

# Investigating volatile compounds in the vapour phase of (1) a hot water infusion of rhizomes, and of (2) rhizomes of *Siphonochilus aethiopicus* using head space solid phase microextraction and gas chromatography with time of flight mass spectrometry

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## Highlights

- *Siphonochilus aethiopicus*, a traditional remedy for the treatment of allergic asthma.
- Extraction of compounds in vapour phase of a hot water infusion of rhizomes by SPME
- Eucalyptol (1,8-cineole) is the main compound present in vapour phase.
- Eucalyptol reportedly controls asthma.
- Eucalyptol may be key in *S. aethiopicus* as a traditional treatment of asthma.

## ABSTRACT

Wild ginger, *Siphonochilus aethiopicus*, is a traditional remedy for the treatment of allergic asthma and other conditions. Preparations include hot infusions of rhizomes and steaming of the rhizomes and inhalation of the vapour. Volatile compounds in the vapour phase (representing the fraction that is inhaled) of both (1) a hot water infusion of fresh and air dried rhizomes and of (2) rhizomes were concentrated by head space solid phase microextraction (H/S-SPME) and analysed by gas chromatography with time of flight mass spectrometry (GC-TOFMS). Eucalyptol (1,8-cineole) was the major

compound present in the vapour phase of a hot water infusion of fresh and dried rhizomes, and was also present as one of three major compounds in the vapour phase of fresh rhizomes. The drying of rhizomes caused a significant loss of eucalyptol and other compounds. Eucalyptol reportedly controls airway mucus hypersecretion and asthma. As such the presence of eucalyptol in the vapour phase of hot preparations may contribute to the anecdotal effectiveness of *S. aethiopicus* as a decongestant and traditional remedy for the treatment of allergic asthma.

*Keywords:* African (Wild) Ginger; *Siphonochilus aethiopicus*; Eucalyptol (1,8-cineole); asthma; solid phase microextraction (SPME); volatile compounds; gas chromatography with time of flight mass spectrometry (GC-TOFMS)

## 1. Introduction

*Siphonochilus aethiopicus* (Schweinf.) B. L. Burtt, commonly known as wild or African ginger, is a member of the family Zingiberaceae (Holzapfel et al., 2002; Street and Prinsloo, 2013). Its distribution is restricted to southern Africa (Holzapfel et al., 2002). In South Africa it grows in Mpumalanga and the Northern Province (Street and Prinsloo, 2013). Due to the popular use of wild ginger in traditional medicine it has become extinct in KwaZulu-Natal (Viljoen et al., 2002; Street and Prinsloo, 2013). Freshly cut rhizomes and roots are primarily used for mild asthma, colds, influenza and sinus problems (Fouché et al., 2013). Preparations include chewing on the fresh rhizomes, hot and cold infusions of the rhizomes and roots, steaming of the rhizomes and inhalation of the vapour (Fouché et al., 2013). Fouché et al. (2013) found that “S.

*aethiopicus* has anti-inflammatory and immune-suppressing properties *in vitro*, supporting anecdotal accounts of its effectiveness against allergic asthma.” Viljoen et al. (2002) reported the composition of the essential oil of the roots and rhizomes of *S. aethiopicus*. The authors found that the main compounds in the essential oil of both the roots and the rhizomes are a furanoterpenoid siphonochilone, a monoterpenoid eucalyptol (1,8-cineole), and the monoterpenes *cis*- $\beta$ -ocimene and *cis*-alloocimene. The application of siphonochilone and extracts containing siphonochilone from dried *S. aethiopicus* were patented for use in formulations treating asthma and allergic conditions (Horak et al., 2009).

As one of the traditional uses is adding hot water to rhizomes and inhalation of the vapours as a decongestant and for treating asthma, understanding the chemical profile of the vapours could provide insight and substantiation of the customary use. Volatile compounds in the vapour phase of fresh and air dried rhizomes of *S. aethiopicus* and their respective hot water infusions were concentrated by head space solid phase microextraction (H/S-SPME), analysed by gas chromatography with time of flight mass spectrometry (GC-TOFMS) and are reported here for the first time.

