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Isolation and Analysis of Essential Oils from Spices

BACHELOR'S THESIS

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Declaration

I hereby declare that I have done this thesis entitled “Isolation and Analysis of Essential Oils from Spices” independently, all texts in this thesis are original, and all the sources have been quoted and acknowledged by means of complete references and according to Citation rules of the FTA.

In Prague ……..

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Markéta Vostarková

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Abstract

Herbs and spices have been used in traditional medicine for thousands of years. Knowledge of the chemical composition of herbaceous plants is desirable in pharmacy for the development of new drugs and dietary supplements. The main aim of this bachelor thesis was chemical analysis of active compounds contained in the essential oil of 4 kinds of spices. Samples of black cumin, ginger, cardamom and cinnamon (*C. cassia*) were imported from Jaipur, India. The literature review part describes botanical information, traditional use and active compounds with their positive health effects for each of the spice, as well as explanation of methods used for the extraction and analysis of samples. In the experimental part, essential oils were extracted using the Soxhlet extraction apparatus and analysed by the gas chromatography - mass spectrometry (GC-MS).

Main and trace components were identified, and their mass spectra were compared with the National Institute of Standards and Technology (NIST) library. On average, 95% of the substances were identified. Results were compared with literature data as well as with other samples. The presence of main compound thymoquinone (TQ) in black cumin and (E)-cinnamaldehyde in cinnamon was confirmed, however the results from analysis of cardamom probably correspond to the chemical composition of different species from the same family. The minor variations in the chemical composition of ginger can be affected by the age of the sample, the country of origin and the quality of storage.

Key words: *Nigella sativa*, ginger, cardamom, *Cinnamomum cassia*, GC-MS analysis, active compounds, Soxhlet extractor

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List of the abbreviations used in the thesis

GC-MS - Gas chromatography-mass spectrometry

TQ - Thymoquinone

BC - Before Christ

SFE - Supercritical fluid extraction

KI - Kovats retention index

RT - Retention time

RI – Retention index

RI calc – Retention index calculated

RI lit – Retention index from literature source

MW - Molecular weight

HP – High performance

1. Introduction

Some of the medicinal plants are nowadays known for their culinary use as a spices and additives rather than as natural medicine. However, these plants also contain a wide range of active compounds and have been traditionally used in the past to naturally cure and prevent a wide range of health problems. The demand for herbal medicines is constantly growing due to their presumed safety, accessibility and less side effects compared to chemical drugs. In pharmacy herbaceous plants and their active compounds are often used for the development of new drugs and dietary supplements.

This Bachelor Thesis focuses on the chemical analysis of 4 species of spices, typical for Southeast and Southwest Asia. For each of the selected spices a different part of the plant is usually used. Thus, in the experimental part of the thesis, essential oil was extracted from the seeds of black cumin, ginger rhizome, fruit capsules of cardamom and cinnamon bark. All the samples were collected on the local market in Jaipur, India where they are used to treat common gastrointestinal disorders, cold and respiratory problems (Siripoltangman & Chickos 2019; Tambe & Gotmare 2019. They are also an integral part in preparation of traditional Indian dishes like curry and Garam Masala. The literature review describes each of the selected herbs in detail and provides information about its history, botany, traditional use and active compounds.

The extracted oils where analysed via gas chromatography-mass spectrometry (GC-MS). This method combines separation of molecules in chromatograph and subsequent identification of these molecules by mass spectrometer (Stauffer et al 2008). Obtained data were entered into tables and compared with each other and with literature data.

2. Literature Review

2.1. Black cumin *(N. sativa)*

2.1.1. Introduction

Nigella sativa (*N. sativa*) is an annual herbaceous plant from Ranunculaceae family, which is native to Southwest Asia including India, Iran, Syria and Pakistan (Kooti et al. 2016). It was cultivated more than 3000 years ago in ancient Egypt (Hadi et al. 2016) and for this reason is today widely used in all Mediterranean countries (Gholamnezhad et al. 2016). *N. sativa* is known in different languages by various names, such as Kalonji in Hindi, Habbah Al-Sauda in Arabic, Siah Daneh in Persian, and black cumin, black seed or black caraway in English (Kooti et al. 2016).

2.1.2. Botanical overview

The plant has finely divided leaves and grows to maximal height of 40-70 cm (Amin & Hosseinzadeh 2016). Flowers are predominantly pale blue or white coloured with 5-10 petals (Kooti et al. 2016). Ripe fruit capsules of this plant produce numerous black or dark brown trigonal seeds (see Figure 1). These seeds contain most of the active components which may have positive impact on health (Gholamnezhad et al. 2016).

2.1.3. Traditional uses

Black cumin has an extensive historical and religious background. In Islamic countries is believed that the Holy Prophet Mohammed stated this herb as a cure for all diseases except death (Gholamnezhad et al. 2016). Studies dealing with *N. sativa* have repeatedly mentioned antimicrobial, anti-inflammatory, antibacterial, antifungal and antiparasitic effects of this plant. In the past, seeds and seed oil were traditionally used for treatment of wide range of diseases including rheumatism, asthma, gastrointestinal disorders, diabetes (Kooti et al. 2016), migraine and skin ailments (Gharby et al. 2015).

Seed oil also reduces the growth of microbes and postpones lipid oxidation in food (Elshama 2018). Thanks to preservation abilities and aromatic taste, the seeds are besides medicine used as a spice or additive in various food products (Amin & Hosseinzadeh 2016).

2.1.4. Active compounds

Seed is composed of proteins, carbohydrates, vitamins, minerals and two groups of oils- fixed and volatile. The ratio of components may vary in relation to geographical location, cultivation method and harvest time. Fixed oils contain unsaturated fatty acids, represented by linoleic (50-60%), oleic (20%) and palmitic acid (Dattal et al. 2012). The second group of oils are essential (volatile) oils, which contain most of the active substances responsible for beneficial effects in plant. In essential oil of *N. sativa* can be found especially thymoquinone (TQ), cymene, terpinen-4-ol and longifolene (Amin & Hosseinzadeh 2016).

Thymoquinone (TQ) is usually predominant and majority of the medicinal effects of this plant are attributed to this active ingredient. Research conducted by Woo et al. (2012) describes effect TQ on autoimmune diseases including asthma, arthritis and diabetes. Glucose lowering effect of TQ is issue of many scientific papers and experiments involving diabetic rats. Moreover, studies showed that TQ contained in *N. sativa* has ability to cure and prevent intoxication of drugs including common drugs, chemotherapeutic drugs, antibiotics and insecticides (Elshama 2018). Besides *N. sativa*, which is considered as a major natural source of TQ, it can be also found in several representants of the mint family- Lamiaceae (Taborsky et al. 2012).

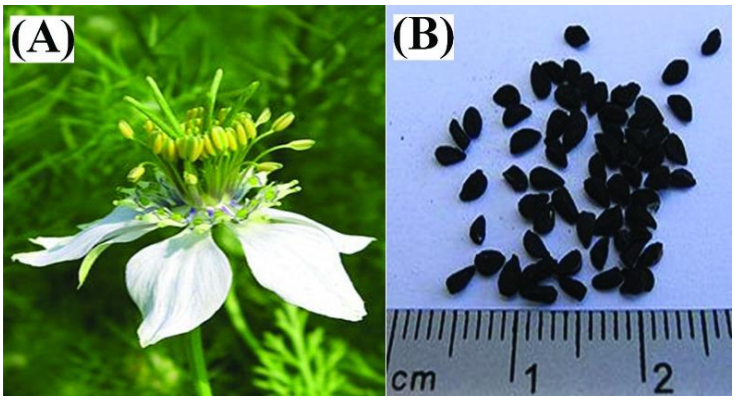

Figure 1: (A) *Nigella sativa* flower (B) *Nigella sativa* seeds

(Dollah et al. 2013)

2.2. Ginger *(Z. officinale)*

2.2.1. Introduction

Ginger (*Zingiber officinale*) is a perennial herb from the family Zingiberaceae. The plant is native to Southeast Asia. In India and China ginger has been used since ancient times. Its generic name Zingiber comes from the Sanskrit word Singabera, meaning "shaped like a horn". In the 1st century of Common Era, ginger was brought into the Mediterranean region by traders and gradually spread all around the world.

2.2.2. Botanical overview

Zingiberaceae family consists of approximately 40 genera and hundreds of species. *Z. officinale* is representant of the genus *Zingiber*. In India according reports, can be found another 14 species of this genus in addition to *Z. officinale*, which are locally used (Zachariah 2008).

