46th APIMONDIA International Apicultural Congress

Canadian Honey Council

Symposium Apitherapy - from Science To Practice
Session: Validation of Apitherapy in Modern Medicine

# Therapeutic inhalation of beehive´s air

## Characterizing the volatile components present in the air of beehive of *Apis mellifera* species


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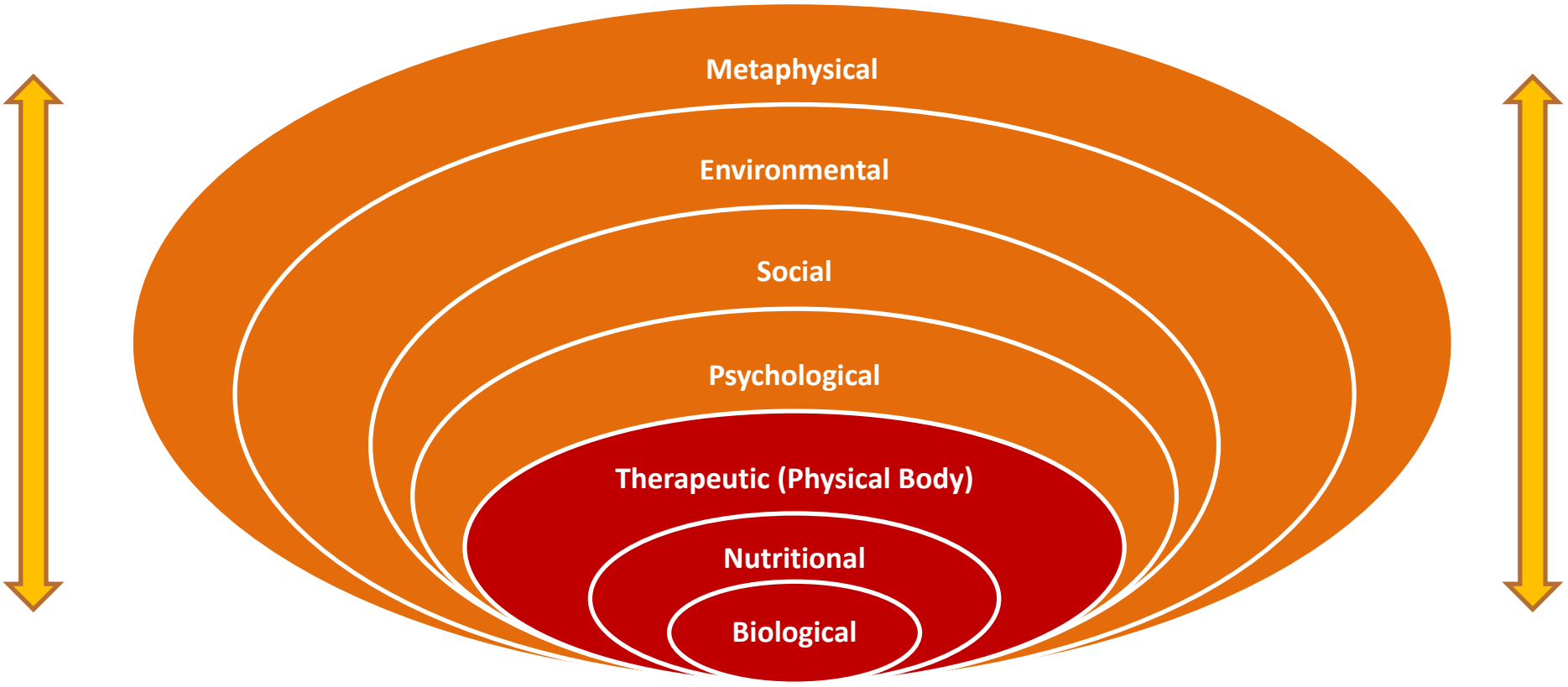
**Montreal, Quebec (Canada)**
**September 2019**

# Recognizing the wholeness of the healing power of honeybees

Validation of Apitherapy in Modern Medicine

# Recognizing the wholeness of the healing power of honeybees




## Validation of Apitherapy in Modern Medicine

# Recognizing the wholeness of the healing power of honeybees

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**Validation of Apitherapy in Modern Medicine**

# Therapeutic inhalation of beehive´s air

Source: Google Images / Apiair Vertrieb / Bienenluft Beehive air inhalation

# Beehive´s Products

**Honey composition** after [75, 80], values in g/100 g

| | Blossom honey | | Honeydew honey | |
|---|---|---|---|---|
| | average | min-max | average | min-max |
| **Water content** | 17.2 | 15-20 | 16.3 | 15-20 |
| **Fructose** | 38.2 | 30-45 | 31.8 | 28-40 |
| **Glucose** | 31.3 | 24-40 | 26.1 | 19-32 |
| **Sucrose** | 0.7 | 0.1-4.8 | 0.5 | 0.1-4.7 |
| **Other disaccharides** | 5.0 | 28 | 4.0 | 16 |
| **Melezitose** | <0.1 | | 4.0 | 0.3-22.0 |
| **Erlose** | 0.8 | 0.56 | 1.0 | 0.16 |
| **Other oligosaccharides** | 3.6 | 0.5-1 | 13.1 | 0.1-6 |
| ***Total sugars*** | ***79.7*** | | ***80.5*** | |
| **Minerals** | 0.2 | 0.1-0.5 | 0.9 | 0.6-2 |
| **Amino acids, proteins** | 0.3 | 0.2-0.4 | 0.6 | 0.4-0.7 |
| **Acids** | 0.5 | 0.2-0.8 | 1.1 | 0.8-1.5 |