## **2. Materials and Methods**

### *2.1 Samples*

Rhizomes of *S. aethiopicus* (labelled as African Ginger “Faraday market type” ECD-MP-0314) were received from the Department of Enterprise Creation and Development (ECD) at the Council for Scientific and Industrial Research (CSIR). Fresh

rhizomes were harvested from cultivation sites in Giyani, Limpopo Province, South Africa. The harvested *S. aethiopicus* specimens were propagated from plants collected from the Faraday Muti Market (Faraday Street, Johannesburg, South Africa). According to the literature the Faraday Muti Market plants are of the wild type (Mander et al., 2007). A voucher specimen from the cultivation site in Giyani could not be deposited for identification due to the unavailability of the required leaves, flowers and fruits. In addition to the fact that only fresh rhizomes were received from the CSIR, major constraints were the scarcity of the plant in the wild, the unavailability of fruits as these mature underground and are difficult to find, and as well as the brief seasonal flowering of African ginger.

## 2.2 Chemical standards

An eucalyptol (1,8-cineole) analytical standard (Fluka) was purchased from Sigma-Aldrich (Pty) Ltd. Kempton Park, South Africa. A working standard was prepared by weighing 0.0163 g into a 25 ml volumetric flask and diluting with *n*-hexane (Analytical grade, Merck). For linear retention index determination *n*-alkanes (C<sub>10</sub>–C<sub>28</sub>) were used (Merck, Pretoria, South Africa).

## 2.3 Head space solid phase microextraction (H/S-SPME)

Fresh rhizomes of *S. aethiopicus* were sliced into small pieces by using a knife. One gram of either fresh or air dried (the sliced, small pieces were air dried at ambient temperature for 14 days) rhizome pieces was weighed into a 24 ml glass vial (Separations, South Africa). For the hot water infusion, 20 ml of boiled water was added to the vial and the rhizomes were left to soak for 20 min. The hot water infusion

was then transferred to another 24 ml glass vial. Vials containing either rhizomes or a hot water infusion were sealed with screw caps with a centre hole of 3.2 mm radius lined with Teflon® septa (Separations, South Africa). Vials containing either the rhizomes or the hot water infusion were immersed in a water bath at 40 °C for 15 min. Sorptive extraction was done with a SPME device which was fitted with a 2-50/30 µm DVB/Carboxen/PDMS StableFlex fibre (Supelco, Sigma-Aldrich (Pty) Ltd. Kempton Park, South Africa). The fibre was exposed to the head space above the rhizomes or hot water infusion for 20 min. After extraction the compounds were desorbed from the SPME fibre for 5 min in the injection port of a GC-TOFMS at 225 °C (2.4). The fibre was conditioned between extractions by heating it in a GC injection port (split flow mode 50:1) for 20 min at 250 °C.

#### *2.4 Gas chromatography time of flight mass spectrometry (GC-TOFMS)*

Analyte separation was done using a LECO Pegasus 4D GC-TOFMS including an Agilent 7890 GC (LECO Africa (Pty) Ltd., Kempton Park, South Africa) on an apolar Rxi-5Sil MS 30 m x 0.25 mm ID x 0.25 µm df (Restek, Bellefonte, PA, USA) capillary column. The carrier gas, helium, was of ultra-high purity grade (Afrox, Gauteng, South Africa) and was set at a flow rate of 1.4 ml min<sup>-1</sup> in the constant flow mode. The SPME fibre was desorbed for 5 min in a SPME inlet liner (Supelco, Sigma-Aldrich (Pty) Ltd. Kempton Park, South Africa) of a GC inlet at 225 °C. The GC inlet was operated in the split mode (100:1 split ratio). The GC oven temperature programme was 40 °C (0.9 min) at 8 °C min<sup>-1</sup> to 280 °C (5 min). The GC run time was 35.9 min. The MS transfer line temperature was set at 280 °C and the ion source temperature was set at 200 °C. The electron energy was 70 eV in the electron impact ionization mode (EI+), the data

acquisition rate was 10 spectra s<sup>-1</sup>, the mass acquisition range was 40–500 atomic mass units (amu), and the detector voltage was set at 1650 V.