Figure 2: *Zingiber officinale* (Gunasena 2014)

Ginger stem grows about a meter high with 15 to 30 cm long leaves organized alternately in two vertical rows. Ginger blooms relatively unfrequently. Flowers are small and usually have pink or yellow colour streaked with purple. Fruits occur rarely, it is a triangular shaped capsule producing black irregular seeds. Main part of the plant that is used for the consumption is the rhizome. The rhizome is a perennial, horizontal stem of a plant placed in the soil and often incorrectly referred as the "ginger root" (White 2007).

2.2.3. Traditional uses

The rhizomes with strong aromatic taste are widely used as spice, medicine and dietary supplements (Krüger et al. 2018). Ginger is one of the elementary ingredients in traditional cuisines all around the world, it can be consumed fresh, as a dried powder, preserved in syrup, crystallized (candy) or like a tea. In Asia is used for preparation of wide range of dishes especially for curry (India), ginger masala, kimchi (Korea) or traditional drinks including spiced masala chai (India) and salabat (Philippines). Western cuisine uses ginger mainly for the preparation of sweet foods and a ginger beer.

Pharmacological properties of ginger include antioxidative, anti-inflammatory, cancer preventive, antimicrobial and antifungal effects (Krüger et al. 2018). Ability to regulate bacterial growth can provide protection for individuals with deficient immune system such as HIV positive patients (Yu et al. 2007). In medicine is applied for treatment and prevention for various conditions, such as the common cold, respiratory disorders (asthma) and gastrointestinal disorders like diarrhoea and nausea (Siripoltangman & Chickos 2019). Ginger also helps to reduce blood sugar levels and regulate insulin response in people with diabetes (Li et al. 2012).

2.2.4. Active compounds

Approximately 4% of the total weight of the ginger rhizome consists of essential oils (Siripoltangman & Chickos 2019). In this oil can be found a wide range of active compounds, which are divided into sesquiterpenes (volatile) and phenyl alkanols (non-volatile) (Roufogalis 2014). Non-volatile compounds are mostly responsible for the pungent taste. These compounds are gingerols and shogaols. Shogaols, which are even more pungent, are found at lower contents and are result of gingerols degradation caused by a storage or a heat processing (Krüger et al. 2018).

The major volatile compounds identified in ginger are α-zingiberene, β-sesquiphellandrene, curcumene, α-farnesene, β-bisabolene, camphene and eucalyptol. α-Zingiberene gives ginger its aroma and his presence makes plant resistant to some species of pests. β-Sesquiphellandrene is associated with anti-cancer effect thanks to ability to reduce growth of cancer cells. Curcumene and β -bisabolene have positive effect against mosquitoes, which cause and spread many tropical diseases (Siripoltangman & Chickos 2019).

2.3. Cardamom *(E. cardamomum)*

2.3.1. Introduction

Cardamom (*Elettaria cardamomum*) also known as green, true or small cardamom belongs to the ginger family- Zingiberaceae (Ashokkumar et al. 2019). This herbaceous perennial plant is indigenous to India, Pakistan, Sri Lanka and Burma, where is called by local name "elaichi" (Gilani et al. 2007). Sometimes considered as "Queen of spices", cardamom is valued spice all around the world (Tambe & Gotmare 2019). It is third in the ranking of the world's most expensive spices, after saffron and vanilla. For this reason, cardamom was an important article of ancient Greek and Roman trade (Chempakam & Sindhu 2008).

2.3.2. Botanical overview

Wild populations of *Elettaria cardamomum* are growing in areas with altitudinal range 600-1300 m above sea level, in smaller isolated spots of wet, evergreen forests (Kuriakose et al. 2008). Bushy herb grows to height of 2-5 m. Stem is formed by alternate dark green leaves, 30-35 cm long and 7-10 cm wide. Inflorescence carrying panicles grow directly from the rhizomes on long floral stalks (Chempakam & Sindhu 2008). Orchid-like flowers are mostly white to lilac and pollen is transferred by cross-pollination. In 120 days from flowering, three sided and 1-2 cm long fruit capsules are completely mature. During the stage of ripening fruit colour turns from green to yellow. In each ripe fruit are 12-32 black, irregularly three-sided and highly aromatic seeds (Ashokkumar et al. 2019).

Figure 3: ***Elettaria cardamomum*** (Terre Exotique 2018)

2.3.3. Traditional uses

Dried cardamom is widely used for culinary purposes. Individual seeds are in cuisine more valued and expensive than complete fruit capsules, because contain higher levels of aroma, however, lose their flavour rapidly. It is typical ingredient in various Indian and Nepali sweet dishes, curry pastes and masala chai. 60% of the total world production of cardamom is exported to Arab countries, where is used to prepare coffee (Chempakam & Sindhu 2008). Seeds are commonly chewed to neutralize breath odours (Suneetha and Krishnakantha 2005) and sometimes used by people drinking large amounts of coffee to detoxify caffeine (Chempakam & Sindhu 2008).

Thanks to ancient Vedic texts from the 4th century BC, is known that Indian Ayurveda used cardamom capsules for treatment of various conditions and diseases (Ashokkumar et al. 2019). Traditional uses include treatment of gastrointestinal disorders (digestive problems, vomiting, dyspepsia), headache, cold, kidney stones, epilepsy and cardiovascular diseases (Gilani et al. 2007). One teaspoon of cardamom powder mixed with honey and taken twice a day has been recommended for patients with high blood pressure (Verma et al. 2009). Moreover, the seeds help treat respiratory problems as coughs or bronchitis and inflammatory diseases like common asthma. Not least among medical uses is prescription of cardamom to people with anorexia, because it stimulates the appetite (Tambe & Gotmare 2019).

2.3.4. Active compounds

Content and ratio of individual substances in cardamom can differ based on the variety, plant part (capsule/seeds), cultivation, age of the product and extraction method. Several substances predominate in all analyses performed so far: α-pinene, sabinene, eucalyptol, α-terpineol, terpinen-4-ol. Eucalyptol (1.8.-cineol) is represented at the highest levels, anti-inflammatory effect of this compound is responsible for treatment of asthma and bronchitis (Juergens et al. 2003).

Another substance contained in cardamom is α-pinene. α-Pinene has been the subject of many studies aimed at its gastroprotective effect. For positive effects in treatment of cardiovascular diseases is probably responsible especially α-terpineol. In the article written by Khaleel et al. (2018) authors describe α-terpineol ability to decrease the arterial pressure in detail. Terpinen-4-ol (which is also main compound of medicinal tee

tree oil) is often attributed to treat skin diseases and together with sabinene and eucalyptol provide aromatic flavour and odours of cardamom. Trace components in cardamom are for example β-pinene, p-cymene, (E)-nerolidol (Ashokkumar et al. 2019), α-thujene and γ-cadinene (Chempakam & Sindhu 2008).

2.4. Cinnamon *(C. cassia)*

2.4.1. Introduction

Approximately 250 species of trees and shrubs all around the world were reported to belong among the genus *Cinnamomum* from Lauraceae family (Rao & Gan 2014). Two of these species were identified as medicinal herbs: *Cinnamomum zeylanicum (C. zeylanicum)* and *Cinnamomum cassia (C. cassia)* (Gruenwald et al. 2010). *C. zeylanicum* is known as Ceylon or true cinnamon and is native to Sri Lanka and South India. *C. cassia* is sometimes called Chinese cinnamon and this name was given to her based on the country of origin. Nowadays is Chinese cinnamon widely cultivated in southern and eastern Asia (Vietnam, India, Indonesia, Myanmar) (Leela 2008). The bark of both mentioned species is one of the most popular spices used for perfumes, cosmetics, medicine and culinary purposes.

2.4.2. Botanical overview

Cinnamon is an evergreen tree which grows to height of 10-15 m. The inner bark is grey (see Figure 4). Leaves are ovate-oblongly shaped, 7-18 cm long and have red colour when they are young (Leela 2008). According to article written by Dominy et al. (2002), plants use red colour of young leaves to protect them against UV radiation damage or some herbivores who are blind to the red part of the spectrum. Flowers are white, arranged in panicles that are similarly long as leaves and have long greenish peduncles. The fruit size is approximately 1cm, blue-purple colour, round shaped and contains only one single seed (Leela 2008).

