Cleaning flax fibre; extracting and identifying antimicrobials and measuring water absorption of plant stems

by

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Thesis Abstract

Decorticated flax contains a significant amount of shive content, which limits applications of flax fibre. Separation of shives from the fibre is essential to improve the quality of flax fibre. Pneumatic method and a Sorter were implemented to meet the above objectives. Terminal velocities of individual flax fibre and shive particles were investigated and their width, length, and mass were recorded. A sorting method was tested for separation of short and long fibre for two grades of fibre: Grade 1 and Grade 2, with initial fibre purities of 51% and 15%, respectively. The results of the pneumatic tests showed that the length of fibre particles did not influence the terminal velocity. For shives, the increase in mass and width showed an increasing trend in terminal velocity. The ranges of terminal velocities for shive and fibre particles were 1.13 to 4.09 m/s and 0.51 to 1.07 m/s, respectively, which were significantly different. Fibre purity of approximately 80% for Grade 1 and 66% for Grade 2 were recorded from sorting, which were a significant improvement when compared to the initial purities. This study demonstrated the potential of the pneumatic and sorting methods for improving fibre quality.

With the increase in resistant strains of microorganisms to antibiotics, researchers have started to explore plant parts to discover new antimicrobials. Since medieval times all portions of plants were used medicinally. Plant tissues, including stems, possess secondary metabolites (SMs), which have known antimicrobial properties. The purpose of this study was to investigate: the presence of antimicrobial compounds in stem extracts of canola, flax, hemp, and sweet clover; and study sorption-desorption behavior of their powdered stem material. GC-MS analysis of all extracts showed the presence of many SMs, including fatty acids, terpenoids, steroids, and sterols, etc. Many of the SMs found in the extracts have previously shown antimicrobial activity against a broad spectrum of organisms according to literature. Water sorption isotherms of stems

showed a typical International Union of Pure and Applied Chemistry (IUPAC) Type II sigmoid curve similar to natural fibres. Equilibrium moisture content (EMC) of canola and sweet clover was significantly higher than flax and hemp at 95% RH. However, EMC of all stems was higher than the fibre saturation point of wood (27%). The preliminary investigation via GC-MS showed promising results with the presence of many antimicrobial compounds and water absorptivity results of hygroscopic stems can be used as the initial key property for a variety of applications.

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List of Abbreviations

ALA	Alpha-linolenic acid
SWM	Schweitzer-Mauduit
d.b.	Dry basis
STLC	saw type lint cleaner
LA	Linoleic acid
PAHs	Polycyclic aromatic hydrocarbons
WHO	World Health Organization
SMs	Secondary metabolites
C	Carbon
FDA	Food and Drug Administration
GPC	Gel permeation chromatography
SEC	Size-exclusion chromatography
LC	Liquid chromatography
TLC	Thin-layer chromatography
HPLC	High-performance liquid chromatography
GC	Gas chromatography
AC	Adsorption chromatography
PC	Partition chromatography or liquid-liquid partition chromatography
IEC	Ion-exchange chromatography
NMR	Nuclear magnetic resonance
MS	Mass spectrometry
IR	Infrared
UV-Vis	Ultraviolet-visible
GC-MS	Gas chromatography - mass spectrometry
LC-MS	Liquid chromatography - mass spectrometry
LC-NMR	Liquid chromatography - nuclear magnetic resonance
MIC	Minimum inhibitory concentration
ERH	Equilibrium relative humidity
EMC	Equilibrium moisture content
OH	Hydroxyl group
MC	Moisture content
RH	Relative humidity
IUPAC	International Union of Pure and Applied Chemistry
DVS	Dynamic vapor sorption
CIC	Composites Innovation Center
NIST	National Institute of Standards and Technology

1.0 General Introduction

1.1 Introductory Comments

Plant material has been used in many industries such as automobiles, aerospace, and composites. The use of plant material in biomedical industry is still limited. For instance, flax fiber has been implemented into a wound dressing (Czemplik et al. 2011) and as a surgical mesh (Michel et al. 2014). However, the purity of flax fibre required for this application is hard to achieve by the current fibre processing technologies. The medicinal use of many parts of the plant has been explored at a large scale as a remedy for many illnesses. There is a growing concern over the absence of resistant antibiotics against the ever-growing strains of newly adapting microorganisms every day. With ever-increasing demand of plant-based products due to their biodegradability and environmental friendliness, most companies strive for developing new products from plants and plant fibres. The first step was to obtain pure fibre and explore the biomedical properties of plant material.

The first part of this thesis focused on cleaning flax fibre. Flax fibre obtained via hammer mill decortication produces high amounts of chaff and dust with a low fibre purity and yield (Chen et al. 2004). This decortication method is consistently used in industry due to its low initial cost and high throughput (Xu et al. 2012). Therefore, the decorticated flax contains a lot of shives and other unwanted particles and hence, extensive cleaning is required to meet requirements of biomedical applications of flax fibre. In this study, the potential of pneumatic method was investigated based on terminal velocities of individual shives and fibre particles. Effectiveness of cleaning was also evaluated using a sorting method to sort long and short fibres for two grades of decorticated flax mixtures.

For the second part of this thesis, stems of canola, flax, hemp, and sweet clover were investigated for the presence of antimicrobial compounds. The presence of secondary metabolites, which are primarily responsible for defense mechanism, in all parts of the plants, possess antimicrobial, antioxidant, antiviral properties, etc. (Bourgaud et al. 2001). Their existence in the stems would mean the presence of similar properties. Therefore, to explore this area, chromatographic separation and identification of the components in stem extracts (extracted with hexane) was carried out via GC-MS to look for antimicrobial compounds.

Water sorption characteristics of the powdered stem material of the aforementioned plants were also tested. Knowing water absorptivity of a hygroscopic material such as stem is a key property before finding an end application of a material (Zaihan et al. 2009). The moisture uptake and release properties at different relative humidities and temperatures are essential to avoid the growth of microbes while storing (Stencl et al. 2010) and to be able to use them in a variety of different applications.

Several applications encompass all of the elements of this thesis including cleaning of fibre, studying sorption-desorption behavior of the stems, and determining the presence of antimicrobials in the stem extracts. For instance, a wound dressing can be envisaged of being composed of cleaned fibres with known concentration of antimicrobials and the capacity to diffuse the antimicrobial compounds into the wound. The highly pure and long fibres can be obtained from the combination of the two tests from the first part of this thesis. The fibres (from the stems) are likely to contain similar antimicrobial compound(s) as the stems. The concentration of the antimicrobials in the stems can be determined by running the authentic standards (compounds) against the GPC fractions of each extract in GC-MS again. The powdered stem material has shown appropriate desorption behavior to remove water during

desorption cycle in the RH range from 95 to 0%. Similarly, the sorption-desorption behavior of fibres (hemp and flax) has also been studied in literature showing their ability to absorb or desorb water (Hill et al. 2009; Xie et al. 2011). Such behaviour is useful because wound dressings with high moisture capabilities can remove pus and exudates from the wound (Aramwit et al. 2013). However, the extracts in the state explored here cannot be used in contact with skin due to their non-polar nature and potential immiscibility in polar environment within the human body, and their capacity to diffuse antimicrobials would need to be tested using a disk diffusion susceptibility test.

A second potential application of this thesis work mentioned here is in composite cleaning products, such as scrub pads, which can be made from stem fibres, ground stems and a resin base, and will provide a natural source of antimicrobials. Scotch-Brite™ has a line of "greener" clean products in which 50% of scrubbing fibres were obtained from agave plant. These products are biodegradable and scratch-resistant. Scotch-Brite sponges have the ability to efficiently clean up spills and sanitize it at the same time. Similarly, the extracts can be used as an ingredient in cleaning or sanitizing solutions to work against the commonly found bacteria in non-sanitary areas.