Identification of eucalyptol in the samples was done by comparison of retention times and mass spectra to that of an authentic reference standard (0.5 µl of the eucalyptol working standard was injected in the split mode (100:1)), and as well as mass spectra comparison to that of the National Institute of Standards and Technology (NIST08) library. All other compounds were tentatively identified by comparison of the sample mass spectra to that of the NIST08 library (Table 1). Experimental linear retention indices (RI<sub>exp</sub>) (Table 1) were determined by analysing *n*-alkanes (*n*-C<sub>10</sub> to *n*-C<sub>28</sub>) and were calculated according to the method of Van den Dool and Kratz (1963).

### **3. Results and discussion**

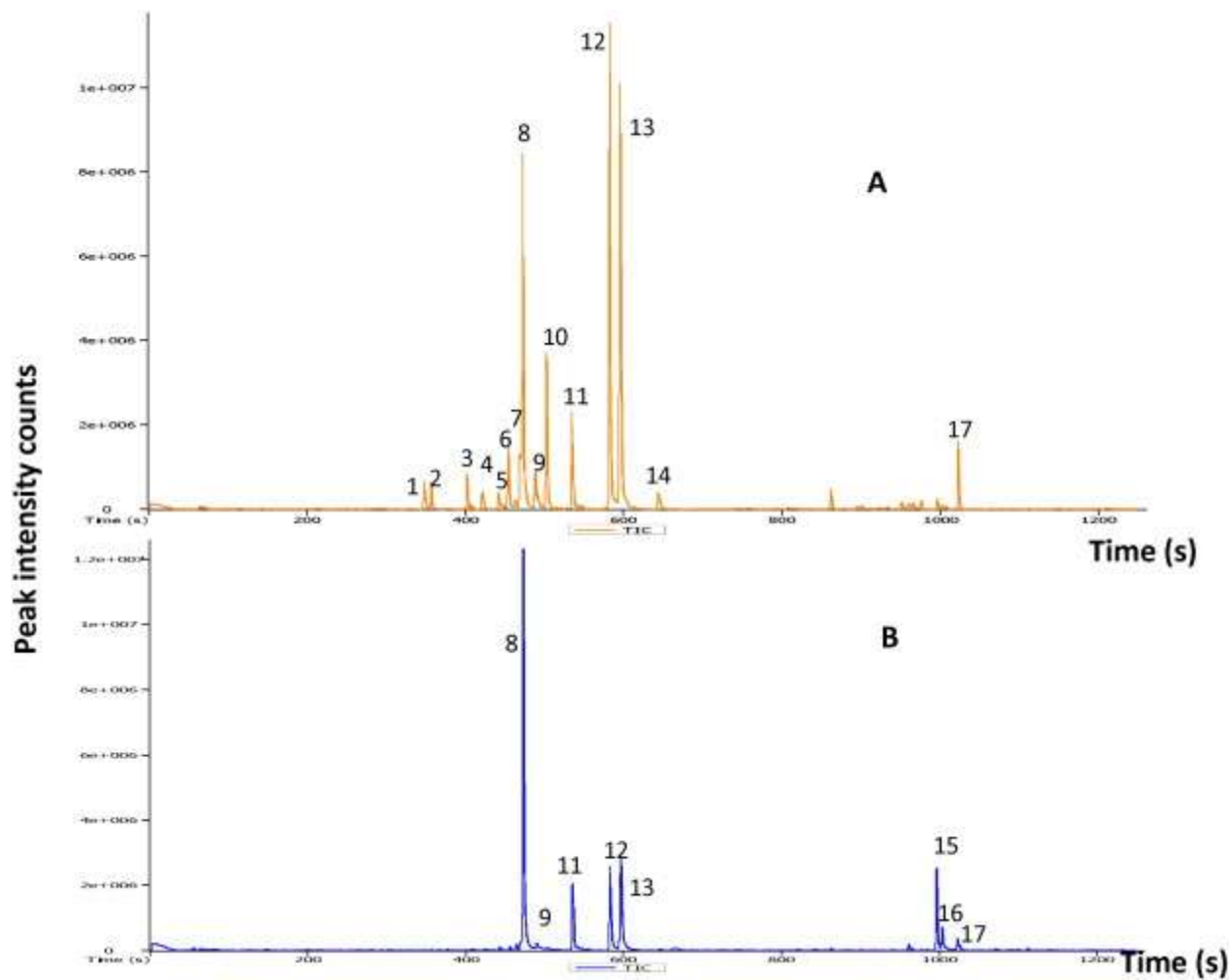
Viljoen et al. (2002) tentatively identified (tentative identification was based on calculated relative retention indices and mass spectral comparison to a library) compounds in the essential oils of roots and rhizomes of *S. aethiopicus*. However, traditional preparations include the hot infusions of rhizomes of *S. aethiopicus* and inhalation of the resulting vapours as a decongestant and for treatment of mild asthma (Fouché et al., 2013). Hence, we report a first investigation of the composition of the