Figure 4: *Cinnamomum cassia* (Encyclopaedia Britannica 2019)

2.4.3. Traditional uses

The volatile oils can be obtained from several plant parts (bark, leaf, root bark), however dried bark of this herb is the most often used part for commercial, culinary or medical purposes (Gruenwald et al. 2010). Bark sticks and milled bark of cinnamon is an essential ingredient in traditional cuisines all around the world. It can be added to various sweets, pastry, desserts and beverages (coffee). Asian, Arabian and Latin American cuisine use cinnamon in preparation of meat dishes. In India is mostly accompanied by cardamom and used in savoury food (Garam Masala, curry pastes). Together with ginger and cardamom is also one of the ingredients of spiced masala chai.

Besides food preparation, cinnamon is popular in cosmetics and perfumery for its characteristic aroma. In pharmaceutical industry is sometimes used to improve unpleasant taste of some drugs. Medicine has been using cinnamon for centuries and activities including antimicrobial, antioxidant and antidiabetic effects are commonly associated with this herb. *C. cassia* has positive blood pressure-lowering effect, as coagulant prevents bleeding but also can increase the blood circulation to improve tissue regeneration. Powder has been used traditionally to treat dental problems, oral microbiome and added to chewing gums for its ability to refresh and eliminate bad breath odour (Rao & Gan 2014). Is commonly used for gastrointestinal disorders, rheumatism and cold. Extensive studies are showing positive outcomes in treatment of both types of diabetes and cancer (Gruenwald et al. 2010). Momtaz et al. (2017) in his article mentions optimistic prospect for use of cinnamon as possible cure for Alzheimer's disease.

2.4.4. Active compounds

The chemical composition is significantly different depending on specie and part of the plant. Dried bark is composed of moisture, crude fibre, carbohydrate, protein, fixed and volatile oil (Leela 2008). The last one mentioned is the most important one for commercial and other purposes. The volatile oil of *C. cassia* is predominantly composed of (Z)-cinnamaldehyde followed by coumarin, α-cubebene, γ-amorphene, δ-cadinene, (−)-calamenene, o-methoxycinnamaldehyde and τ-muurolol (Deng et al. 2014).

Cinnamaldehyde which is the main component in all cinnamon species brings the characteristic taste and aroma. It is widely used as food flavouring but also as safe insecticide against mosquitos and as additive in anti-corrosion sprays. However, the most

discussed active compound of *C. cassia* is coumarin. This component can be found in other cinnamon species (*C. zeylanicum*) as well, but in very low quantities. Because of frequently reported side effects and possible toxicity, coumarin is subject of many controversial studies. Long term exposure to coumarin in rodents has been found to cause hepatotoxicity and various liver and lung tumours. In humans such effects are rare and associated only with very high doses (Dinesh et al. 2015). Medical doses were reported to be safe (Momtaz et al. 2017), however, excessive amounts may negatively affect liver function in humans, for this reason it would be desirable to determine the maximum recommended dosage of this herb for patients (Dinesh et al. 2015).

2.5. Extraction methods

Together with some other factors (part of the plant, cultivation, storage, etc.), extraction method is one of the crucial aspects affecting levels and quality of essential oils obtained from plants. Commonly employed techniques for extracting essential oils include hydro-distillation, steam distillation and solvent distillation (Kokoska et al. 2008; Zhang et al. 2018). In addition to these conventional techniques, new alternative methods have been developed lately. One of them is Supercritical Fluid Extraction (SFE), this method uses a special state in which the temperature and pressure of supercritical fluid is above its critical point and differences between liquid and gas phases disappear. Other alternatives include microwave-assisted extraction, ultrasonic-assisted extraction and controlled pressure drop process (Stratakos & Koidis 2015).

In order to select the most appropriate method the characteristics of both the method and the sample should be considered. The decisive aspects may include time duration, financial costs, water solubility of the essential oil, sample amount and heat sensitivity.

2.5.1. Soxhlet extraction method

The Soxhlet extraction apparatus was firstly described in 1879 by German chemist Franz Ritter von Soxhlet. The scientist who was born in the Czech Republic (Brno) dedicated his career to analyse the properties of milk. The Soxhlet apparatus was originally invented for the extraction of fat from the milk solids (Sella 2007). The method was used as a standard technique for over a century and the efficiency of newly developed extraction methods was measured by comparison with the Soxhlet extractor (De Castro & Priego-Capote 2010).

The sample is placed in an extraction thimble. From the bottom it is attached to the distillation flask and from the top to the condenser (see Figure 5). The distillation flask is filled with the solvent used for extraction and placed to the heat source. As the solvent begins to evaporate, it rises up the apparatus in the form of vapour. When the vapour enters the condenser, it cools down, flows out the bottom of the condenser in liquid state of matter and begins to fill the extraction thimble. When the thimble is completely full, liquid flows back through the siphon into the distillation flask (De Castro

& Priego-Capote 2010). The process continues for several hours until the extraction is complete and active compounds from the sample are dissolved in the distillation flask. The essential oil is gained by subsequent evaporation of this liquid (Charles & Simon 1990).

Advantages of this method are following: no further action is needed once the extraction is running except the regular control of the apparatus, simple methodology requiring little training, no filtration is needed, complete extraction and high efficiency (De Castro & Priego-Capote 2010).

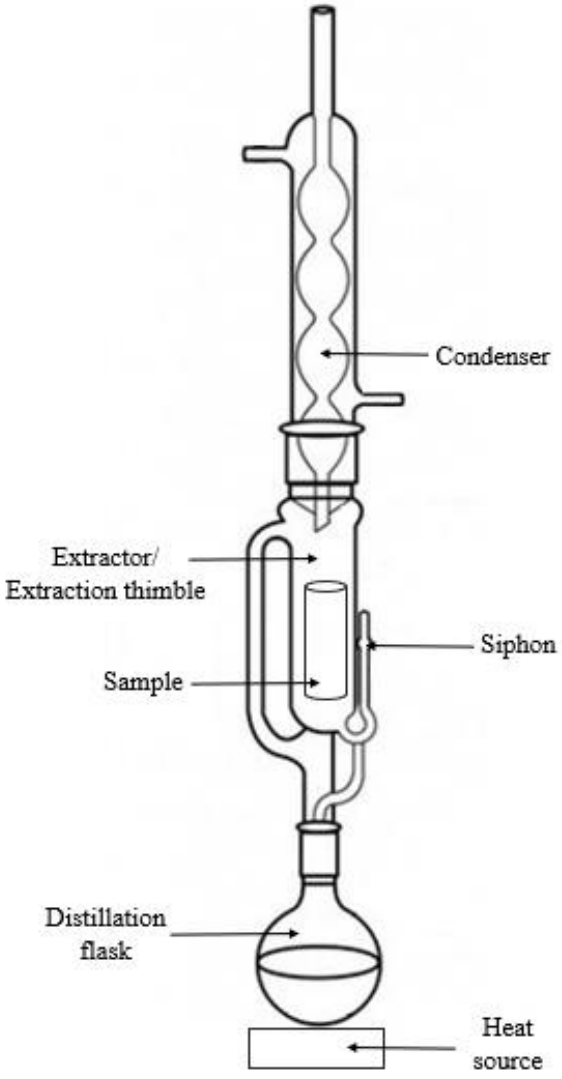

Figure 5: Soxhlet extraction apparatus

(De Castro & Priego-Capote 2010; Author of the Thesis)

2.6. Chromatography

Chromatography is the most widely used method for separating components of a mixture in a laboratory. The process is based on their interactions with two phases: a mobile phase and a stationary phase (see Figure 6). The mobile phase is fluid and moves through the system while carrying sample components. The stationary phase in the form of a porous bed or film is held within a system and does not travel. After sample injection, components pass through the system. The components that have strong interaction with the stationary phase move more slowly than components with weak interaction. Differences in travelling speeds of constituents lead to their separation (Hage 2018; Poole 2000).