1.2 General Objectives

The objectives of the first part of this thesis were to:

(1) measure the terminal velocities of individual flax fibre and shive particles, and

(2) investigate the fibre purity and yield resulting from a Sorter for two different grades of decorticated flax.

The objectives of the second part of this thesis were to:

(1) investigate the stem extracts of canola, flax, hemp, and sweet clover via GC-MS for the

presence of antimicrobials, and

(2) determine sorption-desorption characteristics of powdered stem material of the aforementioned plants.

1.3 Thesis Structure

This thesis has been structured in paper format. General Introduction and General Literature Review are presented in Chapters 1 and 2, respectively. Chapter 3 talks about the cleaning of decorticated flax using a Sorter and pneumatic method. Chapter 4 explores antimicrobial compounds via GC-MS analysis of stem extracts of canola, flax, hemp, and sweet clover; and presents study of water sorption characteristics of their powdered stem material. Both these chapters were presented at separate conferences in 2014 and 2015, respectively. Chapter 5 summarizes the generalized conclusions for the entire thesis and potential future work that can be carried out based on the results of Chapters 3 and 4. Lastly, Chapter 6 contains the references for the first two chapters.

2.0 Literature Review

2.1 Flax Plant

2.1.1 General

Flax (*Linum Usitatissimum*) is an ancient crop, which has been grown since pre-Roman times. Flax crop is grown for seeds and fibre production (FCC 2013). North American flaxseed is used for producing different oilseed varieties. Popularity of flax in food products rose due to the presence of omega-3 fatty acid and high fibre content (FCC 2013).

2.1.2 History of Flax

Flax is the one of the oldest crop known to man (FCC 2013) and the oldest fibre used for clothing and along with hemp and wool held a principal position in Europe until cotton production in North America in the 18th century (Salmon-Minotte and Franck 2005). Flax has played a vital role in Canadian agricultural history. It is one of the five major crops of Canada including wheat, barley, oats, and canola. Since 1994, Canada has been the world's largest producer and exporter of flax. According to Statistics Canada, 500,000 tons of flax was produced in a poor crop year of 2004-05 whereas 1.035 Mt was produced in 2005-06. Major importers of flax from Canada is the US (30%), Europe (60%), Japan (4%), and South Korea (FCC 2013). Three major breeding programs: Agriculture and Agri-Food Canada program at Morden Research Center, Manitoba; the Crop Development Center program at University of Saskatchewan; and the Agricore United program at Morden Research Center were developed for the production of flax and solin in Canada. Solin is a flax derivative with less than 5% alpha-linolenic acid (ALA) content. However, flax has high levels of ALA content (FCC 2013).

2.1.3 Cultivation

Flax grows on medium to heavy-textured soils, which can retain water (MAFRI 2013). MAFRI (2013) lists all the information required for an optimum yield of flax such as sowing conditions, seeding rate, seeding depth, etc. Cultivation for flax fibre requires the following operations in sequence: ground preparation, planting, anti-weed spray, pulling, de-seeding, turning, lifting, drying, and stocking (Salmon-Minotte and Franck 2005).

2.1.4 Varieties

The following flax varieties are grown in Manitoba: Hanley, CDC Bethune, AAC Bravo, Lightning, Prairie Sapphire, Prairie thunder, etc. (Seed Manitoba, 2013).

2.1.5 Uses of Flax Plant

Flax is used as a fibre source, textiles, linseed oil, and reinforcement in composites (Salmon-Minotte and Franck 2005). Flax fibre is used in the production of special types of papers such as cigarette paper, currency, bibles, prayer books, etc. (Kiron 2013). Flax Council of Canada lists the following uses of flax fibres and straw. Flax processing plant in Canora, Saskatchewan, uses flax to replace fibreglass in making dashboards and headliners. Flax fibre can be potentially used to replace weaker fibre (virgin wood fibre), which is presently added to recycled paper pulp to give it strength. Flax fibre is already being utilized for insulating walls and ceilings, and particleboard. The strengthening property of flax has been utilized to make fine bond papers, car-door panels, plant pots, and retaining mats. Schweitzer-Mauduit (SWM) Canada, based in Winkler, Manitoba, is the major processor of flax and it uses flax shives for bio-fuel production, and mulch. The flax straw has been used as animal bedding, duck nesting sites, lining for drainage ditches, and as a fuel source in "bale burners".

2.1.6 Properties

Flax fibres have been a popular choice in textiles because they absorb moisture, allow breathability, become softer after washing, have very low elasticity, and possess thermo-regulating, non-allergenic, and antistatic properties (Kiron 2013). Flax fibres possess rapid moisture absorption and desorption, and high crystallinity because of high cellulose content which leads to high creasability and lustre of flax fabrics, low extensibility, and high tenacity of yarns, reduced abrasion resistance, and good quality drapes (Salmon-Minotte and Franck 2005). The electrical resistance of flax fibre decreases by 10 times after 10 minutes of wetting. However, good moisture retaining and absorption properties of flax can be disadvantageous to the dimensional stability of the composite (Mustata and Mustata 2013).

2.2 Processing of Flax Fibre

Processing of flax plant for fibre and seed includes all the operations post-cultivation from pulling stalks, retting, and drying to scutching, which are performed through pullers and turners. Pulling the stalks takes place when the flax plants mature which is indicated by their yellow-brown color (Salmon-Minotte and Franck 2005).

2.2.1 Retting

Retting is a natural process in which pulled straws are left in the field to allow microorganisms to break the pectin bond between the fibres and the straw and the retting duration depends on the moisture and temperature of the media (Meijer et al. 1995). There are two types of retting namely: dew retting and water retting.

Dew retting depends heavily on the weather (dew, sun, or rain) (Meijer et al. 1995) as the straws are left on the ground for several weeks. The degree of retting is evaluated with the ease at which the fibres are able to separate from the shives. Turning of the straw is required so

that same degree of retting occurs on either side of the swathe. Under retting and over-retting can cause difficulties with the decortication of the straw and hence, lead to poor fibre quality (Salmon-Minotte and Franck 2005).

In water retting, large bundles of straw are submerged into a small water body such as a pond or a small river for a few days. The straws are left in the field to dry before being stored. It is faster and a more controlled process than dew retting but it is more laborious and requires treatment of the water body pre-retting (Salmon-Minotte and Franck 2005).

2.3 Scutching or Decortication

Decortication is the mechanical process, which separates the flax straw into fibre (outer layer of the stalk) and shives (inner woody cores). In the early 1900s, decortication was carried out manually by beating the straw, turning it, breaking the bundle in half, and finally separating fibre and stem, which was washed with water to remove the impurities (Carter 1913). Presently, it is typically done via biological, physical, or mechanical processes. Biological decortication essentially involves the process of retting (either dew- or water-retting) discussed above, which breaks the pectin bond between the fibre and the shives. Physical decortication can be seen as an amalgamation of biological and physical processes (Hann 2005). Traditionally, the physical decortication method was similar to the water retting procedure described above. However, this approach has been modified such that the temperature, moisture, enzyme, and microbes used for retting are user selected. This allows prevention of degradation of flax straw fibres (Hann 2005).

The mechanical decortication equipment includes scutching equipment, cutterheads, crushing rollers (Hobson et al. 2001), ball mills (Baker et al. 2010), and hammer mills, etc. to subject the straw to compression, shear, and impact forces. Only the strongest of the plants such as flax and hemp can handle such high stresses.

Decortication of flax has mostly been limited to the use of hammer mill due to its low initial cost and high throughput (Xu et al. 2012). The objective of hammer mill is to break down shives into shorter chunks due to impact forces created by rotating hammers. Hammer mills are favored because they have a high capacity but they do not have a high fibre yield due to the generation of more chaff and the end product typically contains loose fibres and fibres-bound-to-cores (Chen et al. 2004). Münder et al. (2004) used an advanced decortication technology based on hammer mill decortication that yielded 18% long fibres from linseed. By-products of decortication are tow (short fibres), shives, and other waste (dust, etc.). This separation increases the market value of both products, fibres and shives, and as a result opens up possibilities for manufacturing of varieties of products.