**Table 1**

Compounds present in the vapour phase of rhizomes and in the vapour phase of a hot water infusion of rhizomes of *S. aethiopicus*. The presence of Eucalyptol was confirmed with an authentic reference standard. All other compounds are reported as tentative based on the similarity of spectra to that of a mass spectral library and the use of retention indices.

Peak Number <sup>a</sup>	Compound	MW <sup>b</sup>	Formula	Similarity <sup>c</sup>	RI <sub>exp</sub> <sup>d</sup>	%RPA <sup>e</sup> Rhizomes <sup>f</sup>	%RPA Hot Water Infusion
1.	α-Pinene	136	C <sub>10</sub> H <sub>16</sub>	834	961	1.3	<1
2.	β-Pinene	136	C <sub>10</sub> H <sub>16</sub>	933	965	1.2	-
3.	Sabinene	136	C <sub>10</sub> H <sub>16</sub>	912	985	1.3	-
4.	β-Myrcene	136	C <sub>10</sub> H <sub>16</sub>	904	994	1.1	-
5.	α-Terpinene	136	C <sub>10</sub> H <sub>16</sub>	817	1008	3.1	<1
6.	Limonene	136	C <sub>10</sub> H <sub>16</sub>	874	1014	1.9	-
7.	α-Phellandrene	136	C <sub>10</sub> H <sub>16</sub>	871	1015	3.1	-
8.	Eucalyptol (1,8-cineole) <sup>g</sup>	154	C <sub>10</sub> H <sub>18</sub> O	921	1016	19.7	44.8
9.	cis-β-Ocimene or trans-β-Ocimene	136	C <sub>10</sub> H <sub>16</sub>	945	1024	1.6	1.0
10.	β-Phellandrene	136	C <sub>10</sub> H <sub>16</sub>	840	1029	7.8	0.4
11.	Terpinolene	136	C <sub>10</sub> H <sub>16</sub>	945	1044	4.5	7.5
12.	β-Ocimene	136	C <sub>10</sub> H <sub>16</sub>	946	1065	19.7	10.4
13.	α-Ocimene	136	C <sub>10</sub> H <sub>16</sub>	936	1071	20.3	13.3
14.	cis- or trans-Sabinene hydrate	154	C <sub>10</sub> H <sub>18</sub> O	805	1092	1.3	-
15.	Epiglobulol	222	C <sub>15</sub> H <sub>26</sub> O	760	1539	<1	9.2
16.	Globulol	222	C <sub>15</sub> H <sub>26</sub> O	803	1546	<1	3.0
17.	Unknown	204?		900	1568	3.0	1.4
	Total					90.8	90.9