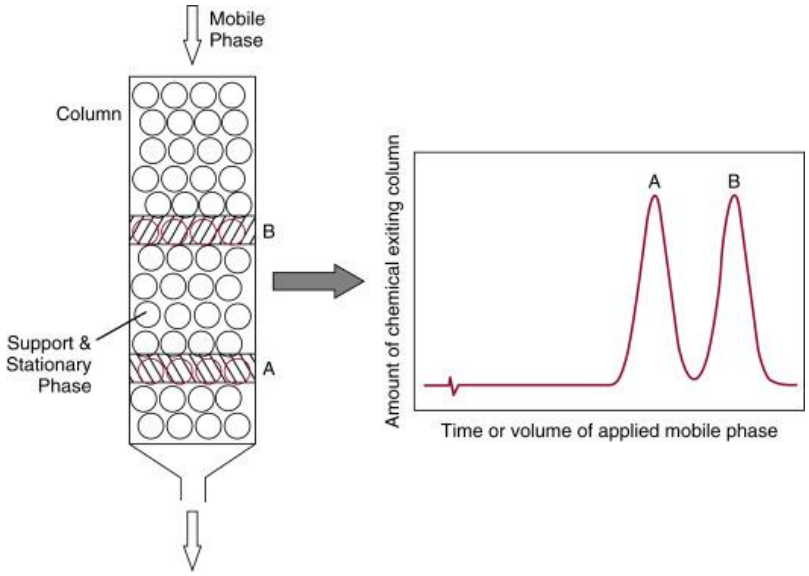

Figure 6: The general components of a chromatographic system

(Hage 2018)

Based on the purpose a chromatography can be preparative or analytical. The preparative chromatography focuses on separation of sufficient quantities of a substance from the sample. Main purpose of analytical chromatography is to separate and analyse substances in order to create compact profile of the sample (Shaffer 2019).

Technique of the analysis differs according to the physical state of mobile phase, it can be gas or liquid. Other types of chromatography can be distinguished according to the shape of chromatographic bed (stationary phase) into column, paper and thin layer. The example of column chromatography is in the Figure 6. Substance in mobile phase

passes through the stationary phase inside the column. Differences in the substances are present in the form of different retention times (time which it takes for a substance to pass through the column). In paper chromatography paper which is made of cellulose is used as stationary phase, identification of compounds is done thanks to colour changes. Last type is similar however, instead of paper, a thin layer of silica gel, alumina or cellulose absorbed on an inert substrate is used.

2.6.1. Gas chromatography

In gas chromatography the mobile phase is a gas, usually helium (He) and the stationary phase in the form of liquid is placed inside the column (Li & Liu 2019). Main components of the gas chromatograph consist of carrier gas, flow controller, sample injector, column, oven and detector (see Figure 7).

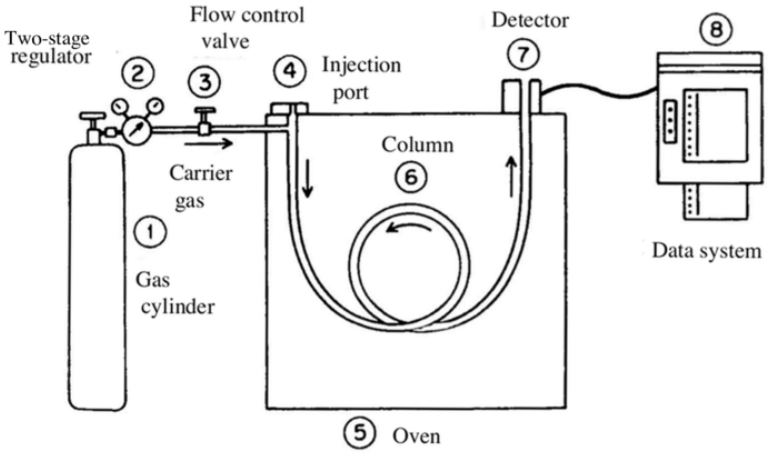

Figure 7: Gas chromatograph

(Kuyper 2014)

At first vial with the mixture is placed in an autosampler which transfers sufficient amount of the sample into the injector (inlet). Via injection port it is subsequently injected into the machine. The temperature in the oven is so high that molecules of the mixture instantly vaporize and are carried by the carrier gas through the column, where the stationary phase is located. As the sample travels through the column, individual components are separated and enter the detector one by one. Identified components are printed in the form of corresponding peak on a final diagram (Stauffer et al 2008).

2.7. Mass Spectrometry

Mass spectrometry is an analytical instrument for identification of molecules within a sample. It is always used in a combination with previous primary separation of molecules which can be done by various techniques like chromatography, capillary electrophoresis or ion mobility. Combination of gas chromatography and mass spectrometry (GC-MS) is probably the most used (Naylor & Babcock 2010).

The mass spectrometer consists of three main parts: an ionizer, a mass analyser and a detector. The process can be divided in 4 stages:

1) Ionisation

   The sample is vaporised and passed into the ionisation chamber, where molecules are bombarded by a stream of electrons. This may lead to separation of one or more electrons and creation of positively charged ions.

2) Acceleration

   The ionisation chamber is also positively charged so these ions are repelled and subsequently accelerate towards negative plates into a stream.

3) Deflection

   The stream of ions is then deflected by a magnetic field depending on their masses (lighter ions are deflected more than heavier ones) and the number of positive charges (the more the ion is charged, the more it gets deflected). These properties are known as a mass/charge ratio (m/z), where the mass sis divided by the positive charge of the ion.

4) Detection

   Finally, when the stream of ions passes through the detector, their different values of the mass/charge ratio (m/z) are recorded and generate a mass spectrum (Downard 2004).

3. Objectives

3.1. Main objective

To provide the chemical analysis of volatile compounds in essential oils extracted from black cumin, ginger, cardamom and cinnamon.

3.2. Specific objectives

1) Literature review focused on traditional use of the spices and beneficial effects of contained active compounds.
2) Comparison of the results between samples and with literature data.

4. Materials and methods

4.1. Samples

All samples were collected in October 2019 from a local market in Jaipur, India. Preparation of the samples was done using the same approach. The only difference occurred in the case of ginger, the sample was already purchased in milled form and the stage of grinding was skipped. Laboratory conditions allowed the construction of two apparatus for this reason two samples were always extracted simultaneously on isolated extraction apparatuses.

4.2. Extraction

Dry sample was milled into powder by using a laboratory scale tube mill. 10 g of this powder were placed into porous cellulose thimble and placed in the extraction thimble on a Soxhlet extraction apparatus. Distillation flask with 200 ml of hexane and 3 pieces of boiling chips was attached to the bottom part of apparatus. Boiling chips were added into the flask to ensure a smooth boiling process. The final stage was to connect the condenser and place the assembled apparatus in a water bath consisting of distilled water. Water bath temperature was set to 80°C. The process was carried out for 2 hours under constant supervision.

After 2 hours of extraction, distillation flask with obtained extract was removed and allowed to cool. Cooling was followed by evaporation of hexane in a vacuum rotary evaporator BÜCHI R-200 Rotavapor. Water temperature of heating bath was 40 °C and applied pressure of 300 mbar. Obtained essential oil was collected in a vial and dissolved in hexane. Concentration of samples were 1 μl/ml.

4.3. GC-MS analysis

Gas chromatography-mass spectrometry analysis was performed on Agilent Technologies 5977A MSD with a HP-5 column (5%-phenyl)-methylpolysiloxane, 30 m length, 250 μm internal diameter and 0.25 μm film thickness. Each sample was analysed three times, so in total the GC-MS was used for 12 analysis. Device is equipped with an autosampler using 1 μl volume injection. Temperature of the inlet injection port was 220° C. The oven temperature program applied for analysis was 70 °C (2 min) to 280 °C at 10 °C/min. Helium was used as carrier gas at a flow rate of 1 mL/min. The electron energy of the MSD transfer line was 70 eV with temperature held at 250 °C. Mass spectra were obtained using a scan time of 1 s in the mass range from m/z 30 to 600.

Results from gas chromatograph were analysed via MassHunter Workstation Software Qualitative Analysis Version B.07.00. Compounds contained in samples were identified by comparing their mass spectra with data in NIST2.2 MS database. Majority of compounds could be determined by this method. In ginger and cinnamon 3-4%, in *N. sativa* 7-8% and in cardamom 5-6% of substances remained unidentified.

The accuracy of the identification has been confirmed by comparison of their Kovats retention indices (KI) calculated using n-alkanes (C8-C22) and KI listed in literature databases (Adams 2007; NIST 2019). Some substances could not be confirmed by KI comparison, because not all retention indexes were available.

5. Results

On average 95% of compounds were identified during 3 times performed GC-MS analysis of 4 essential oils (black cumin, ginger, cardamom, cinnamon). The most frequently occurring substances were pinene, sabinene, eucalyptol, α-terpineol, cyclosativene, copaene and nerolidol. The results are shown in 4 separate tables. Chromatograms and mass spectrums of major compounds which are not included directly in the results can be found in appendices (see Appendix 1, 2, 3 and 4).