2.4 Cleaning

In the cotton industry, "cleaning" refers to removal of dirt and trash content (leaves, bracts, vegetative matter) (Anthony and Mayfield 1994) whereas in the hemp and flax industries, cleaning refers to post-decortication processes which includes separation of hurds and shives, respectively, from the decorticated output. Cleaning is necessary to refine the decorticated product (i.e. fibre) and further increase its market value. Cleaning has been performed by various methods over the years (Parvin et al. 2010).

2.4.1 Cleaning through sieving

Passing the material through a particular type of sieve or screen is one of the traditional methods to classify the materials according to size. Sieving depends primarily on the particle shape and screen size (Ludwick and Henderson 1968). Sieving is advantageous if the material has different applications for different particle sizes.

Hidaka (1991) reported the use of grizzlies, revolving screens, sifters, and vibratory

screens in industry with the latter being the most common. In conventional screening techniques, mesh imparted same frequency to all the particles on the screen (Dass 2004). Modern techniques and equipment, such as the Ultimate Screener (Dass 2004) and Sweco separator (Vorster et al. 2002), imparts multiple frequencies so that the vibration effect on individual particles is different which helps ease the separation. A study performed by Liu (2009) concluded that efficiency and performance of the sieving equipment is influenced mostly by tapping (percussions). Sieving has been used for flax cleaning too. Kymäläinen et al. (2001) used a drum separator, a screen with a stream of air, and a Tuka sieve vibrator to sort fibres, coarse shives, and fine shives, respectively.

Although sieving is a very inexpensive and easy method but there are various disadvantages such as: difference in particle parameters (shape, surface, hygroscopicity, and electrostatic charge), problems with mesh size, and sieving procedure (sample size, duration, mesh blockage, screen vibration, and wet or dry sieving) (Ludwick and Henderson 1968). All these drawbacks have decreased the legitimacy of using sieving as the sole method for separation. However, it is still used in industry to segregate particles as a sub-step during the process of cleaning.

2.4.2 Cleaning through a pneumatic method

Pneumatic methods use the density difference between the particles to separate them. The key parameter for pneumatic methods is the aerodynamic property called terminal velocity, which is defined as the velocity at which the particle is about to leave the surface of contact under the influence of horizontal or vertical air stream. Another way of understanding terminal velocity is when the velocity of a free-falling object under the influence of airflow becomes equal to the gravitational pull and resists the fall and stops acceleration of the particle. Another parameter for measuring aerodynamic behavior is drag coefficient, which is also related to the

velocity of the particles suspended in an air stream.

Joshi et al. (1993) found terminal velocity of pumpkin seeds as a function of moisture content (dry basis) and showed that their size and shape varied with the moisture content. However, terminal velocity for an agricultural product can be efficiently described when all physical dimensions such as area, form, size, shape, and volume are taken into consideration (Uhl and Lamp 1966). Aerodynamic properties for several grain or straw materials have been measured to further clean the materials (Uhl and Lamp 1966; Gorial and O'Callaghan 1990). Agricultural products vary in shape, size, density, etc. which makes their center of gravity, drag, and pressure non-coaxial (Hemmat et al. 2007). These parameters would affect the orientation of the particle when the air stream influences it. Many studies have been undertaken to study the effect of varying particle sizes (Bilanski and Lal 1965) and Bilanski (1971) reported that many replicates are required to approximate aerodynamic behavior of agricultural materials.

Many studies to observe the behavior of the particles in a vertical air stream for grain (Gorial and O'Callaghan 1990), separation of grain and chaff (Farran and Macmillan 1979), and threshed materials (Bilanski and Lal 1965) have been undertaken. Kymäläinen et al. (2001) used an air stream to separate coarse and fine shives for the hammer mill decorticated product of hemp and flax straws but they did not mention the use of different terminal velocities. Innocentini et al. (2009) stated that it is important to know the terminal velocities of grain and impurity particles for better separation and the design of a pneumatic apparatus. Therefore, it is essential to know the terminal velocity of both shives and fibre to test the efficiency of pneumatic method to separate decorticated flax.

2.4.3 Cleaning through a flotation method

Flotation methods use the difference in density of the particles in the mixture in

comparison with water. Water acts as layer separating the denser material that sinks down and the lighter particles that stay afloat. A floatation method is successful if one of the particles is hydrophobic and the other one is hydrophilic (Guney et al. 2013) when used for water treatments, specifically, for separating different varieties of plastics (Guney et al. 2013). Matis and Gallios (1993) reviewed many flotation methods for sorting out valuable minerals.

For agricultural products, Parvin et al. (2013) successfully used a flotation method to clean hemp fibres and improved the fibre purity from 55% to 90%. This method has not been used for cleaning flax fibre. However, the primary disadvantage of using floatation method is its inability to eliminate the fibre-fibre bond and fibre-shives entanglements, which is the result of pectins still keeping these bonds intact in unretted stalks. Salmon-Minotte and Franck (2005) suggested placing the fibres in warm water at 60°C to diminish the effect of pectins. This approach is typically used for wet spinning the tow (short) and line (long) fibres for production of yarns. Wet processes have another drawback that they might change the chemical nature of the fibres and make them unfit for composite manufacture (Prasad et al. 2005).

2.4.4 Cleaning through a ball mill

Ball mills are also used for decortication purposes. Baker et al. (2010) used a planetary ball mill for decortication of hemp fibre. Prasad et al. (2005) defines ball milling as a mechanical process where collective energy is released due to the collision of balls and centrifugal forces within the mill. The parameters for ball milling are milling speed, milling time and milling load or sample size. The material is placed over the grinding media in the grinding bowl. The grinding balls are rotated around a horizontal axis (Prasad et al. 2005) whereas the grinding bowl rotates about its own axis, which makes the centrifugal forces act in both parallel and opposite directions resulting in a frictional effect of the balls against the inner surface of the bowl and impact effect

when the balls hit the opposite wall (Fritsch 2005). Higher speeds lead to higher lifting of the balls inside the mill resulting in greater impact when they hit the opposite wall or the bottom of the wall (Prasad et al. 2005).

Use of ball mills in size-reduction has been applied to a wide variety of applications. Size reduction of powder containing completely inactive influenza virus was made ready for nasal delivery by a micro-ball mill (Garmise et al. 2006). Khan et al. (2009) used a planetary ball mill for size reduction of hemp to study the fineness of hemp fibre bundle. Prasad et al. (2005) explored the use of heating prior to milling hemp fibres. Csiszár et al. (2013) used the ball mill for size reduction of flax fibres to test various properties such as water sorption, degree of polymerization, copper number, and hydroxyl number. This principle of crushing flax shives to sieve them out was used in this project to obtain the flax fibres. However, all the preliminary tests showed no visible separation between shive and fibre particles for dry milling. For wet milling, end product was a soft paste with no visible separation seen after air-drying the samples. The cleaning of flax fibre via ball milling has not been investigated.

2.4.5 Cleaning through carding

Carding is a mechanical process in which the stripping action between the teeth of the rollers provides the cleaning mechanism. Carder was initially used in cotton industry to disentangle fibres and unwanted particles to obtain a web of fibre (Lee and Ockendon 2006). A Drum card is the simplest carding device, which consists of two cylinders: main cylinder and a licker-in. The decorticated product is laid down on the feeding tray to allow good in-feed to the licker-in roller and when the product goes from the licker-in to the main cylinder, the rollers' teeth provide the stripping action which results in elimination of shives, fines, and opens up entanglements. Parvin et al. (2013) used a drum card for hemp fibre cleaning which increased the

overall yield and fibre purity from 55% to 70%. Preliminary investigation using a drum card was carried out in this project, which resulted in an increase in fibre purity from 51% to 60%. However, the yield was significantly low.