<sup>a</sup>Peak number-see Figure 1 <sup>b</sup>Molecular weight <sup>c</sup>Mass spectral similarity to a NIST08 library <sup>d</sup>Experimental Retention Index on a Rxi-5Sil MS capillary column <sup>e</sup>% Relative peak area (only compounds ≥1 % RPA are reported) <sup>f</sup>Fresh, not dried <sup>g</sup>Confirmed with an authentic reference standard, all other compounds are reported as tentative identification (section 2.4).

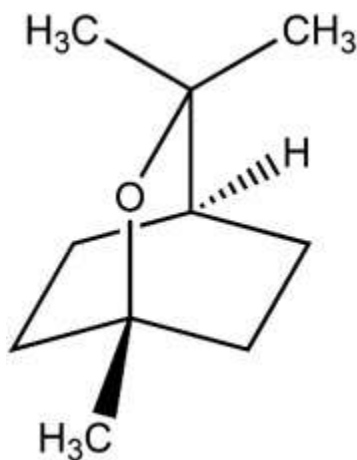


**Figure 1.** Total ion chromatograms (TIC) of compounds in the vapour phase of fresh rhizomes (A) and in the vapour phase of a hot water infusion (B) of fresh rhizomes of *S. aethiopicus*. See Table 1 for compound names.

vapour phase of such a hot water infusion and comparison to the vapour phase of the untreated rhizomes. Figure 1 illustrates the comparison of total ion chromatograms (TIC) of compounds in the vapour phase of fresh rhizomes (A) and in the vapour phase of a hot water infusion (B) of fresh rhizomes of *S. aethiopicus*. Two distinctly different profiles were obtained for each of the two preparation types. The TIC of the vapour phase of the fresh rhizomes showed fifteen compounds, while the vapour phase of the



hot water infusion of fresh rhizomes contained predominantly eight compounds (Table 1). Eucalyptol (identity was confirmed with an authentic reference standard), with a relative peak area (RPA) of 45% of the total chromatogram was the main compound detected in the vapour phase of the hot water infusion of fresh rhizomes. In contrast, eucalyptol in the vapour phase of the fresh rhizomes was present with a RPA of 20% and was one of three major compounds. This finding is in agreement with Viljoen et al. (2002) who reported eucalyptol (Figure 2) as one of the major compounds present in essential oil from the roots and rhizomes.