In total 20 components (93%) were identified in the sample of **black cumin**, these results are shown in **table 1**. Oil from the seeds contained fixed oils rich in unsaturated fatty acids like 40.91% oleic acid and 19.88% linoleic acid and essential oil compounds like 1.23% α-thujene, 5.52% p-cymene, 1.53% longifolene. Presence of the TQ as major active compound was confirmed, this substance accounted for 10.80% of the oil. Figure 8 represents the chromatogram of *N. sativa* and Figure 9 shows mass spectrum of the TQ.

Table 2 shows all 31 identified compounds of the essential oil of **ginger**, their percentages together accounted for 96.5% of the sample. The results of most commonly presented compounds in ginger and their percentages were following: α-curcumene (16.45%), camphene (0.24%), β-bisabolene (9.88%), β-sesquiphellandrene (7.35%) and eucalyptol (0.82%). The content of α-zingiberene, which is generally considered in studies to be the substance with the highest content in ginger rhizomes, represented only 9.19% in our sample. The sample contained a significant amount of germacrene D, making up 8.03% of the sample and shogaol in the amount of 11.22%. The sample also contained 0.23% of anethole, strongly aromatic compound which was not identified in any of the compared literature sources.

Table 1: Chemical composition of the oil from black cumin

Compound	RI calc	RI lit	Relative area – average [%]	MW
α-Thujene	921	931	1.23	136
α-Pinene	930	939	0.27	136
Sabinene	968	976	0.14	136
β-Pinene	972	980	0.30	136
p-Cymene	1017	1026	5.52	134
Limonene	1021	1031	0.53	134
γ-Terpinene	1054	1062	0.23	136
(Z)-4-methoxy thujane	1088	-[a]	0.14	168
α-Thujone	1110	1105	0.85	168
Terpinen-4-ol	1170	1177	0.11	154
Dihydrocarvone	1194	1193	0.18	152
Thymoquinone	1241	1249	10.80	164
Thymol	1287	1290	0.73	150
α-Longipinene	1347	1351	0.29	204
Longifolene	1405	1402	1.53	204
Thymohydroquinone	1550	1554	0.37	166
Ethyl hexadecanoate	1985	1993	3.61	284
Linoleic acid	2140	2150	19.88	282
Oleic acid	2157	2161	40.91	280
β-Monoolein	2681	-[a]	5.28	356

[a] ……. Retention index was not found

Source: Author of the Thesis

Figure 8: Chromatogram of black cumin

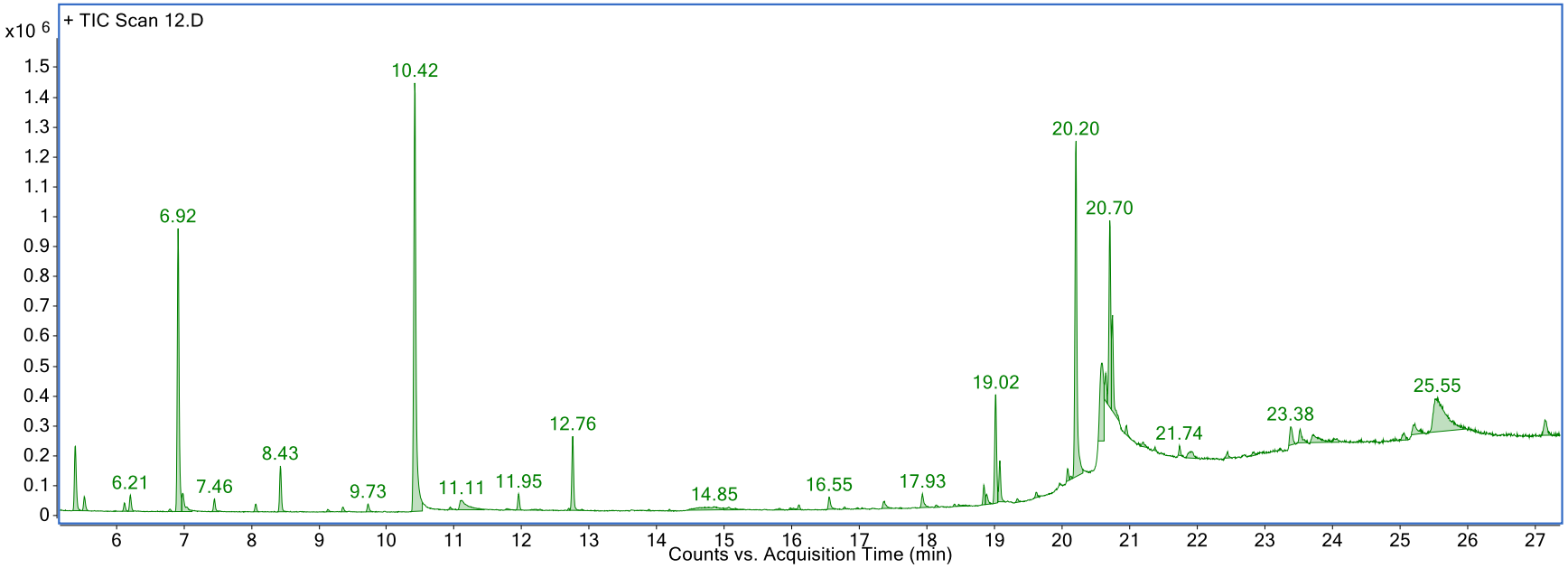

Figure 9: Mass Spectrum of TQ

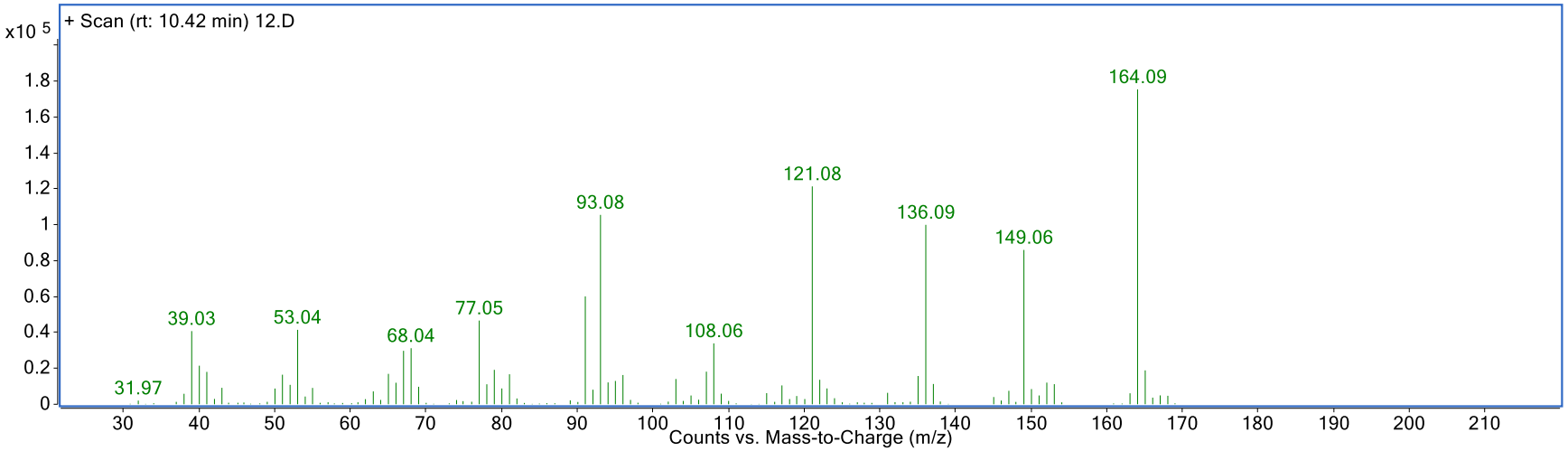

Table 2: Chemical composition of the oil from ginger

Compound	RI cacl	RI lit	Relative area – average [%]	MW
Camphene	957	953	0.24	136
Eucalyptol	1041	1033	0.82	154
m-Cymenene	1090	1082	0.47	132
Linalool	1111	1098	0.29	154
Borneol	1190	1177	0.83	154
L-α-Terpineol	1214	1197	1.23	154
Bornyl acetate	1295	1285	0.30	196
Anethole	1300	1289	0.23	148
Cyclosativene	1377	1368	0.49	204
Copaene	1390	1381	0.47	204
Caryophyllene	1466	1454	0.41	204
Germacrene D	1483	1480	8.03	204
α-Curcumene	1486	1483	16.45	202
γ-Curcumene	1498	1505	6.88	204
α-Zingiberene	1504	1495	9.19	204
α-Farnesene	1511	1508	4.51	204
β-Bisabolene	1519	1509	9.88	204
β-Sesquiphellandrene	1547	1544	7.35	204
Selina-3,7(11)-diene	1564	1558	0.28	204
Guaia-3,9-diene	1565	1556	0.33	204
Nerolidol	1574	1564	0.94	222
8-Cedren-13-ol	1638	1630	0.22	220
γ-Eudesmol	1648	1636	2.50	222
epi-α-Cadinol	1658	1653	0.25	222
(Z)-Eudesm-6-en-11-ol	1695	-[a]	2.79	222
Geranyl-α-terpinene	1998	-[a]	2.16	272
10,13-Octadecadienoic acid, methyl ester	2135	-[a]	0.88	290
Shogaol	2305	2294	11.22	276
10-Gingerol	2384	-[a]	0.95	350
6-Gingerdiol 3,5-diacetate	2537	2536	3.99	380
[10]-Shogaol	2755	-[a]	1.87	332