**Trace elements in honey,** after [10]

| Element | mg/100 g | Element | mg/100 g |
|---|---|---|---|
| Aluminium (Al) | 0.01 - 2.4 | Lead (Pb)* | 0.001 - 0.03 |
| Arsen (As) | 0.014 - 0.026 | Lithium (Li) | 0.225 - 1.56 |
| Barium (Ba) | 0.01 - 0.08 | Molybdenum (Mo) | 0 - 0.004 |
| Boron (B) | 0.05 - 0.3 | Nickel (Ni) | 0 - 0.051 |
| Bromine (Br) | 0.4 - 1.3 | Rubidium (Rb) | 0.040 - 3.5 |
| Cadmium (Cd)* | 0 - 0.001 | Silicium (Si) | 0.05 - 24 |
| Chlorine (Cl) | 0.4 - 56 | Strontium (Sr) | 0.04 - 0.35 |
| Cobalt (Co) | 0.1 - 0.35 | Sulfur (S) | 0.7 - 26 |
| Floride (F) | 0.4 - 1.34 | Vanadium (V) | 0 - 0.013 |
| Iodine (I) | 10 - 100 | Zirkonium (Zr) | 0.05 - 0.08 |

*- elements regarded as toxic, can be partially of anthropological origin

**Composition of wax**, after[46]

| | | Number of components in fraction | |
|---|---|---|---|
| **Component** | **Quantity %** | **Major** | **Minor** |
| Monoesters | 35 | 10 | 10 |
| Diesters | 14 | 6 | 24 |
| Triesters | 3 | 5 | 20 |
| Hydroxy monoesters | 4 | 6 | 20 |
| Hydroxy polyesters | 8 | 5 | 20 |
| Acid esters | 1 | 7 | 20 |
| Acid polyesters | 2 | 5 | 20 |
| Hydrocarbons | 14 | 10 | 66 |
| Free acids | 12 | 8 | 10 |
| Alcohols | 1 | 5 | ? |
| others | 6 | 7 | ? |
| **total** | **100** | **74** | **210** |

Table 1: **Composition of bee venom dry matter,** after [1, 4, 18, 21]

| Substance Group | Component | % of dry weight |
|---|---|---|
| Proteins (Enzymes) | Phospholipase A2 | 10-12 |
| | Phospholipase B | 1 |
| | Hyaluronidase | 1-2 |
| | Phosphatase | 1 |
| | α - Glucosidase | 0.6 |
| Peptides | Melittin | 40-50 |
| | Apamine | 2-3 |
| | MCD peptide | 2-3 |
| | Secapine | 0.5-2 |
| | Pamine | 1-3 |
| | Minimine | 2 |
| | Adolapine | 0.5-1 |
| | Procamine A, B | 1-2 |
| | Protease inhibitor | 0.1-0.8 |
| | Tertiapine, cardiopep, melittin F | 1-2 |
| Phospholipids | | 1-3 |
| Biogenic amines | Histamine | 0.5-2 |
| | Dopamine | 0.2-1 |
| | Noradrenalin | 0.1-0.5 |
| Amino acids | Aminobutyric acid, α-amino acids | 1 |
| Sugars | Glucose, fructose | 2-4 |
| Volatiles (pheromones) | Complex ethers | 4-8 |
| Minerals | P, Ca, Mg | 3-4 |






**HONEY**

**WAX**

**PROPOLIS**

**ROYAL JELLY**

**POLLEN**

**BEE´S SECRETIONS**

**Composition of royal jelly** after[57]

| | Fresh | lyophilized |
|---|---|---|
| Water % | 60 - 70 | < 5 |
| Lipids % | 3 - 8 | 8 – 19 |
| 10-hydroxy-2-decenoic acid % | > 1,4 | > 3,5 |
| Protein % | 9 – 18 | 27 – 41 |
| Fructose + glucose+ sucrose % | 7 – 18 | |
| Fructose % | 3 – 13 | |
| Glucose % | 4 – 8 | |
| Sucrose % | 0,5 – 2,0 | |
| Ash % | 0,8 – 3,0 | 2 – 5 |
| pH | 3,4 – 4,5 | 3,4 – 4,5 |
| Acidity (ml 0.1N NaOH/g) | 3,0 – 6,0 | |

**Pollen composition** after[5]

| **Main Components** | **Content Minimum – Maximum g/100g dry weight** |
|---|---|
| Proteins | 10-40 |
| Lipids | 1-13 |
| total Carbohydrates* | 13-55 |
| Dietary fibre, Pectin | 0,3-20 |
| Ash | 2-6 |
| undetermined | 2-5 |
| **Minerals, trace elements** | **mg/kg** |
| Potassium | 4000-20000 |
| Magnesium | 200-3000 |
| Calcium | 200-3000 |
| Phosphorus | 800-6000 |
| Iron | 11-170 |
| Zink | 30-250 |
| Copper | 2-16 |
| Manganese | 20-110 |
| **Vitamins** | **mg/kg** |
| β-Carotene | 10-200 |
| B1; Thiamin | 6-13 |
| B2; Riboflavin | 6-20 |
| B3; Niacin | 40-110 |
| B5; Pantothenic acid | 5-20 |
| B6; Pyridoxin | 2-7 |
| C; Ascorbic acid | 70-560 |
| H; Biotin | 0.5-0.7 |
| Folic acid | 3-10 |
| E: Tocopherol | 40-320 |