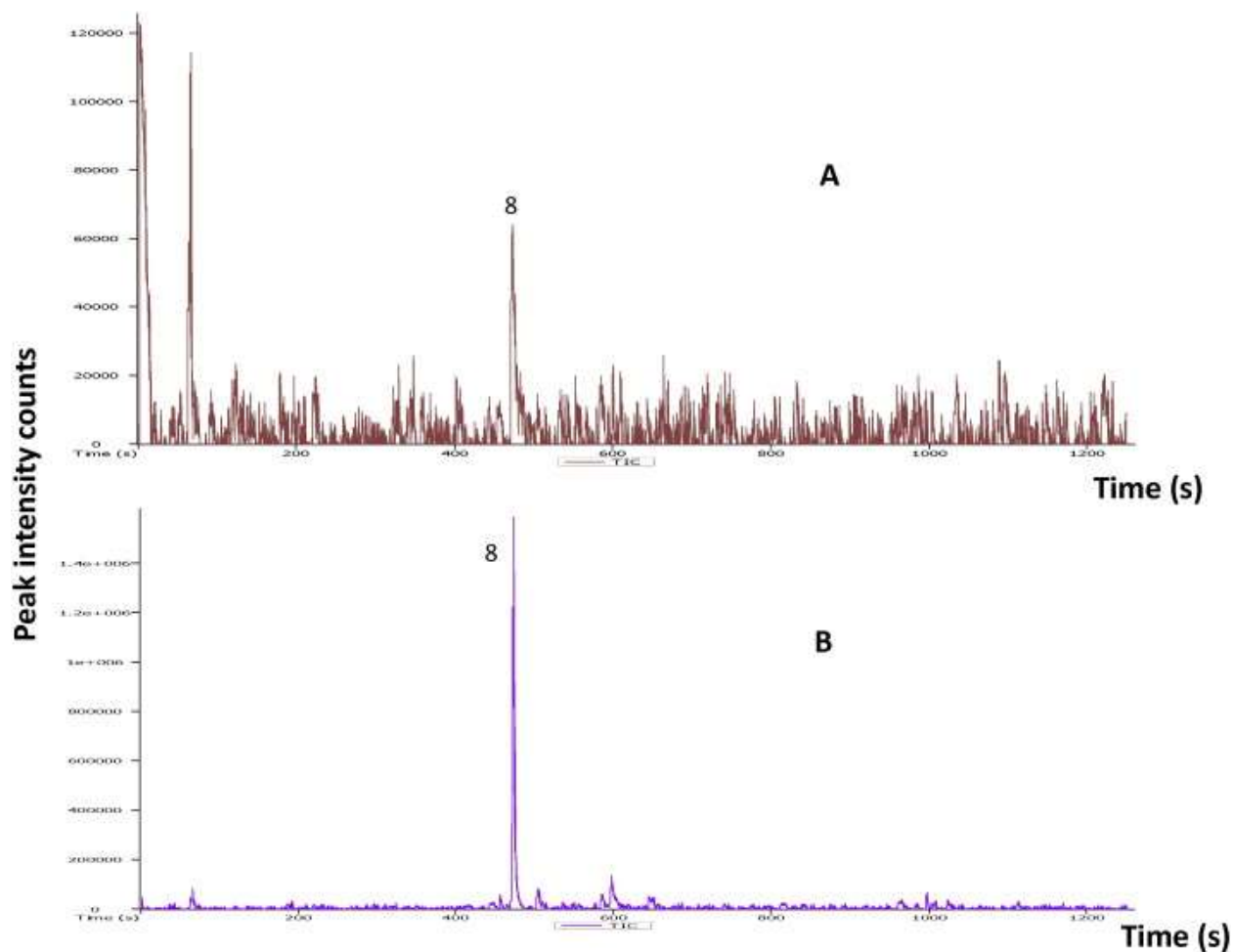


**Figure 2.** Structure of eucalyptol (1,8-cineole).

Secondary compounds reported in Table 1 were assigned tentative identities based on their retention indices determined on an Rxi-5Sil MS GC capillary column and sample mass spectral comparison to that of a NIST08 library. Retention indices (RIs) reported in the literature is typically determined on a 5% phenyl 95% dimethylsiloxane phase (Rxi-5, DB-5, etc.). Although the phase structure of an Rxi-5Sil MS GC capillary column is not the same as that of a 5% phenyl 95% dimethylsiloxane phase, in general