Research has been performed on the effects of carding parameters such as licker-in speed on the cleaning efficiency (Gangwar 2009), fibre elongation (Göktepe et al. 2003), fibre length, fibre strength, and the overall cleanliness (Göktepe et al. 2003). Fibre openness and cleaning efficiency increases with the increase in licker-in speed (Gangwar 2009), but Göktepe et al. (2003) reported that too high licker-in speeds resulted in a reduction in fibre length and fibre strength, and an increase in short-fibre content. Das et al. (2012) studied the impact of orientation of fibres in carded and drawn slivers. Lee and Ockendon (2006) investigated the transfer of fibres between carding machine surfaces (different cylinders) via modeling. The above studies on the parameters of carding prove the continuous and high use of carding in industry.

2.4.6 Cleaning through animal fibre processing equipment

There are many animal fibre-processing plants in North America. Some of them manufacture the processing equipment and the others process the fibre according to our needs. One such manufacturer of cottage industry spinning equipment is Belfast Mini Mills Ltd. They manufacture a wide range of equipment ranging from tumbler, washing system, picker, separators, carders, rug yarn maker, felt maker, spinners, etc. There are certain differences between animal fibres and plant fibres. Animal fibres are mainly composed of proteins. For instance, wool is made up of keratin and silk is made up of fibroin and sericin. However, plant fibre is mainly composed of cellulose, which is not a protein but a polymerized glucose molecule (Salmon-Minotte and Franck 2005). Apart from the compositional differences, animal fibres tend to be significantly longer in length than plant fibres.

Animal fibre processing equipment has not been incorporated for natural fibre processing due to the aforementioned differences. However, there is one study by Panigrahi et al. (2012) which used different sequences of Tumbler, Picker, Dehairer, Cutter, and Carder for processing retted flax fibres for use in biocomposites. The weight retained after the two sequences ranged from 8.9 % to 12.9%, which amounts to a very small yield. The fibre purity was not measured. Therefore, for the implementation of animal fibre processing equipment for flax fibre processing, fibre purity of each machine in the sequence will need to be investigated.

2.4.7 Cleaning through cotton gin machinery

Many types of equipment have been developed for cotton cleaning for the removal of lint, motes, leaves, and other trash content. The following sections describe the different types of equipment that have been used for cotton cleaning and their modification for flax fibre cleaning.

Cotton gin machinery includes cylinder cleaners, stick machines, impact cleaners, trashmaster cleaners, fibre cleaner, and saw-type lint cleaner (STLC) (Anthony and Mayfield 1994). Cylinder cleaners possess the mechanical aggressiveness to remove shives from flax straw by removing finely divided particles for fibre length less than 5.1 cm (Anthony 2002). Stick machines exert centrifugal force from saw cylinders to extract burs and sticks from seed cotton whereas an STLC uses air current, centrifugal force, and scrubbing action between saw cylinder and grid bars to remove leaf particles, motes, grass, etc. post cleaning, extracting, and ginning (Anthony 2002).

2.4.7.1 Cylinder cleaner and saw-type lint cleaner

A study by Anthony (2002) concluded that the most effective combination of machines for flax fibre decortication and cleaning was three cylinder cleaners followed by one STLC, which had a fibre yield of 13.7% at a purity of 86.1%. STLC was used when the pre-cleaning

equipment (stick machine, trashmaster, impact cleaner and different sequences of cylinder cleaner) did not achieve the required purity of 80% (Anthony 2002). Anthony (2005a) built flax fibre processing machine based on their successful results in Anthony (2002). The main problems with cylinder cleaner was low final flax fibre purity, low efficiency (ton per hour), and unsuitability for unretted flax (Anthony 2002, 2005a). In addition, the elimination of more trash content lead to less overall fibre yields.

2.4.7.2 Shaker to remove shives from fibre

A study by Akin et al. (2005) changed the width of the modules for flax fibre processing to provide flexibility to the processing sequence in comparison to a single-run machine. Other modifications were also made to the Top Shaker, which was used to remove shives from the fibre. Top shaker contains a series of metal prongs vibrating within the fibre mat where the sample is pulled along the pinned apron at 720 cycles per min. The top shaker was not effective for unretted flax as it lost 45% of the initial mass after processing two times each with 9-roller crusher and top shaker. Processing two times, each with scutching wheel and top shaker resulted in recovery of 29% of the initial starting material. Top shaker was unsuccessful because unretted samples had more shives bound to the fibre than the retted samples.

In another study, Anthony (2005b) added a secondary saw cylinder in an experimental pilot plant to reduce the waste content because the longer fibre will latch on to the secondary saw cylinder and hence more fibre will be retrieved at the end. This plant retrieved more fibre in comparison to conventional 16-D lint cleaner and had similar quality of fibre to 16-D or 24-D lint cleaner.

To summarize, flax is an important crop to us with its fibre being utilized in a variety of applications. However, current decortication techniques of flax produce the end product having

many shives (by-product). Fibre and shives have different properties and therefore, the application of decorticated product as-is requires further cleaning. To clean decorticated flax, this study explores the use of pneumatic method and Sorter to obtain high yield and purity of fibres.

Bridge between Part 1 and Part 2

The focus of the first part of this thesis is on cleaning decorticated flax to improve fibre purity and yield and hence, its market value to readily use them in biocomposites, aerospace, automobiles, etc. The second part of the thesis describes research on four crops, namely; canola, flax, hemp, and sweet clover, for the presence of antimicrobial compounds in their stem extracts via GC-MS analysis, and water sorption characteristics of their powdered stem material. The literature review for the second part begins with the history of medicinal use of plants and the current applications of the four plants used in this study. Next, the presence of secondary metabolites and their properties in various parts of plants is reviewed. Our hypothesis is that some of properties possessed by secondary metabolites such as antimicrobial, antiviral, anti-inflammatory, antioxidant, etc. would also be present in plant stems. For exploring antimicrobial compounds in the stems of the aforementioned plants, conventional extraction techniques followed by partitioning, separation, and detection techniques are reviewed. Then, the common methods employed to determine water sorption isotherms are reviewed.

2.5 History of use of plant parts

Plants have been used as a medicine for centuries. Researchers believe that approximately 350,000 plant species exist and the chemical composition of many of them is unknown (Brielmann et al. 2006) which limits the applications of these species. Parts of the plants were used as-is for different remedies ranging from an illness to curing pain for different parts of the body. With the known applications of plant parts, it was possible to isolate the compounds responsible for the medicinal action. The intermixing of herbs along with the drugs became common to reduce the toxic effect of the herbs (Dharmananda et al. 2015). Moreover, with the progress in isolating compounds, new compounds are being biosynthesized. The transition from plants to synthetic drugs is only a few hundred years old and it was only possible due to the compounds found in the plants (Karuppusamy 2009). For instance, the less irritating effect of acetylsalicylic acid (aspirin) on the stomach than salicyclic acid, which was derived from a plant (Dharmananda et al. 2015), potentially led the way for the transition towards synthetic drugs. Approximately 61% of the drugs known worldwide are based on plants (Brielmann et al. 2006). Three decades of research has revealed the potential of compounds present in fruits and vegetables to reduce the chronic diseases related to age and help in the reduction of other chronic and inflammatory conditions such as stroke, different types of cancers, allergy, asthma, etc. (Kaufman et al. 2006).

2.5.1 Medicinal plants

A lot of research has been performed on medicinal plants. This includes information regarding traditional medicinal uses, origin and distribution, isolation of chemical constituents, and performing pharmacological and clinical trials for in-depth analysis (Liu 2011). For instance, different parts of Kudzu (*P. montana)* has been used as staple food, vegetable, starch, and fibre.