Table 2 A: **Composition of raw poplar propolis** after [4, 6, 16, 28, 46, 55, 68]

| | **Substances** |
|---|---|
| **BALSAM**<br>40 - 70 %<br>Ethanol soluble<br>Poplar origin | *Phenolics*<br>Phenols, phenolic acids, esters, flavanons, dihydroflavanons, flavons, flavonols, chalkones, phenolic glycerides ;<br>*Others*:<br>Aliphatics: acids, alcohols, esters, aldehydes, ketones, benzoic acid and esters |
| Essential oils<br>3-5 %<br>ethanol soluble<br>poplar origin | Mono-, and sesquiterpenes |
| **NON-BALSAM**<br>Ethanol insoluble<br>Wax: 20-35 %<br>Beeswax origin | Beeswax components |
| Others: ca. 5 %<br>partly ethanol soluble<br>bee and pollen origin | Mainly minerals average ash content 2.1 %<br>Polysaccharides:2 %<br>Proteins, amino acids, amines and amides: 0. 7 %<br>Traces of carbohydrates, lactones, quinones, steroids, vitamins |

Table 2 B: **Composition of raw Baccharis propolis** after [11, 15, 33, 44, 45, 60]

| | **Substances** |
|---|---|
| **BALSAM**<br>45 - 70 %<br>Ethanol soluble<br>Baccharis origin | Mainly cinnamic acid and derivatives, coumaric acid, prenylated compounds, artepillin C<br>Minor quantities of phenolics as flavonoids, benzoic acid, aliphatic acids and esters |
| **NON BALSAM** | |
| 10-15 %<br>Ethanol insoluble<br>Baccharis origin | prenylated compounds, alkanes and terpenoids |
| 15-25 %<br>Ethanol insoluble<br>Beeswax origin | Beeswax |
| ca. 5 %<br>partly ethanol soluble<br>Bee and pollen origin | 2.5 – 4.5 % minerals<br>1-2 % of carbohydrates: fructose, mannose, inositol, erythrose<br>1-2 %: glycerol, lower aliphatic acids, amino acids, amines |

# Volatile compounds isolated from the bee´s products are classified in a variety of chemical categories

| WAX | HONEY | POLLEN | PROPOLIS | ROYAL JELLY | VENOM |
|---|---|---|---|---|---|
| HYDROCARBON (C20-C35) | ALDEIDS | PHENOLIC COMPOUNDS | PHENOLIC COMPOUNDS | AMPHIPATHIC ACIDS | MELITINE |
| FATTY ACIDS | KETONES | AMINO ACIDS | FLAVONOIDS | PHENOLIC COMPOUNDS | PHISPHLIPASE A2 |
| ESTERS (C40-C50) | ACIDS | HYDROCARBONS (C21-C35) | TERPENES | SUGARS | APAMINA |
| ALCOHOLS (C24 -C34) | ALCOHOLS | AMPHIPATHIC ACIDS (C16-C18) | ESTER | PROTEINS | HISTAMINE |
| DIOLS | HYDROCARBONS | ESTERS | SUGARS | FATTY ACIDS | PEPTIDEOS |
| PALMITIC ACID | NORISOPRENOIDS | ALCOHOLS | HYDROCARBONS | MINERALS | HYALURONIDASE |
| | TERPENES AND DERIVATIVES | SUGARS | MINERALS | CARBONILED COMPOUNDS | |
| | BENZENES AND DERIVATIVES | FATTY ACIDS | ALCOOIS | | |
| | PHENOLIC COMPOUNDS | | | | |
| | CARBONILED COMPOUNDS | | | | |
| | ESTERS | | | | |
| | FURAN | | | | |
| | PYRAN | | | | |
| | SUGARS | | | | |


Some References:

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# Research on the Inhalation of Volatile Compounds of Bees Products

Kamaruzaman et al. BMC Complementary and Alternative Medicine 2014, 14:176
http://www.biomedcentral.com/1472-6882/14/176

BMC Complementary & Alternative Medicine

RESEARCH ARTICLE Open Access

## Inhalation of honey reduces airway inflammation and histopathological changes in a rabbit model of ovalbumin-induced chronic asthma

Nurfatin Asyikhin Kamaruzaman[1], Siti Amrah Sulaiman[2], Gurjeet Kaur[3] and Badrul Yahaya[1*]

**Abstract**

**Background:** Honey is widely used in folk medicine to treat cough, fever, and inflammation. In this study, the effect of aerosolised honey on airway tissues in a rabbit model of ovalbumin (OVA)-induced asthma was investigated. The ability of honey to act either as a rescuing agent in alleviating asthma-related symptoms or as a preventive agent to preclude the occurrence of asthma was also assessed.

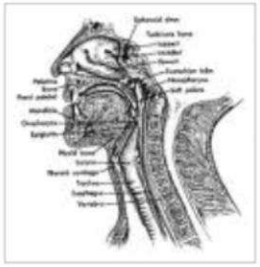
**Methods:** Forty New Zealand white rabbits were sensitized twice with mixture of OVA and aluminium hydroxide on days 1 and 14. Honey treatments were given from day 23 to day 25 at two different doses (25% (v/v) and 50% (v/v) of honey diluted in sterile phosphate buffer saline. In the aerosolised honey as a rescue agent group, animals were euthanized on day 28; for the preventive group, animals were further exposed to aerosolised OVA for 3 days starting from day 28 and euthanized on day 31. The effects of honey on inflammatory cell response, airway inflammation, and goblet cell hyperplasia were assessed for each animal.

**Results:** Histopathological analyses revealed that aerosolised honey resulted in structural changes of the epithelium, mucosa, and submucosal regions of the airway that caused by the induction with OVA. Treatment with aerosolised honey has reduced the number of airway inflammatory cells present in bronchoalveolar lavage fluid and inhibited the goblet cell hyperplasia.