there was good agreement between the experimental RIs and those reported in the literature. For example, the retention index of  $\alpha$ -terpinene was calculated as 1008 (Table 1) on an Rxi-5Sil MS phase, while a literature value of 1012 is reported for a DB-5 phase ([http://www.flavornet.org/f\\_kovats.html](http://www.flavornet.org/f_kovats.html)). The monoterpenes (except for  $\alpha$ - and  $\beta$ -ocimene), *cis*- or *trans*-sabinene hydrate, and epiglobulol reported in Table 1 were also found by Viljoen et al. (2002) in an essential oil of *S. aethiopicus*. The authors reported the presence of *cis*- and *trans*-alloocimene (tentative identification). However, we have tentatively assigned peaks 12 and 13 (Figure 1) as  $\beta$ -ocimene and  $\alpha$ -ocimene respectively (Table 1). The calculated RIs (on an Rxi-5SilMS) of 1065 and 1071 (Table 1) do not match that of *cis*- or *trans*-alloocimene (1129 and 1142 (Adams, 1995)). Rather, the calculated RIs are closer to literature values for  $\beta$ -ocimene (1052 on a DB-5 phase) and for  $\alpha$ -ocimene (1056 on a DB-5 phase) ([http://www.flavornet.org/f\\_kovats.html](http://www.flavornet.org/f_kovats.html)).

Given that the emphasis of this study was the identification of the major compound present in the vapour phase of a hot water infusion (representing the fraction that is inhaled) of rhizomes of *S. aethiopicus* only those compounds with a %RPA  $\geq 1$  are reported in Table 1. However, sesquiterpenes were detected (%RPA < 1) in the vapour phase of fresh rhizomes. These were tentatively identified as  $\beta$ -elemene,  $\gamma$ -elemene,  $\delta$ -elemene,  $\alpha$ -selinene,  $\alpha$ -caryophyllene ( $\alpha$ -humulene) and germacrene-D and were also reported by Viljoen et al. (2002). Of the sesquiterpenes present in the vapour phase of fresh rhizomes, only  $\gamma$ -Elemene was present in the vapour phase of a hot water infusion of fresh rhizomes.



**Figure 3.** Total ion chromatograms (TIC) of the vapour phase of air dried rhizomes (A) and the vapour phase of a hot water infusion (B) of air dried rhizomes of *S. aethiopicus*. Peak 8: eucalyptol.

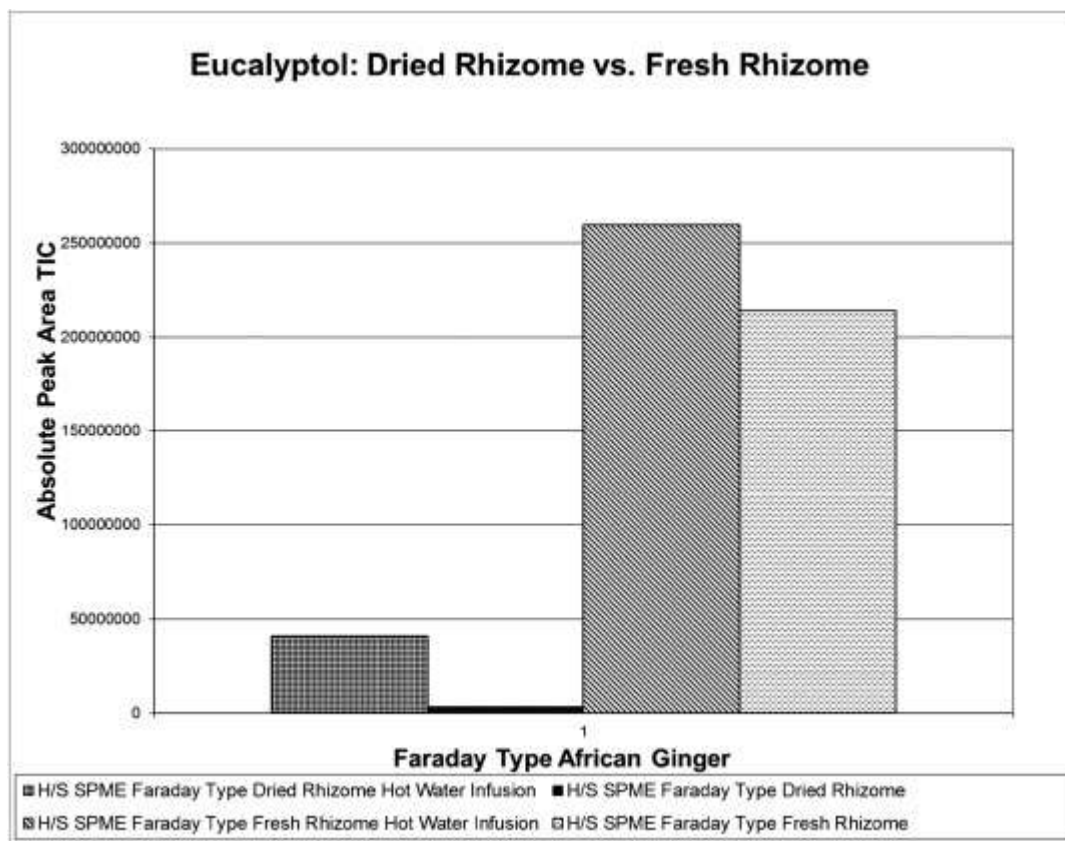
In view of the fact that previous studies on the efficacy of *S. aethiopicus* for the treatment of asthma were done using dried rhizomes and roots (Fouche et al., 2011), the effect of drying the rhizomes may provide more insight into the volatile compounds that may contribute to its efficacy. Figure 3 illustrates the comparison of total ion chromatograms of compounds in the vapour phase of air dried rhizomes and in the vapour phase of a hot water infusion of air dried rhizomes of *S. aethiopicus*. Of the