Traditionally, its flowers, leaves, roots, seeds, and stems were used to cure dysentery, diarrhea, diabetes, and to reduce pain and stiffness in different parts of the body. Clinical trials have indicated its prospects in treating alcoholism, cardiovascular disease, and several hormone-related diseases such as cancers of breast, prostate, and uterus (Kaufman et al. 2006). Another widely medicinal plant used in ancient India is neem with almost every part of it being implemented as a medicine. Isolation of compounds from neem has allowed the commercialization of neem products into toothpastes, shampoos, soap bars, etc. (Kaufman et al. 2006). The above two examples provide a brief overview of the studies involving medicinal plants. The following sections report the traditional medicinal use (if applicable), modern uses, and other current uses of the plants and their stems, which were used in this study, namely; canola, flax, hemp, and sweet clover.

2.5.2 Canola

The seeds of a crop of genus *Brassica* can only be categorized as canola if they contain "less than 2% erucic acid in its fatty acid profile and the solid component shall contain less than 30 micromoles of any one or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3 butenyl glucosinolate, and 2-hydroxy- 4-pentenyl glucosinolate per gram of air-dry, oil-free solid" (CCC 2015). The canola seeds contain approximately 44% oil, which contains two major fatty acids: alpha-linolenic acid (ALA) and linoleic acid (LA). ALA lowers bad cholesterol and protects against stroke and heart attack and LA is essential for the brain, and growth and development in infants (CCC 2015). Canola has the least amount of "bad fats" in comparison to other vegetable oils in the market with no cholesterol and a good source of vitamin E. Canola is used as animal feed for cattle, fish, poultry, and swine (CCC 2015).

Canola straw has been researched as a potential source of fuel. Chico-Santamarta et al.

(2011) studied the microbial changes in canola straw bales and pellets during storage and concluded that pellets were a better combustion fuel due to less microbial deterioration and low moisture content. The same group (Chico-Santamarta et al. 2013) compared the potential of baled and pelletized straw for combustion related properties and it was concluded that the amount of sulphur, nitrogen, and chlorine does not play a significant role as a potential combustion fuel applicant.

The xylo-oligosaccharides found from fractionation of lignin and sugars in carbohydrates from canola straw by Pronyk and Mazza (2012) have a huge potential in the pharmaceutical and food industries (Moure et al. 2006). Recently, canola straw was investigated for the manufacture of medium density fibreboard (Yousefi 2009) but the straw lacked some of the properties compared to material typically used to make fibreboard.

Many applications of canola straw as a non-wood material have also been explored, especially, in the paper and pulping industry (Kiaei et al. 2014). Enayati et al. (2009) concluded that the lignin content of canola straw was comparable to the other non-wood materials used in the pulping industry. Hosseinpour et al. (2010) explored the role of canola straw in chemimechanical pulping process by determining its dimensional properties and chemical composition and concluded that tensile and tear indices, and brightness of paper-sheets made from canola straw were better than bagasse and similar to wheat straw.

2.5.3 Flax

Apart from the industrial uses of flax (*Linum usitatissimum)* which were discussed in section 2.1.5, flax has also been used as a medicine. Flax seed or linseed contains oil (30-45%) and protein (25%) along with wax, resin, sugar, phosphates, xanthophylls, vitamins and linamirin, and mucilage (6%) (Chauhan et al. 2009). It is rich in α-linoleic acid, which lowers

the risk of stroke and other cardiovascular diseases (Chauhan et al. 2009). Medicinal uses of linseed oil include treatment of piles, use as a laxative, removal of irritant drugs; mucilage cough syrup, anti-inflammatory, decrease in blood platelet aggregation, and lowering of blood pressure (Chauhan et al. 2009). Arectal oil (1:1 linseed oil and limewater) has been used to treat burns and scolds (David and Toms 2006). Flaxseeds contain bioactive carotenoids, catechins, and lignans, and are a very good source of podophyllotoxin, deoxypodophyllotoxin, and other pharmaceuticals (Kaufman et al. 2006).

The necessity to utilize flax straw for pulping making was questioned by Schafer and Bray (1929) and to this day, we are exploring its applications. Phytochemicals in flax mostly consist of lignans, terpenoids, phenolic acids, and flavonoids, which have known antioxidant properties and an inhibitory effect against carcinogenic induced tumors (Czemplik et al. 2011).

Other potential uses of flax straw include animal bedding, heating fuel, horticultural mulch, insulation board, loose-fill insulation, particleboard, and plastic composite filler (FCC 2013). Flax shives have also been researched for the application of an activated-carbon absorbent (Cox et al. 1999), composite cement additive (Khazma et al. 2008), and biochemical and extraction feedstock (Jacobs et al. 2003).

2.5.4 Hemp

Benet (1975) listed a variety of traditional uses of hemp (*Cannabis sativa*). It has been popularly used in folk medicine in Europe and Asia due to its psychoactive properties; soothing, and tranquilizing action. It has been mentioned in many ancient texts such as Atharvaveda (1400 B.C) and Zend-Avesta as a medicinal plant. Traditionally, hemp sprigs were used to prevent convulsions. It has been used to alleviate toothache. In central Asia, it has been used to cure chronic alcoholics, and other uses mostly involved some form of concoction taken in various

forms for pleasure. Modern uses include the use of hemp seed pills for alleviation of functional constipation (Cheng et al. 2011). Ross (2007) has an extensive list of traditional medicinal uses of hemp particular to a country from Afghanistan and China to Yugoslavia and Zimbabwe. The book also lists the chemical constituents in *cannabis sativa* with its potential pharmacological activities and clinical trials.

Hemp has been implemented in many market products. Hemp fibre has been used in several BMW models for truck liners and press-molded airbag parts due to its lightweight and renewability (Fortenbery and Bennett 2003). Few of the industrial uses include wood fibreboards, as reinforcement in composites, geotextiles, and insulation materials (Merfield 1999). Moreover, fibres have been used for making paper and carpets, hurds for animal bedding and feed, and as a good source of fuel (Merfield 1999).

Merkel et al. (2014) explored the potential of hemp and pomace fibres in composites and lignocellulosic fibres obtained from hemp straw in the automotive biocomposites. The essential oil of hemp was active at 0.5 mg/ml against *S. aureus* and *S. faecalis* but weak against *P. fluorescens* and *E. coli* (Fournier 1978).

2.5.5 Yellow Sweet Clover

The flowering branches and leaves of yellow sweet clover (*Melilotus officinalis*) have been used as a medicine. Bandages from these parts of the plants were used in ancient Greece for their soothing and cooling effect, blood detoxification, and removal of other toxins (Chorepsima et al. 2013).

Tea from flowers, leaves, and seeds has been used as a remedy for common cold, bronchitis, heartburn (Bilton 2005), insomnia, nervous tension, neuralgia, palpitations, congestive menstruation, flatulence, and intestinal disorders (MDidea 2015). The infused oil was

used for convalescence to provide nourishment, and regain body strength (Bilton 2015). The plant is considered antispasmodic, and induces mucous secretions from lungs and has been used as a diuretic (MDidea 2015). Traditionally, it has also been used to treat inflammations in the softer body parts and presently, it is used in treating conjunctivitis (Herbs2000 2015). It is also used as an emollient, expectorant, and vulnerary (MDidea 2015).

Mamedov et al. (2005) have listed the use of aerial parts of *Melilotus officinalis* in skin wounds, irritations, rashes, and allergies and for the treatment of pyoderma. An extract of sweet clover showed similar anti-inflammatory effect similar to couramin against phagocytosed *E. coli* (Pleşca-Manea et al. 2002). A clinical study performed by Allen et al. (1942) on spoiled sweet clover prolonged the coagulation and prothrombin time of the blood. Chorepsima et al. (2013) reviewed the use of yellow sweet clover in healing diabetic foot ulcers. The above studies provide a strong medicinal background of sweet clover.

Other uses of sweet clover as a natural herbicide have also been explored (Wu et al. 2010). It was concluded that an aqueous extract of overground parts of sweet clover inhibited weed growth similar to alfalfa and hairy vetch. Yellow sweet clover was also used for degradation of potentially carcinogenic polycyclic aromatic hydrocarbons (PAHs) via phytoremediation (Parrish et al. 2005).