**Conclusion:** In this study, aerosolised honey was used to effectively treat and manage asthma in rabbits, and it could prove to be a promising treatment for asthma in humans. Future studies with a larger sample size and studies at the gene expression level are needed to better understand the mechanisms by which aerosolised honey reduces asthma symptoms.

Thursday, May 24, 2007

## Propolis an Option for Treating Upper Respiratory Tract Infections



**Propolis Antimicrobial Activity: What's New?**

Le infezioni in medicina, 2007 Mar;15(1):7-15

Propolis is a hive product that bees manufacture from balsamic resins actively secreted by plants on leaf buds and barks. Propolis composition is highly variable, depending on the plant species and on the season of collection. However, propolis essentially contains resins, balsams, essential oils, flavonoids, vitamins, minerals and pollen, albeit at different concentrations.

Although more than 300 constituents have been identified in propolis samples, biological activity is mainly due to few substances, such as flavonoids, terpens, caffeic, ferulic and

**Specific studies are still needed in order to identify the volatile components present the air obtained directly from the beehives as it is used in natural clinical practices**

# Goals

- Develop a methodology for collecting and analyzing the air from beehives
- Characterize the volatile compounds present in the air of the beehives (*Apis mellifera* species)

# Methods - Collecting the beehive´s air

The beehive´s air samples ware collected in the Dito Pintado Apiary (Brazil)

# Methods - Beehive Preparation

**Prepared Langstroth hive cover**
- Opening
- Metal screen for protection

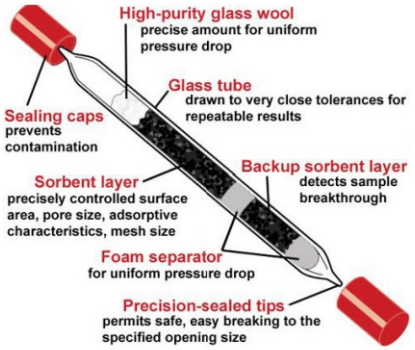
**Air circulation**
- Mini-Fan (Cooler)
- Batteries

**Air conduction**
- Funnel
- Hose

# Methods - Sampling and Storing

## 1 – Air sampling manifold bottle

## 2 - Activated carbon tube
## 3 – Tenax tube



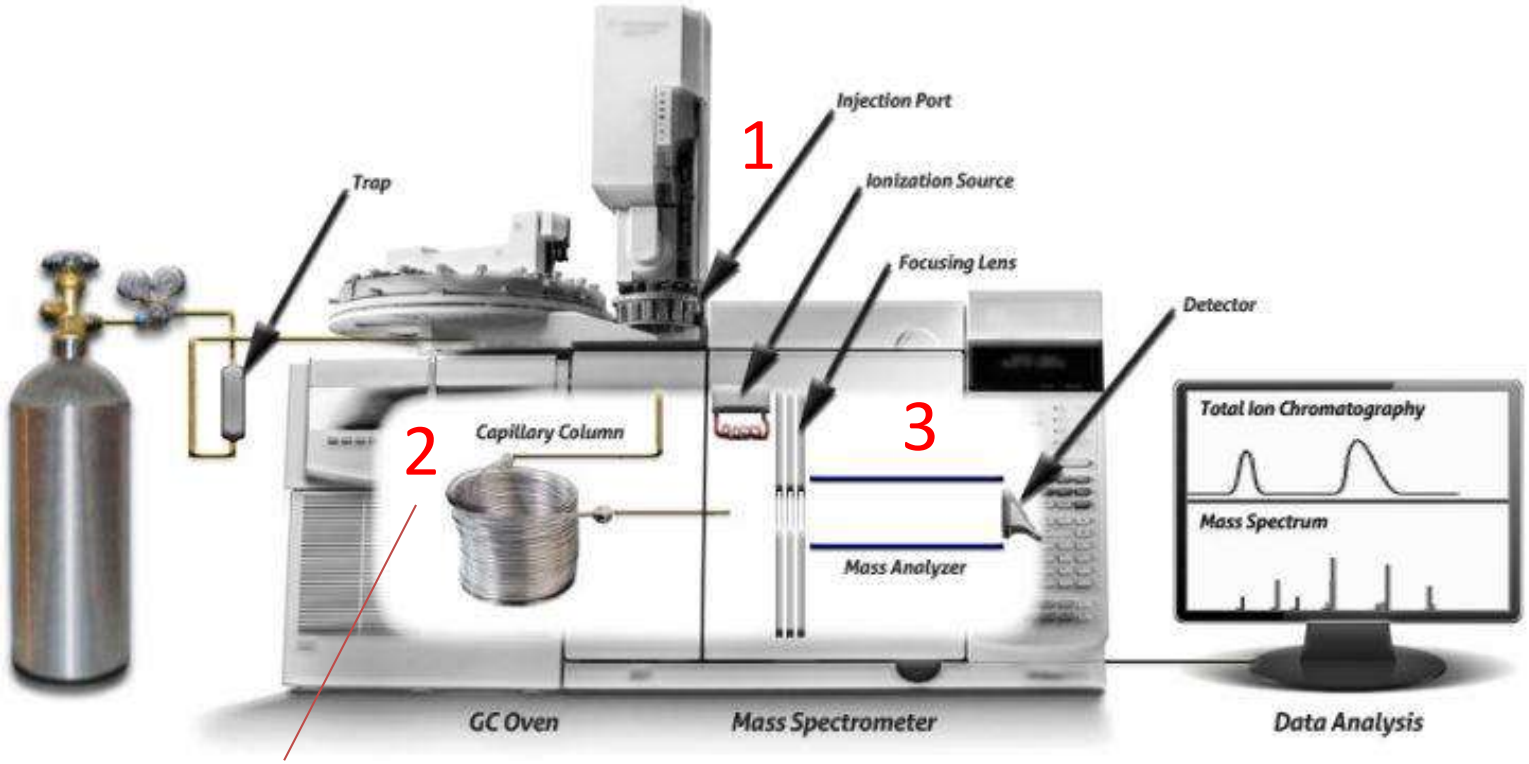
# Methods - Sample Preparation / Extraction