seventeen compounds detected in the head space of fresh rhizomes (Figure 1A and 1B), eucalyptol was the only compound that was detected at trace levels in the head space of air dried rhizomes (Figure 3A and 3B). The results show a complete absence of the volatile compounds which were detected in the fresh rhizomes indicating that the drying process results in a significant loss of the volatiles compounds. However, it should be noted that trace compounds, not detected in the head space of air dried rhizomes under the specific operating conditions, may be detected when performing a splitless injection as opposed to a split injection. Head space of the fresh rhizomes was injected with a split ratio of 100:1 to prevent overloading the system. Therefore, in order to directly compare the profiles of fresh and air dried rhizomes the head space of the air dried rhizomes was also injected with a split ratio of 100:1.

Figure 4 illustrates a comparison of eucalyptol in the vapour phase of air dried and fresh rhizomes and in their respective hot water infusions. Drying of the rhizomes caused a striking loss of eucalyptol. For the hot water infusion a relative loss of 84% of eucalyptol occurred when using dried rhizome, while in the vapour phase of the dried rhizomes the relative loss of eucalyptol was 98%. Levels of eucalyptol were higher in the vapour phase of the hot water infusions due to the hot water extracting the compound from the rhizomes.

In a double-blind placebo-controlled trial Juergens et al. (2003) reported that eucalyptol controlled airway mucus hypersecretion and asthma. The potential contribution of eucalyptol from fresh rhizomes as a decongestant and a treatment for asthma has to be taken into consideration in efficacy studies. Eucalyptol in the vapour

phase, representing the fraction that is inhaled, likely contributes to the reported efficacy of *S. aethiopicus* as a decongestant and a treatment for asthma.



**Figure 4.** Eucalyptol (absolute peak area) in the vapour phase of fresh and air dried rhizomes and in their respective hot water infusions. H/S SPME: head space solid phase microextraction. TIC: total ion chromatogram.

#### 4. Conclusion

Eucalyptol is the major compound present in the vapour phase of a hot water infusion of fresh or air dried rhizomes and is one of three major compounds present in the vapour phase of fresh rhizomes of *S. aethiopicus*. The drying of rhizomes produced

a distinct loss of eucalyptol and other compounds. The presence of eucalyptol in the vapour phase (representing the fraction that is inhaled) of hot preparations may be a key factor in the anecdotal effectiveness of *S. aethiopicus* as a decongestant and traditional remedy for the treatment of asthma.

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