Various parts of canola, flax, hemp, and sweet clover have been used as a medicine. This part of the thesis keeps its focus on testing the stems. Our dependence on traditional medicine, which comprises of secondary metabolites from plant extracts, is overwhelming. World Health Organization (WHO) estimated that number to be 80% (Winston 1999). Stems like the other parts of the plants also possess secondary metabolites for defense mechanism (Linga Rao and Savithramma 2011; Hussain et al. 2012). Therefore, the entire main branch of the plant

along with the fibres was considered for this project. The stems of the plants also contain a good amount of cellulose and lignin, and secondary metabolites, which could open up a new market for potential natural antimicrobials. Therefore, it is necessary to understand the plant secondary metabolites, their synthesis, functions, and their importance for medical applications.

2.6 Plant Secondary Metabolites

Plant Secondary metabolites (SMs) are the compounds produced by plants that are primarily responsible for providing defense mechanism against herbivores (Wink 1999a). The compounds responsible for basic functions such as photosynthesis and respiration, etc. are called primary metabolites and they include sugars, amino acids, chlorophylls (Harborne 1999), phytosterols, and acyl lipids (Crozier et al. 2006a). SMs have been known for a very long time. The earliest examples of SMs include morphine and codeine isolated from *Papaver somniferum* and salicylic acid from bark of willows, Salix (Theis and Lerdau 2003). Historically, it was believed that SMs provided no usefulness and were called 'end products' by Czapek (Bourgaud et al. 2001). The diversity of phytochemicals in plants include nitrogen-free (such as terpenes and saponins, etc.) and nitrogen-containing constituents (such as alkaloids and amines, etc.) which are both produced in various parts of the plants at high concentrations (Wink 1999a).

2.6.1 Classification

The most common classification of SMs is based on biosynthetic origin, which includes alkaloids, phenolics, and terpenoids (Harborne 1999). There are approximately 25,000 terpenes, tens of thousands of polyphenols, and about 250 sterols, which do not exist in food or traditional medicines (Crozier et al. 2006b). These classes are described in the following sections.

2.6.1.1 Alkaloids

Alkaloids are a group of nitrogen containing metabolites, which are mostly derived from amino acids (Zulak et al. 2006). There are over 12,000 alkaloids, which have been exploited for a variety of applications such as pharmaceuticals, stimulants, narcotics, and poisons (Wink 1998). Alkaloids have a constrained distribution for all its sub-classes in higher plants (Harborne 1999). The most prominent subclasses of alkaloids are amaryllidaceae, betalain, indole, isoquinoline, and peptide (Harborne 1999). Their low availability in plants is attributed to the limited supply of nitrogen to the plants. Although, the presence of alkaloids might be higher in roots, fruits, and seeds (Harborne 1999).

2.6.1.2 Phenols

Phenols have a hydroxyl group attached to an aromatic ring (Crozier et al. 2006). Its major subclasses are anthocyanins, anthochlors, benzofurans, chromones, coumarins, minor flavonoids, flavones and flavonols, isoflavanoids, lignans, phenols and phenolic acids, phenolic ketones, phenylpropanoids, quinonoids, stilbenoids, tannins, and xanthones (Harborne 1999). Crozier et al. (2006) classified phenolics into flavonoids (polyphenolic compounds with two aromatic rings connected by a 3-C bridge) and non-flavonoids.

2.6.1.3 Terpenoids

Terpenes or terpenoids is the most diverse class of SMs. These compounds are classified according to the presence of branched five - carbon units from simple molecules such as monoterpenoids (C_{10}) to molecules containing over 20 C atoms such as triterpenoids, nortriterpenoids, and carotenoids, etc. (Harborne 1999). Terpenoids serve as defensive chemicals located throughout the tissue system with high variability and are released or travel to the site of attack by herbivores or other insects (Theis and Lerdau 2003). Terpenoids are known for their

lipophilic properties and they are mostly present in leaf grandular trichomes, bud exudates, and bark resins. Terpenoids are also used by plants to attract insects and are produced at sub-toxic levels (Theis and Lerdau 2003).

Lipids are classified as fatty acids and their derivatives. Fatty acids are carboxylic acids with a polar hydrophilic head with a long hydrophobic tail. There are over 100 types of fatty acids ranging from small chain (4 C - butyric acid) to long chain (24 C - lignoceric acid) (Brielmann et al. 2006). The derivatives of fatty acids include acyglycerol esters, wax esters, and alcohols (sterols). Most common fatty acids in the plants are oleic acid (unsaturated) and palmitic acid (saturated) (Brielmann et al. 2006).

2.6.2 Functions

SMs have three main functions: defense against herbivores, microbes, and other plants; attraction of pollinating insects, seed dispersing animals, etc.; and UV-protection for the plants (Wink 1999a). They also act as allelopathic (toxic to other plants) agents, and signal molecules (Crozier et al. 2006a). SMs play a major role of adapting plants to diverse environment situations (Bourgaud et al. 2001). Harborne (1999) noted the complexity of establishment of the functions for the SMs. Some serve as antifeedants and insecticides, while insects readily attack some medicinal plants like neem. Some have multiple functions within the plant, and others serve as both secondary and primary metabolite. Their presence in the plants as complex mixtures is another feature that demonstrates their diverse functionality.

2.6.3 Synthesis of SMs

Different developmental stages of plants' growth results in production of different quantities of SMs. SMs produced in either part of the plant helps to establish relationships with the surrounding environment. Defense against microbes and allelopathic function are both active

at early stages of plant life (Chacón et al. 2013).

Most SMs are biologically produced in the cytoplasm, endoplasmic reticulum, or the organelles (Wink 1999b); however, hydrophilic phytochemicals are stored in the vacuole and hydrophobic (or lipophilic) SMs are stored in cuticle, lacticifers, oil cells, or trichomes (Wink 1999a). The factors affecting the production of phytochemicals in fruits and vegetables are method of production, soil and climate, abiotic stress, ripeness, storage, and time of consumption (Crozier et al. 2006b). Many parts of the plant such as plant cells, tissues, and organs have defense functions that increase the presence of some type of SMs (Karuppusamy 2009). SMs are synthesized in the parts of the plant that need protection against outside environment. For instance, a high SM concentration will be felt by a microbe or herbivore at the point of damage on the plant to prevent further damage. There are certain SMs that are transported to the site of damage by phloem producing high concentrations of alkaloids (Wink 1999a). Extensive research of *in vitro* technology of SMs has made it possible to know the location of SMs in parts of the plants (Karuppusamy 2009). Fruits typically contain SMs within the skin while the vegetables contain them in the outer leaves (Crozier et al. 2006b).

2.6.4 Applications of Isolated SMs

Many SMs have been isolated from plants and their properties have been explored in detail. The presence of secondary metabolites in stems would attribute properties such as antibiotic, antifungal, antimicrobial, antioxidant, antiviral, anti-germinative or allelopathic (Bourgaud et al. 2001). There have been many reviews of SMs possessing antimicrobial activity against a wide spectrum of microorganisms. For instance, Compean and Ynalvez (2014) assembled a list of most common SMs (alkaloids, flavonoids, tannins, terpenes, etc.) found in plants and their antibacterial activity against certain microorganisms. Wallace (2004) studied the

antimicrobial properties of saponins and essential oils. Dorman and Deans (2000) successfully tested a few antimicrobial agents from volatile oils of aromatic and medicinal plants against a wide spectrum of bacteria. Rios and Recio (2005) took one-step ahead and successfully tested the medicinal plants as an antimicrobial drug and anti-infection agents.

The above studies and reviews prove that many secondary metabolites are antimicrobial agents. However, to be able to test the antimicrobial activity of plants, pretreatment of plants is required. The next section describes the various extraction methods available to focus the study to non-polar SMs.