| SAMPLE | COLLECTING TIME | STORAGE | EXTRATION SOLVENT |
|---|---|---|---|
| **1 - BOTTLE A** | 10 min | Air / Glass | - |
| **2 - BOTTLE B** | 5 min | Air / Glass | - |
| **3 - BOTTLE C** | 5 min | Air / Glass | - |
| **4 - TUBE D** | 10 min | Activated Carbon | dichloromethane |
| **5 - TUBE E** | 5 min | Activated Carbon | dichloromethane |
| **6 - TUBE F** | 5 min | Activated Carbon | acetonitrile |
| **7 - TUBE G** | 5 min | Tenax | dichloromethane |

# Methods - Analysing the beehive´s air

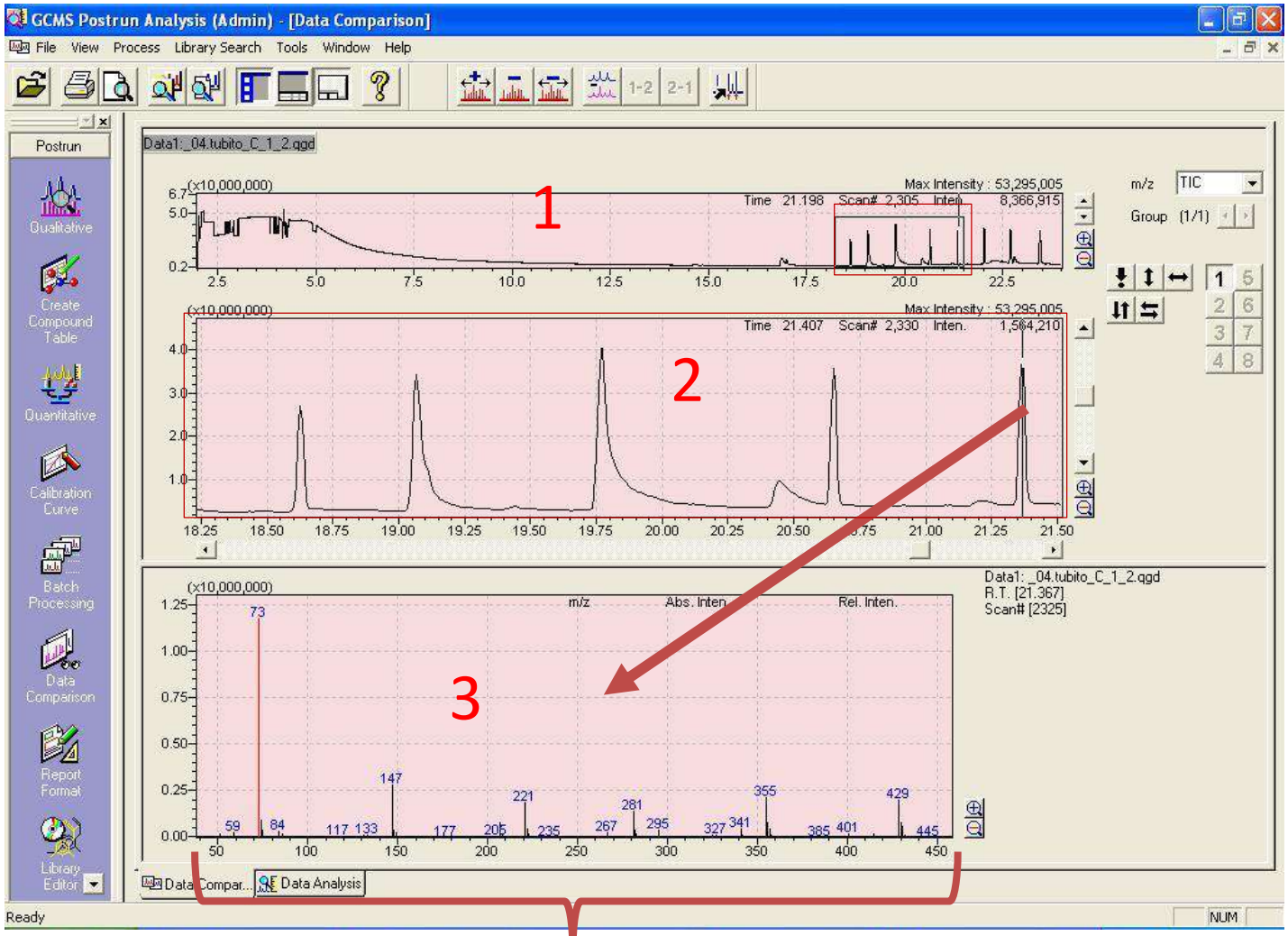
**Gas Chromatography – Mass Spectrometry (GC-MS)** is a combined instrumentation that allows a qualitative and quantitative analysis of complex solutions.

# Methods - Gas Chromatography and Mass spectrometry




40°C to 300 ° C in 24 minutes.

