
| 88 | 17.808 | 1648| cadalene                      | (0.01)     | (0.11)  | (0.01)   | (0.2)    |
|    |        |     | total sesquiterpene hydrocarbons | 0          | 8.1     | 23.6     | 35.9     | 2.8   | 1          |

Numbers placed between brackets represent standard deviation.

Figure 3. Principal component and hierarchical clustering analyses of SPME-extracted volatiles from cough preparations. (a) HCA plot (b) Score plot of PC1 vs PC2 scores. (c) Loading plot for PC1 and PC2 contributing volatiles and their assignments.

Figure 4. OPLS-DA score plot derived from modeling volatiles from Sinawet and Pentamix preparations versus all others (a). The respective S-plot shows the covariance $p[1]$ against the correlation $p[1]$ of the variables of the discriminating component of the OPLS-DA model (b). Cutoff values of $P < 0.05$ were used; selected variables are highlighted in the S-plot, and identifications are discussed in text.
influenced by their menthol content as the most variant volatile component. The same approach can be applied to assess different batch numbers of the same cough product to confirm uniformity in production lines and for quality analysis of raw ingredients prior to its manufacture and inclusion in cough preparations. We do admit that our selection of commercial preparations does not cover all worldwide cough products, but our approach is certainly feasible for analyzing products from such further sources. The same workflow indeed of sample preparation, measurement, and processing can be easily transferred for other metabolome studies of (commercially relevant) preparations containing essential oil ingredients in a complex matrix.

**EXPERIMENTAL SECTION**

**Cough Preparations.** Seven commercial cough products, viz. Alveolin-P, Sinawet, Alveolin, Bebetone, guava, Bronchicum, and Pentamix, were provided from their manufacturers. Details of preparation composition are given in Table 1. Three to four replicates were analyzed for each preparation.

**Chemicals and Materials.** SPME fibers of stableflex coated with divinylbenzene/carboxen/poly-dimethylsiloxane (DVB/CAR/PDMS, 50/30 μm), or PDMS (polydimethylsiloxane) were purchased from Supelco (Oakville, ON, Canada). All other chemicals or standards were obtained from Sigma-Aldrich (Germany).

**Volatile Analysis of Samples.** The HS-SPME volatile analysis was carried out as reported in ref 9 with slight modifications. A total of 1 mL of each cough syrup was placed in SPME screw cap vials (20 mL) and spiked with 10 μg of (Z)-3-hexenyl acetate prepared in deionized water to serve as an internal standard. SPME fiber was inserted manually into the vial containing syrup placed in an oven kept at 50 °C for 30 min. The fiber was subsequently withdrawn into the needle and then injected into the injection port of the gas chromatograph-mass spectrometer. GC–MS analysis was performed using a Schimadzu GC-17A gas chromatogram equipped with a DB-5 column (30 m × 0.25 mm i.d. × 0.25 μm film thickness; Supelco) coupled to a Schimadzu QP5050A mass spectrometer. The interface and the injector temperatures were both set at 220 °C. The gradient temperature program was used for volatile analysis; oven temperature was kept first at 40 °C for 3 min, then increased to 180 °C at a rate of 12 °C min⁻¹, kept at 180 °C for 5 min, and finally ramped at a rate of 40 °C min⁻¹ to 240 °C and kept at this temperature for 5 min. Carrier gas helium was used at a flow rate of 0.9 mL/min. SPME fiber was prepared for the next analysis by placing it in the injection port for 2 min at 220 °C to ensure complete elution of volatiles. Blank runs were made during sample analyses. A HP quadruple mass spectrometer was operated in EI mode at 70 eV; a scan range was set at m/z 40–500.

Quantification of cineole, thymol, and carvacrol levels were carried out using a standard calibration curve prepared by serial dilutions analyzed under the same SPME conditions. The standard calibration curve for the cineole standard was found to be linear over a concentration range of (1, 10, 100, and 1000 μg/mL⁻¹) with a correlation coefficient of 0.999. Equation y = 180002x + 195.33. Assays were carried out in triplicate.

**GC–MS Data Processing and Multivariate Analysis.** Volatile components were identified by comparing their retention indices relative to n-alkanes (C6–C20), mass matching to NIST, WILEY library database, and with standards whenever available, as shown in Table 2. Peaks were first deconvoluted using AMDIS software (www.amdis.net) prior to mass spectral matching. Volatile abundance data were prepared for multivariate data analysis by extraction using MET-IDEA software. Data were then subjected to PCA, OPLS-DA using the SIMCA-P version 13.0 software package (Umetrics, Umeå, Sweden). Markers were subsequently identified by analyzing the S-plot and declared with covariance (p) and correlation (pcor). All variables were mean-centered and scaled to Pareto variance.

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