[a] ……. Retention index was not found

Source: Author of the Thesis

In total 26 compounds identified in the oil of **cardamom** capsules are presented in **table 3**. The major substance contained in the absolute highest values was eucalyptol which reached 60.21%. Followed by substances with significantly lower contents such as α-pinene (1.92%), sabinene (0.92%), β-pinene (6.40%), terpinen-4-ol (2.17%), α-terpineol (8.60%) and nerolidol (3.16%). Presence of α-terpinyl acetate was not detected in the sample, although this compound should form one of the main constituents of the oil. Figure 10 shows the chromatogram of cardamom and Figure 11 shows mass spectrum of the main component- eucalyptol.

Table 4 shows all 18 compounds identified in the oil from the bark of **cinnamon** (*C. cassia)*. In total 76.88% of the sample were represented by (E)-cinnamaldehyde. Other substances contained in lower amounts were α-copaene (4.10%), α-tetralone (3.13%), α-muurolene (2.40%), δ-cadinene (4.11%) and cinnamyl acetate (0.87%). Coumarin was present in the amount of 0.28%.

Table 3: Chemical composition of the oil from cardamom

Compound	*RI calc*	RI lit	Relative area – average[%]	MW
α-Thujene	923	931	0.16	136
α-Pinene	932	939	1.92	136
Sabinene	975	976	0.92	136
β-Pinene	980	980	6.40	136
Eucalyptol	1046	1046	60.21	154
γ-Terpinene	1065	1066	0.66	136
Sabinene hydrate	1087	1097	1.44	154
(Z)-β-Terpineol	1138	1144	0.61	154
p-Menth-2-en-1-ol	1143	1137	0.09	154
Isopinocarveol	1170	1167	0.16	152
δ-Terpineol	1186	1180	0.80	154
Terpinen-4-ol	1196	1206	2.17	154
α-Terpineol	1202	1189	8.60	154
(1S,4R,5R)-1,3,3-Trimethyl-2-oxabicyclo[2.2.2]octan-5-yl acetate	1354	1343	0.24	212
2,4-Dimethoxyphenol	1388	-[a]	0.67	154
β-Elemene	1401	1393	0.16	204
Caryophyllene	1447	1454	0.06	204
4-Methoxy-3-(methoxymethyl)-phenol	1484	-[a]	0.16	168
γ-Cadinene	1505	1513	0.75	204
δ-Guaiene	1507	1508	2.25	204
β-Cadinene	1526	1518	0.19	204
(E)-Nerolidol	1572	1564	3.16	222
Spathulenol	1636	1640	0.37	220
Longifolenaldehyde	1730	-[a]	0.38	220
Ambrial	1863	-[a]	0.25	234
Coronarin E	2144	2135	1.76	284