# Methods - Analysis Example

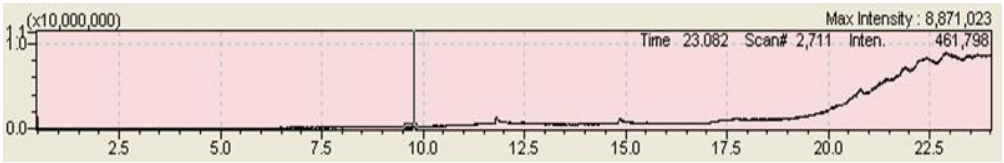



4 The chemical pattern must be compared to a chemical library

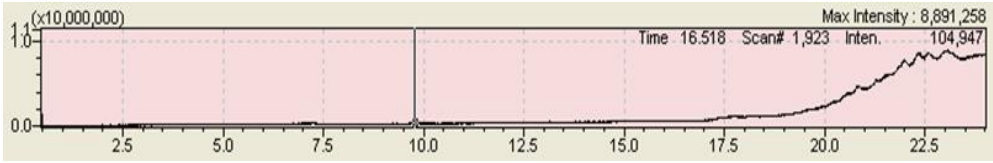
# Results and Discussion

**Result 1** - Using activated carbon glass tubes subjected to constant airflow for 10 minutes and further volatile components extraction in dichloromethane was the most effective method for separating compounds from beehive´s air.

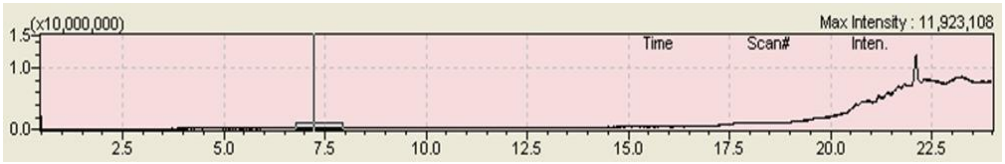
- Bottle 1



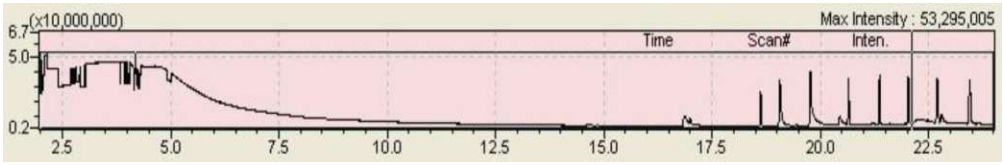

- Bottle 2



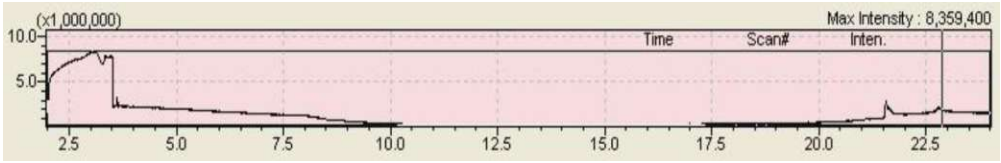

- Bottle 3



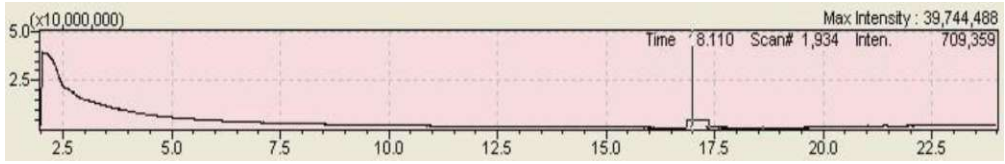

- Tube1



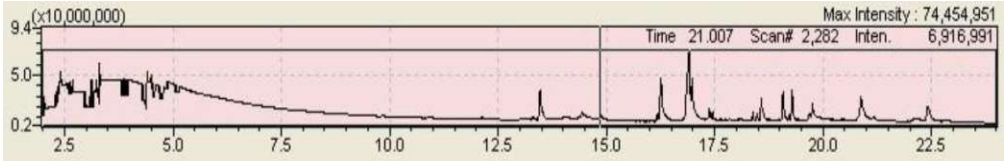

- Tube 2




- Tube 3

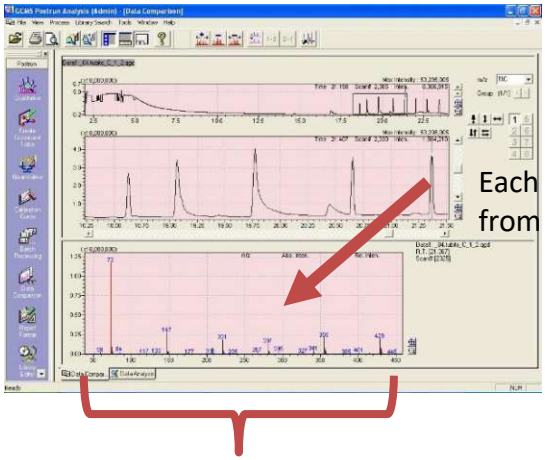



- Tube 4



## Results and Discussion

### Sample - Tube 1



Each peak represents an isolated compound from the beehive´s air

For each peak (compound) there is a detailed molecular information which can be used for chemical identification