2.7 Extraction Methods

There are many extraction methods used by researchers to obtain extracts from different parts of the plants. Most conventional solid-liquid extraction techniques use some form of shaking, soaking, and sonication of plant material in a solvent to get the extracts. Environmental shaker (Tadhani and Subhash 2007) and orbital shaking (Yap 2011) using different solvents have been performed to obtain extracts from leaves and fruits of different plants, respectively. Soaking (Salem et al. 2013) and sonicating (Matu and Staden 2003) have also been used to obtain extracts from parts of the plants.

2.7.1 Soaking

Soaking procedure requires contact of the plant material with the solvent for extended periods. Heat and proper agitation can aid the soaking process but controlled temperature extractions have produced higher yields (Palma et al. 2013). However, its long time requirements, high energy input for separation and potential loss in quality of extracts post-extraction has made it a less popular choice (Palma et al. 2013).

2.7.2 Water or Steam Distillation

Water or Steam distillation is another widely used extraction method to obtain highly volatile phytochemicals. Steam or water is used to heat the solid matrix to retrieve essential oils by vaporization and diffusion, which results in the end product being an immiscible solution of essential oil on the top and hydrolyzed compound at the bottom (Palma et al. 2013). Water, direct steam, and dry steam distillation are the three types, which yield SMs with boiling points below 100, above 100, and above 150°C, respectively (Palma et al. 2013).

2.7.3 Soxhlet Extraction

The principal method used for solid-liquid extraction for many decades is Soxhlet extraction. The sample is held in an extraction chamber, which is located above the flask containing the solvent, and below the condenser coils. During the extraction, the boiled solvent rises up and is condensed back into the extraction chamber. After reaching its maximum level, the solvent drains into the flask along with the extractives. At the beginning of a new cycle, the solute is left behind and only the solvent vapor rises up and condenses back again bringing in more extractives (Palma et al. 2013). The main advantages of Soxhlet extraction are the increased mass transfer rate, contact of fresh solvent with the plant material with every cycle, and no need of filtration post-leaching (Palma et al. 2013). But, it is non-automated, takes a long time, provides no scope of agitation, results in excess use of solvent and waste of extractives, and it can only yield compounds below the boiling point of the solvent used (Luque de Castro and Priego-Capote 2010). Due to the various disadvantages in the conventional apparatus, Luque de Castro and Priego-Capote (2010) reviewed the potential of automation, high-pressure, ultrasound waves, and microwaves; and concluded that ultrasound waves and microwaves were the most probable to overcome the shortcomings in Soxhlet extraction. Many studies comparing the

traditional Soxhlet method to the new alternative methods have been conducted to find nutraceuticals from plants (Wang and Weller 2006), and for extraction from solid samples (Luque de Castro and Garcıa-Ayuso 1998). Hot soxhlet extraction was determined to be better than other methods for lipid extraction (Manirakiza et al. 2001). Despite its disadvantages, Soxhlet is still used widely.

2.7.3.1 FOSS Soxtec Series

FOSS is one of the companies that have taken up the mantle to improve the traditional soxhlet extraction technique. Their Soxtec units follow Randall's modification, which favors the solubility of the extracts in hot solvent by including a new boiling step before rinsing (Anderson 2004). FOSS Soxtec 2050 was used in this study for extraction (Fig. 2.1). Sporring et al. (2005) compared soxhlet extraction method to Soxtec 2055 Avanti system and concluded that 60 minutes steps of both boiling and rinsing steps each produced similar data to Soxhlet with better precision and solvent recovery.

2.7.3.1.1 Working Principle of Soxtec 2050

The air-dried samples were transferred in the cellulose thimble and the solvent was added through a close-loop system in a weighed aluminum extraction cup. Anderson (2004) describes the three cycles of boiling, rinsing, and evaporation. All stages run according to the time allotted to each cycle. In the boiling stage, the thimbles containing the sample were submerged within the solvent inside the extraction cup. The boiling solvent refluxes and condenses through the solvent and into the extraction cup. This stage solubilizes the extract faster in hot solvent making the overall process faster. During the rinsing step, the thimbles are raised and suspended over the boiling solvent resulting in the extractives being flushed out of the sample. The third stage recovers approximately 80% of the solvent. Soxtec extraction units are

an improvement over the traditional soxhlet apparatus in terms of automation, a faster extraction, and reuse of solvent.

Figure 2.1 Soxtec 2050

2.7.3.2 Extraction Parameters

The following pre-treatment of samples is required before extraction via Soxtec 2050.

(1) Preparation of the material: Samples should be (a) pre-dried, (b) devoid of water-soluble components, and (c) finely ground and homogeneous and should pass through 1-mm sieve (Anderson 2004). Reduction in particle size and homogeneity leads to decrease in diffusion amongst the particles, which increases the extraction rate (Palma et al. 2013).

(2) Solvent: Selection of an appropriate solvent is essential to determine the solubility of target compounds and penetrability into the matrix (Palma et al. 2013).

There are three classes of solvents grouped by Food and Drug Administration (FDA) according to the toxicity and their applications (FDA 2012). Class 1 contains benzene, carbon tetrachloride, 1,2-Dichloroethane, etc. These solvents should not be used in manufacturing drugs

because of their high toxicity and hazardous effects to the environment. Class 2 contains acetonitrile, dichloromethane, chloroform, hexane, and methanol, etc. Their use should be limited in pharmaceutical products due to their inherent toxicity. The FDA document provides permissible daily exposures along with the concentration limit for these solvents (FDA 2012). Class 3 contains the solvents, which provide no health hazard or toxicity to humans. This class contains acetic acid, acetone, ethanol, pentane, etc. (FDA 2012).

Many solvents have been used in Soxhlet apparatus for extraction of different phytochemicals. For instance, canola seeds were crushed to force the oil out which followed hexane extraction (Thakor et al. 1995). Hexane and methanolic extracts of non-pungent pepper yielded flavonoids and carotenoids, respectively (Bae et al. 2012). N-hexane was used in this study because it is an excellent solvent to extract non-polar compounds and lipids because of its solubility in oil (Palma et al. 2013).

(3) Others: Temperature, time, and solvent-to-feed ratio also play an important role in extraction. Moderate temperatures should be used for extraction of thermally labile phytochemicals. In some cases, higher time for extraction can break down compounds in non-required format. High solvent to feed ratio tends to work in a 1-step extraction but lower ratio should be altered for a multi-step extraction to get a comparable yield (Palma et al. 2013).

Natural extracts from the plants have been obtained from many plant parts such as seeds, leaves, flowers, berries, barks, and roots, etc. with their applications as nutraceuticals, functional foods, preserving agents, edible oil and fats, drugs, vitamin supplements, and chemical standards (Cavalcanti et al. 2013).

2.8 Fractionation Method

Prior to analyzing the complex components in a plant extract, it is essential to clean-up,

purify, fractionate, or partition the sample to avoid poor separation from separation techniques such as GC or HPLC (section 2.9). These crude extracts can also overwhelm the peaks of the chromatogram due to their complexity and a wide variety of polarities, which is not suitable for chromatography (Van Beek 1999). Therefore, to reduce the complexity of the crude extract and to focus the compounds of interest, it is imperative to implement a fractionation technique. The most widely used partitioning technique is the use of separatory funnel to filter the alkaloids from other classes of secondary metabolites (Van Beek 1999). Some separation techniques (section 2.9) are also used as a pretreatment technique to separate or fractionate the sample prior to chromatographic separation. One such separation technique is gel permeation chromatography.