[a] ……. Retention index was not found

Source: Author of the Thesis

Figure 10: Chromatogram of cardamom

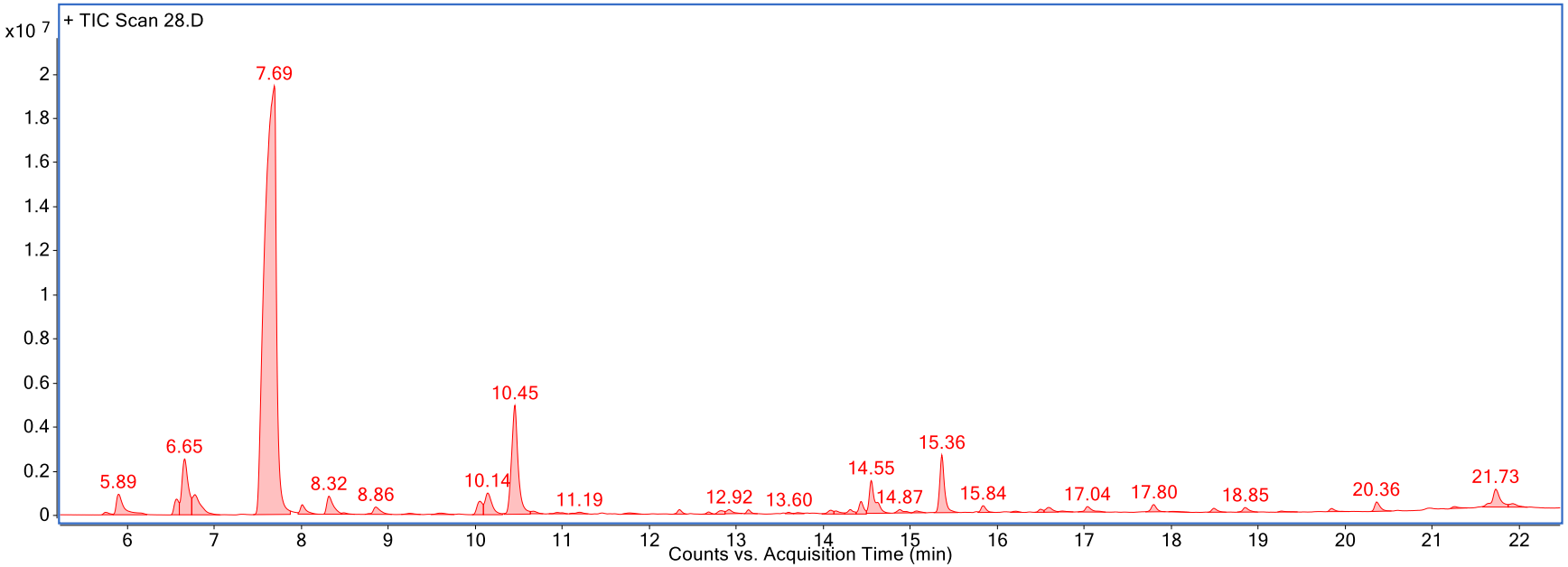

Figure 11: Mass Spectrum of eucalyptol

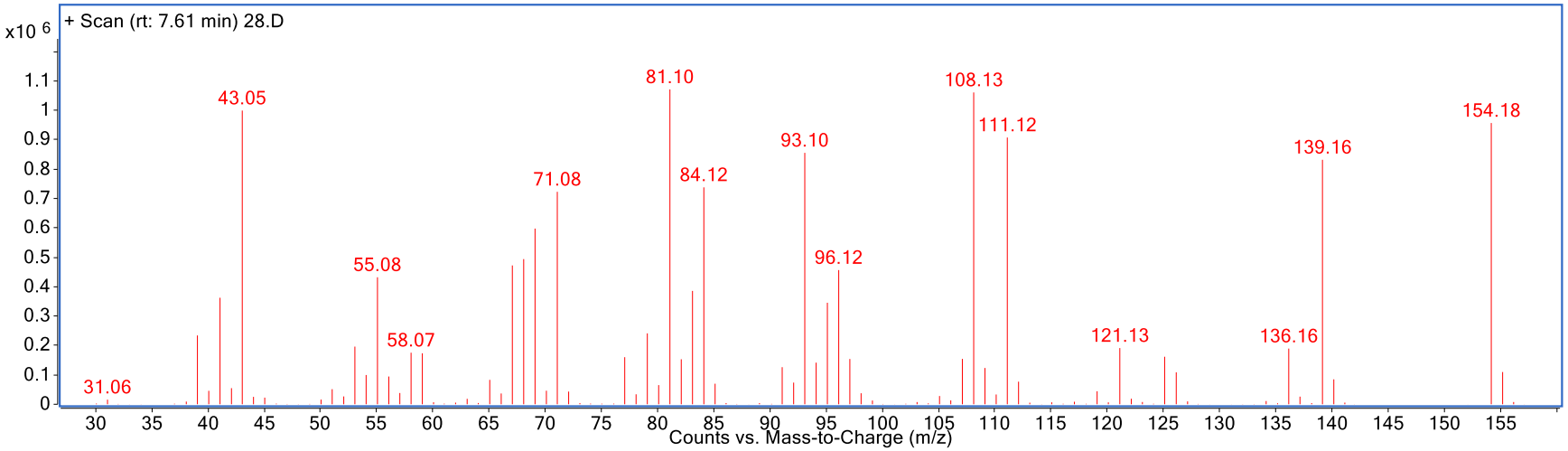

Table 4: Chemical composition of the oil from cinnamon

Compound	RI calc	RI lit	Relative area – average [%]	MW
(E)-Cinnamaldehyde	1265	1268	76.88	132
Cyclosativene	1363	1368	0.53	204
α-Copaene	1369	1376	4.10	204
Aromadendrene	1395	1419	0.17	204
Sativene	1400	1402	0.17	204
Caryophyllene	1415	1418	0.15	204
α-Tetralone	1431	-[a]	3.13	146
Coumarin	1432	1429	0.28	146
Cinnamyl acetate	1448	1454	0.87	176
γ-Muurolene	1466	1477	0.65	204
α-Muurolene	1489	1499	2.40	204
δ-Cadinene	1520	1524	4.11	204
Cubenene	1525	1532	1.28	204
o-Methoxycinnamaldehyde	1528	1505	0.51	162
4.8-Dimethylquinoline	1534	-[a]	0.54	157
Di-epi-1,10-cubenol	1615	1607	0.30	222
t-Muurolol	1631	1642	0.62	222
(4-Tetrazol-1-yl-phenyl)-acetic acid	1644	-[a]	0.11	204

[a] ……. Retention index was not found

Source: Author of the Thesis

6. Discussion

The results were compared with each other and with literature data. Several compounds (eucalyptol, nerolidol, α-terpineol, …) with different amounts occurred in both ginger and cardamom, these 2 plants belong to the same family- *Zingiberaceae*. Same substances in smaller amounts also appeared in comparison of cardamom and black cumin (α-thujene, pinene, sabinene, …). On the contrary, cinnamon differed from other samples by most of the substances contained.

In total 93% of compounds from **black cumin** oil were identified, represented by active compounds such as TQ (10.80%), α-thujene (1.23%), p-cymene (5.52%), longifolene (1.53%). For comparison Gerige et al. (2009) identified 86.7% of compounds in his study and the percentages were: 11.8% TQ, 2.4% α-thujene, 9% p-cymene, 5.7% longifolene. Oil content however depends on the experimental factors, especially sowing (D'Antuono et al. 2002).

In the **ginger** presence of α-zingiberene (9.19%), α-curcumene (16.45%), β-sesquiphellandrene (7.35%) was confirmed. To compare, the essential oil from dried rhizome cultivated in Trivandrum, India contained 30.3% α-zingiberene, 5.8% α-curcumene and 6.6% β-sesquiphellandrene (Sasidharan & Menon 2010). Changes in the chemical composition of oil can occur during storage. Exposure of ginger essential oils to light, air or heat results in decrease in levels of α-zingiberene and β-sesquiphellandrene followed by increase in the amount of α-curcumene. In the past ratio of these three components was used as indicator of the oil quality (Zachariah 2008). The amounts of eucalyptol were lower in comparison with studies performed with extract from fresh ginger rhizome and extracted by both hydro and steam distillation (Sasidharan & Menon 2010; Toure & Xiaoming 2007). Differences between the oils extracted from fresh and dried ginger rhizomes are caused by losses of some volatile components, mostly lower-boiling compounds, during drying. Lower-boiling compounds also include the citrals (neral, geranial ect.) responsible for the citrus aroma and taste of fresh ginger (Zachariah 2008). During our analyse neral and geranial were not identified.

On the contrary, results of our analyse detected relatively high levels of germacrene D (8.03%). Germacrene D is naturally occurring sesquiterpene with antibiotic

and antibacterial properties. In the study by Toure and Xiaoming (2007), germacrene D accounted for 1.55% in the sample from China and 0.43% in the sample from Guinea. Sasidharan and Menon (2010) reported values of 4.2% during the extraction of dried ginger, which was the second highest value of all compared results after our sample. The pungent constituent shogaol is product of the gingerols thermal degradation during solvent extraction methods (Zachariah 2008).

The data from the chemical analysis of **cardamom** were compared with other studies examining the composition of substances from dried fruit capsules. All compared studies reported high content of α-terpinyl acetate, for example Amma et al. (2015) reported a ratio of 28.82% eucalyptol and 44.46% α-terpinyl acetate in the oil from India. In paper written by Omanakutty and Joy (2007) α-terpinyl acetate represents even 47.42% of the sample extracted by hydro distillation. Subsequent comparison with the results of other chemical analyses revealed that the present results might correspond to the chemical compositions of *Amomum subulatum*, a perennial herb from the same family (zingiberaceae) also known as large or false cardamom. The compounds such as α-pinene (1.92%), sabinene (0.92%), β-pinene (6.40%), α-terpineol (8.60%) and nerolidol (3.16%) were present in the *E. cardamomum* study by Amma et al. (2015) in amounts of 1.59% α-pinene and 3.77% sabinene. The rest of mentioned compounds was not present. On the contrary, Kumar et al. (2012) provided GC-MS analyse of *Amomum subulatum* with following results: 4.60% α-pinene, 1.22% sabinene, 7.23% β-pinene, 10.15% α-terpineol and 2.06% nerolidol. The analysis revealed that a sample of cardamom obtained at the local spice market in Jaipur, India is probably *Amomum subulatum*, a herbaceous plant used to treat digestion disorders, liver complaints, throat and lung diseases (Kumar et al. 2012) and sometimes used as a substitute for true cardamom with a lower market price.

The GC-MS analyse of **cinnamon** confirmed the presence of assumed major compound (E)-cinnamaldehyde in the amount of 76.88%. Compared studies dealing with variations of *C. cassia* bark reported standard values of (E)-cinnamaldehyde between 66-77% (Li et al. 2013; Deng et al. 2014). The other compounds like α-muurolene (2.40%), γ-muurolene (0.65%), δ-cadinene (4.11%), α-copaene (4.10%) and cinnamyl acetate (0.87%) were also compared with scientific papers. The values reported by Deng et al. (2014) within a bark sample extracted by hydro distillation were 0.05% α-muurolene, 1.01% γ-muurolene and 4.06% δ-cadinene. In the paper by Li et al. (2013) the amounts

were 1.0-1.8% α-muurolene, 0.6-3.9% α-copaene and 0.4-0.9% cinnamyl acetate. Although high doses of *C. cassia* are sometimes considered as dangerous, because of the possible toxicity of coumarin, our oil extracted from 10g of *C. cassia* bark contained only 0.28% coumarin. Chang et al. (2013) reports 1.25% of coumarin, Li et al. (2013) and Deng et al. (2014) report that coumarin was not present at all.

7. Conclusions

The main objective of this Bachelor Thesis was to provide the chemical analysis of volatile compounds in essential oils extracted from black cumin, ginger, cardamom and cinnamon. In the experimental part of the thesis, samples collected in Jaipur, India were extracted in Soxhlet extraction apparatus and then identified via GC-MS analysis. The obtained data on identified components and their content in percentage were subsequently entered into tables.

In the literature review, individual species and their historical origin, botanical description, traditional methods of use and contained active substances were described in detail. Therefore, the first specific objective of the work was also met.

The second specific objective of this thesis was to compare the results of GC-MS analysis between samples and with official literature data. The results have confirmed the presence of main compound TQ in black cumin (*N. sativa*) and (E)-cinnamaldehyde in cinnamon (*C. cassia*). GC-MS analysis of ginger (*Z. officinale*) revealed lower amounts of substances with propensity to degradation: α-zingiberene and β-sesquiphellandrene. The decrease in sample quality was probably caused by external factors such as heat or light in the time of storage. The analysis of last spice showed that the final composition of the purchased sample did not correspond to the composition of *Elletaria cardamomum*. After additional comparison with the literature data, the sample was identified as *Amomum subulatum*, a plant from the same Zingiberaceae family known as a false cardamom.

The comparison of results between samples showed recurrence of some substances, although the samples were from different herbs and parts of the plant. Most similarities in chemical composition were detected for *Z. officinale* and *A. subulatum*, which botanically belong to the same family. On the other hand, the oil obtained from *C. cassia* bark differed the most in its composition from the rest of samples.