**Result 2 -** Chemical profile of each one of those 15 volatile compounds that we could separate from beehive´s air

Table 1 - List of compounds with their respective molecular information (mass charge ratio (m/z) and fragmentation)

| Compound | Retention Time (min) | m/z | Ìon Fragmentation |
|---|---|---|---|
| 1 | 14,6 | 236 | 50, 57, 67, 84, 91, 95, 109, 123, 133, 137, 149, 163, 165, 180, 193, 205, 221, 236 |
| 2 | 14,8 | 415 | 50, 73, 86, 117, 131, 147, 159, 191, 207, 221, 253, 281, 327, 341, 383, 415 |
| 3 | 16,8 | 211 | 53, 55, 70, 73, 84, 97, 99, 111, 129, 142, 155, 167, 173, 186, 191, 211 |
| 4 | 17 | 401 | 55, 73, 84, 99, 131, 147, 173, 191, 207, 221, 249, 267, 281, 313, 327, 355, 359, 385, 401 |
| 5 | 18,6 | 446 | 59, 73, 89, 117, 147, 161, 191, 207, 221, 249, 267, 281, 311, 325, 355, 369, 401, 415, 429, 446 |
| 6 | 19 | 355 | 50, 57, 76, 104, 121, 132, 149, 167, 189, 205, 223, 254, 263, 281, 295, 355 |
| 7 | 19,45 | 355 | 51, 57, 76, 104, 121, 149, 173, 180, 205, 223, 236, 243, 261, 282, 355 |
| 8 | 19,7 | 445 | 57, 73, 93, 104, 147, 149, 191, 205, 221, 249, 267, 281, 323, 341, 355, 385, 401, 429, 445 |
| 9 | 20,4 | 355 | 50, 71, 76, 104, 121, 132, 149, 167, 176, 193, 219, 237, 250, 269, 291, 341, 355 |
| 10 | 20,6 | 443 | 59, 73, 87, 117, 147, 148, 191, 207, 221, 249, 267, 281, 323, 341, 355, 369, 401, 429, 443 |
| 11 | 21,2 | 447 | 59, 73, 89, 117, 147, 161, 191, 207, 221, 249, 267, 281, 311, 327, 355, 369, 401, 415, 429, 447 |
| 12 | 22 | 434 | 59, 73, 87, 117, 147, 148, 191, 207, 221, 249, 267, 281, 323, 341, 355, 369, 401, 429, 434 |
| 13 | 22,7 | 443 | 61, 73, 87, 117, 131, 147, 177, 207, 221, 249, 267, 281, 295, 327, 355, 357, 385, 415, 429, 443 |
| 14 | 22,8 | 429 | 57, 71, 84, 113, 132, 149, 167, 194, 221, 240, 252, 279, 295, 325, 341, 355, 401, 429 |
| 15 | 23,5 | 443 | 59, 73, 87, 117, 145, 147, 175, 207, 221, 249, 267, 281, 295, 327, 355, 357, 385, 401, 429, 443 |

# Results and Discussion

**Mass spectrum library**
**NIST62.LIB**

**Software**
**GCMS PostRun Analisys**

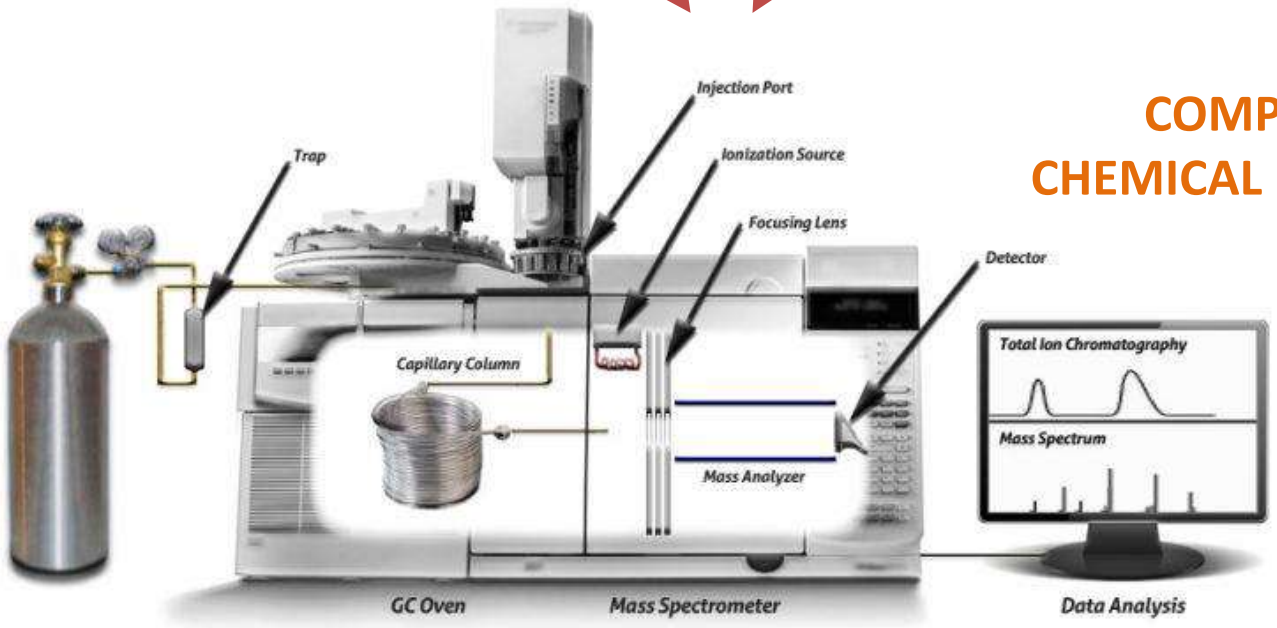
**Result 3** - A list of the most likely volatile substances we separate from the bee hive

| Compound | Retention Time (min) | Ions Fragmentation | Suggested Substances | | | |
|---|---|---|---|---|---|---|
| | | | % Similarity | Formula | Name | m/z |
| 1 | 13.1 | 51, 57, 69, 84, 91, 109, 123, 138, 151, 163, 179, 194, 207 | 74 | C4H3Cl | 1,2,3-Butatriene, 1-chloro- $$ Butatriene, chloro- $$ Chlorobutatriene | 86 |
| | | | 74 | C4H3Cl | 1-Buten-3-yne, 2-chloro- $$ 2-Chlorobuten-3-yne | 86 |
| | | | 74 | C4H3Cl | 1-Buten-3-yne, 1-chloro-, (Z)- $$ cis-1-Chlorobuten-3-Yne $$ cis-1-Chlorobutenyne | 86 |
| 2 | 14.6 | 51, 57, 67, 84, 86, 107, 123, 137, 149, 165, 166, 177, 193, 205, 220, 236 | 68 | C2H4N4 | 3-Amino-s-triazole $$ 1H-1,2,4-Triazol-3-amine $$ .delta.2-1,2,4-Triazoline, 5-imino- $$ s-Triazole, 3-amino- $$ Aminotriazole (plant regulator) $$ Amitrol $$ Amitrol 90 $$ Amitrole $$ Amizol $$ Azaplant $$ ATA $$ Cytrol $$ Cytrole $$ ENT 25445 $$ Herbidal Total $$ Weedazol $$ 3-Amino-1,2,4-triazol $$ 3-Amino-1,2,4-triazole $$ 3-Amino-1H-1,2,4-triazole $$ 3-Aminotriazole $$ 3-AT $$ 1,2,4-triazol-3-ylamine $$ x-all Liquid $$ Amerol $$ Aminotriazol-spritzpulver $$ Aminotriazole $$ Amitolamitril $$ Amitril T. | 84 |
| | | | 67 | C15H17NS | Pyridine, 3-(tert-butylthio)-4-phenyl- | 243 |
| | | | 67 | C4H3Cl | 1-Buten-3-yne, 2-chloro- $$ 2-Chlorobuten-3-yne | 86 |
| 3 | 14.8 | 51, 73, 84, 117, 135, 147, 177, 207, 221, 281, 327, 341, 383, 415 | 74 | C6H13NO | Cyclopentanemethanol, 1-amino- $$ 1-Aminocyclopentanemethanol $$ Amino-1 methylol-1 cyclopentane $$ 1-Amino-1-cyclopentanemethanol | 115 |
| | | | 73 | C4H3Cl | 1-Buten-3-yne, 2-chloro- $$ 2-Chlorobuten-3-yne | 86 |
| | | | 73 | C4H3Cl | 1,2,3-Butatriene, 1-chloro- $$ Butatriene, chloro- $$ Chlorobutatriene | 86 |
| 4 | 16.8 | 51, 55, 70, 84, 99, 100, 111, 129, 142, 149, 155, 171, 173, 186, 191, 207 | 70 | C8H17N | 2-Buten-1-amine, N-butyl-, (Z)- | 127 |
| | | | 69 | C7H14N2O | N-Methyl-3-piperidinecarboxamide | 142 |
| | | | 68 | C8H17N | 2-Buten-1-amine, N-butyl-, (E)- | 127 |

# Identifying the Volatile Compounds

NEW BEEHIVE´S AIR SAMPLES

SUGGESTED SUBSTANCES SAMPLES




COMPARE CHEMICAL PROFILES

# Chemical categories of the beehive´s air suggested substances, match to the chemical categories of the beehive´s products

## Beehive´s air suggested substances

## (Chemical categories)

- oxygenated hydrocarbons,
- nitrogenous hydrocarbons, alkaloids,
- iodinated compounds,
- chlorinated compounds,
- chlorinated hydrocarbons,
- fatty acids,
- lipids,
- steroids,
- carbohydrates
- miscellaneous of natural products.

## Substances of beehive´s products (previous researches)

## (Chemical categories)

- hydrocarbons,
- fatty acids,
- esters,
- alcohols,
- nitrogenous compounds,
- aldehydes,
- terpenes,
- carbohydrates and
- phenolic compounds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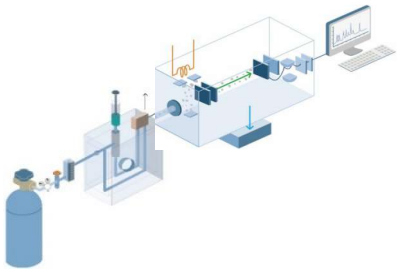
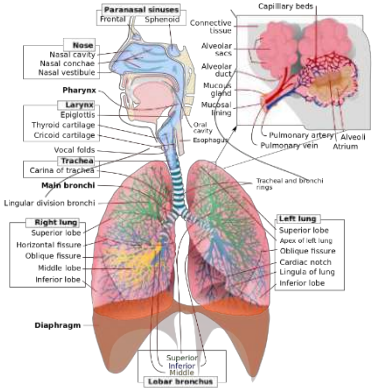
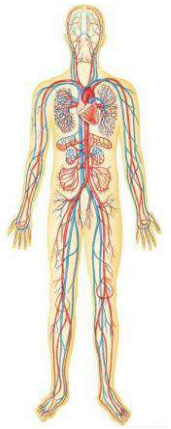
# Conclusion and Final Considerations

**Getting a chemical profile of the volatile compounds of the beehive´s air is the first step towards the identification of the beehive´s air composition, which is essential for building a solid scientific knowledge about the pharmacologic and therapeutic effect of beehive´s air inhalation.**





# Open for Collaborations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