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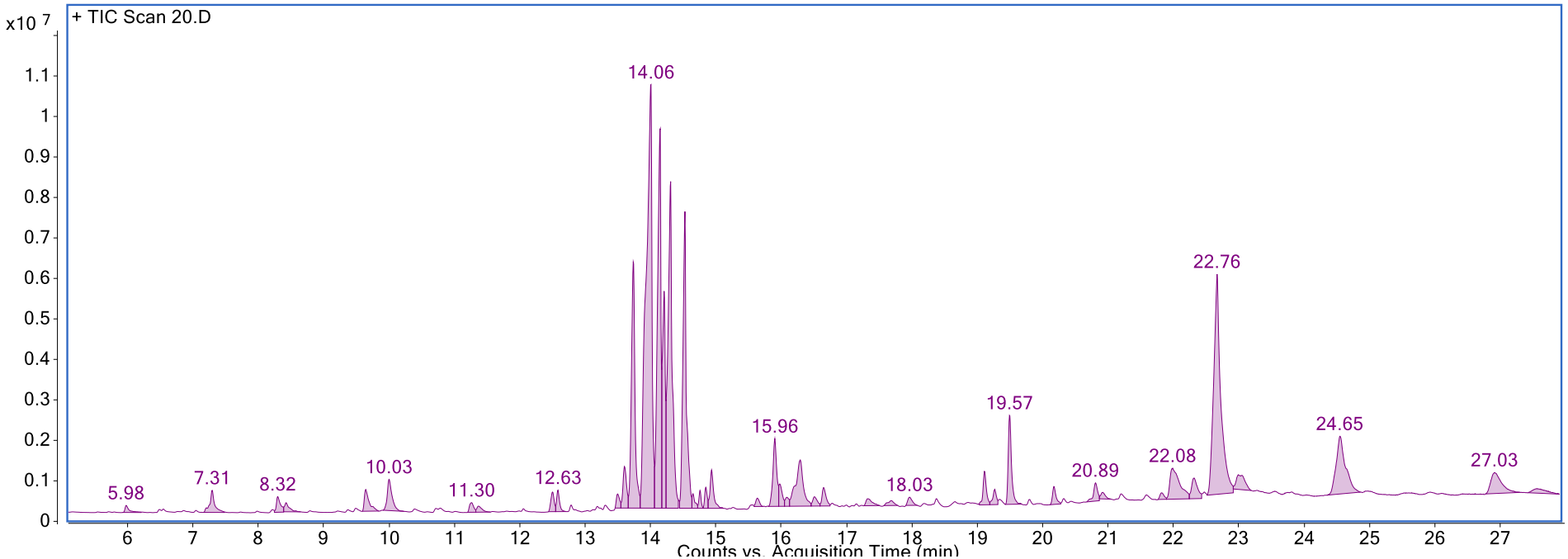
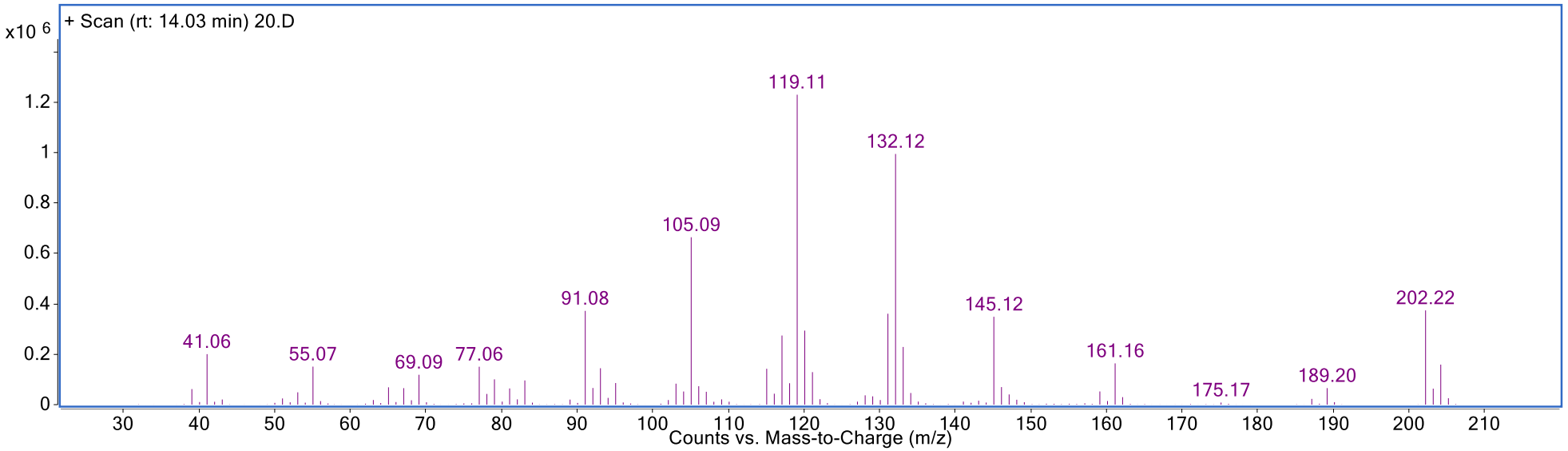
Appendices

List of the Appendices:

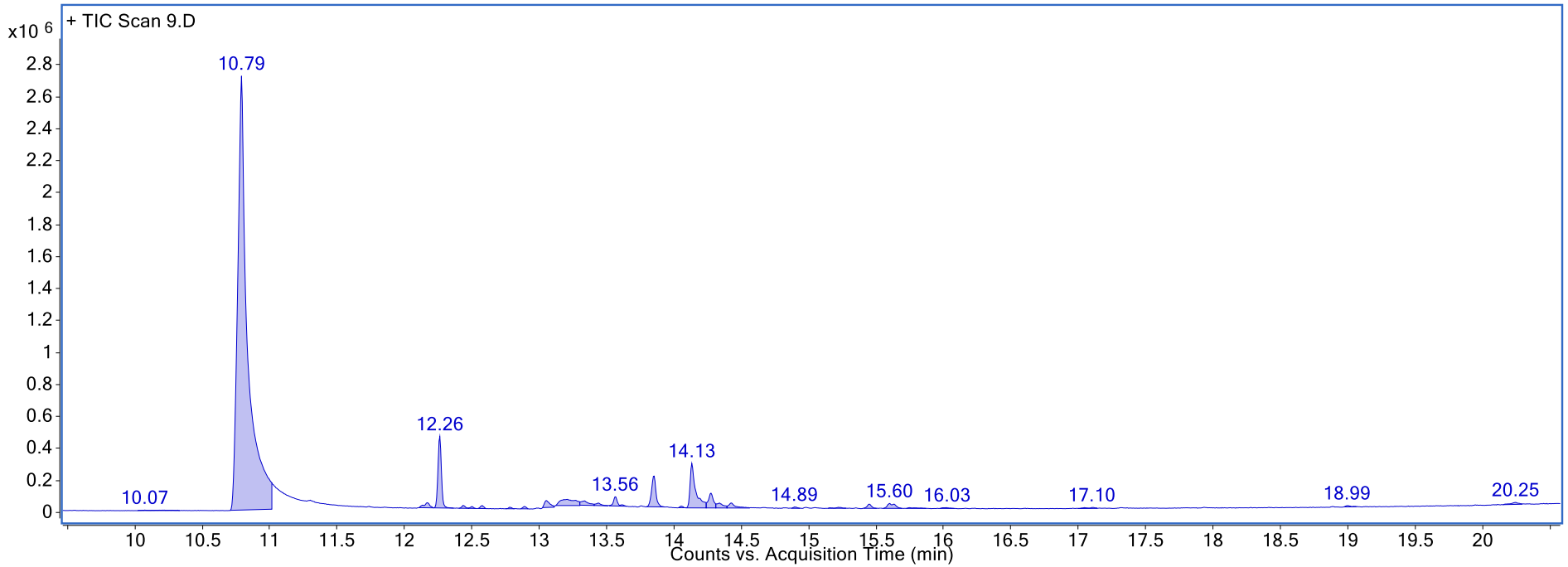
Appendix 1: Chromatogram of ginger



Appendix 2: Mass Spectrum of α-curcumene



Appendix 3: Chromatogram of cinnamon



Appendix 4: Mass Spectrum of (E)-cinnamaldehyde