2.8.1 Gel Permeation Chromatography

Gel Permeation Chromatography (GPC), also known as size-exclusion chromatography (SEC) or gel filtration chromatography, is most often used for polymer separation, characterization, and sample pretreatment and cleanup (Majors 2013). GPC separates the components by molecular weights through a gel-packed column (Fig. 2.2) (Bly 1970) such that the heavy molecules cannot enter the small pore size of the gel and pass directly through the column whereas smaller molecules take a more convoluted path and are eluted at the end (Majors 2013). Heavy molecules such as oils, lipids, and hydrocarbons can be easily separated using GPC (Majors 2013). A filtered solution of the mixture is typically poured from the top of the column to avoid clogging up the gel. Navarro et al. (2006) compared the three purification methods, namely; solid phase extraction, microwave assisted saponification, and GPC, before analyzing PAHs via GC-MS, and concluded that GPC had the cleanest extracts. Therefore, it is an essential purification step before running the samples in GC-MS equipment to avoid

cluttering the chromatogram with too many peaks.

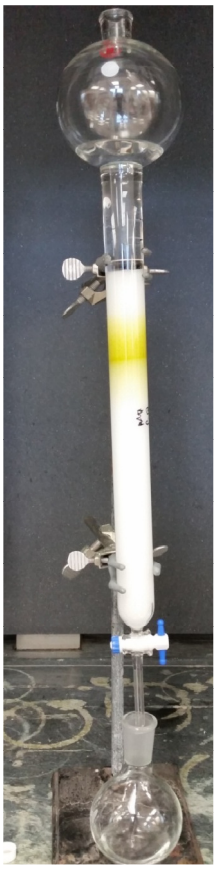


Figure 2.2 GPC Fractionation Column

2.9 Separation Techniques

The analytical methods typically used to separate components in a plant extract include liquid chromatography (LC), thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), fast protein liquid chromatography, and gas chromatography (GC) (Cseke et al. 2006). Chromatography distributes the components of a mixture (analytes) between a stationary phase and a mobile phase (Van Beek 1999; Cseke et al. 2006). The type of chromatographic separation method used depends on the sample involved. Chromatography involves five major separation criterions: adsorption chromatography, liquid-liquid partition chromatography, ion-exchange chromatography, size-exclusion chromatography, and gas

chromatography (Cseke et al. 2006).

2.9.1 Adsorption chromatography

Adsorption chromatography (AC) separates the components based on their polarity when the mobile phase (organic solvent) is adsorbed on the surface of stationary solid phase and equilibrium is established (Analytical Methods 2015; Cseke et al. 2006). The typical adsorbents are silica gel, florisil, and alumina. Different AC techniques have been employed depending on the pore size of the material (Cseke et al. 2006).

2.9.1.1 Thin layer chromatography

Thin layer chromatography (TLC) separates the components based on the rate of their suction. Typically an adsorbent (stationary phase - silica gel and alumina) is coated on one side of the plate and the sample to be examined is spotted on the same plate and the rate of suction of the analytes via capillary action is the criterion for separation (Cseke et al. 2006).

2.9.1.2 High Performance Liquid Chromatography

HPLC pumps the mobile phase through the stationary phase (uses small particle size) present in a column because of the accumulation of large pressure (Cseke et al. 2006). A typical HPLC apparatus consists of the following instrumentation: solvent reservoir, injection system, column, pump, detector, and a computer station. Many advanced systems have more than one pump and detector, which allows changing the polarity of the mobile phase with a larger separation range and it takes less time for separation (Cseke et al. 2006).

2.9.2 Partition Chromatography

Partition chromatography (PC) is also known as liquid-liquid partition chromatography due to both stationary and mobile phases being liquid. PC separates the components based on

their solubility in the two immiscible phases. The stationary phase solvent is adsorbed on an inert solid supporting matrix (e.g. paper or a column) (Cseke et al. 2006). PC is used for analytical separations and not for pretreatment purposes.

2.9.3 Ion-Exchange Chromatography

Ion-Exchange Chromatography (IEC) separates the ionized functional groups based on the interactions of the ions present in the mixture with the resin in the column (Analytical Methods 2015). The stationary phase is oppositely charged to the ions in the sample to be examined whereas the mobile phase is an aqueous buffer where pH and ionic strength can be both altered to control elution time (Cseke et al. 2006). IEC is commonly used for separation of fruit acids.

2.9.4 Size-Exclusion Chromatography

Size-exclusion chromatography (SEC) separates the components in a mixture based on size. A type of SEC (GPC) was discussed in section 2.8 as part of the fractionation technique.

2.9.5 Gas Chromatography

Gas Chromatography (GC) is used to separate highly volatile organic compounds and essential oils due to the differences in the boiling points of the volatiles in the mobile gas phase and the stationary liquid phase (Cseke et al. 2006). The gas chromatograph has an injection port, a column, programmable oven, and a detection system (typically Flame Ionization Detector). The dimensions and the phase properties of the GC column provide variability for the samples to be analyzed over a wide temperature range (Sahil et al. 2011; Cseke et al. 2006).

2.10 Characterization Techniques

The next step after separating the components of the plant extracts is to characterize or

identify them. Nuclear magnetic resonance (NMR), mass spectroscopy (MS), infrared (IR), and ultraviolet-visible spectrophotometric (UV-Vis) methods are the most common for characterization of natural products (Vogler and Setzer 2006).

2.10.1 Nuclear Magnetic Resonance

NMR spectroscopy provides a fingerprint region for the compound in the extract. It also allows an atomic level study of the compound and its chemical environment. The fingerprint region is obtained by studying the spin states of the nuclei using a magnet. Various 1-D and 2-D methods have been employed within NMR spectroscopy (Vogler and Setzer 2006).

2.10.2 Ultraviolet-visible Spectroscopy

Ultraviolet-visible (UV-Vis) spectroscopy produces an absorbance versus wavelength plot in the wavelength range of 1900 to 800 nm. The absorbance spectra along with the maximum wavelength are used for characterization. All organic compounds including solvents have their signature absorbance spectra. Therefore, the solvents' spectra should be taken into consideration during analysis (Vogler and Setzer 2006).

2.10.3 Infrared Spectroscopy

The vibrations of chemical bonds and functional groups when the IR radiation interacts with the material help identify the unknown compounds. A typical IR spectrum is a plot of light intensity (for e.g. absorbance) versus property of light (wavenumber or wavelength) (Smith 2011). IR spectroscopy produces a fingerprint region for a functional group, which is useful to identify compounds (Vogler and Setzer 2006). It is a very useful tool for characterization of compounds due to the high correlation of the maximum absorbance band and the functional groups (Vogler and Setzer 2006).

2.10.4 Mass Spectrometry

Mass spectrometry (MS) provides better sensitivity in the identification of natural products than NMR. The interpretation of the chromatogram is more complex than NMR spectra. MS breaks down the ions from the components to be analyzed and then uses electric and magnetic fields to determine the molecular weights of the compounds (Vogler and Setzer 2006). These compounds are then compared to the library database to obtain more information. Based on the variation in the working principle of ion generation and fragmentation, different MS techniques have been used (Vogler and Setzer 2006).

2.11 Hyphenated Techniques

Hyphenated techniques group the separation and identification (section 2.9 and 2.10) techniques together. The hyphenated techniques were introduced when GC and MS systems were coupled for the first time (Sahil et al. 2011). There can be many combinations of hyphenated techniques depending on the interface that links the two instruments, one for separation and the other one for detection (Sahil et al. 2011). The most common techniques used for natural products are GC-MS, LC-MS, and LC-NMR (Vogler and Setzer 2006).

2.11.1 GC-MS

GC-MS (Fig. 2.3) as a hyphenated technique provides universality, sensitivity, and specificity (Van Beek 1999). The coupling of GC and MS reduces of the error of detection of a molecule and a compound. It has become a powerful analytical tool for detection and identification of essential oils and other organic volatiles (Vogler and Setzer 2006). The samples injected into GC along with the inert gas stream (typically helium) are separated based on differential absorptive interaction with the GC column, which are then ionized, fragmented, and detected by MS (Vogler and Setzer 2006). Low volatile mixtures like phenols require some form

of derivitization step to make them more volatile to be analyzed in GC-MS (Stalikas et al. 2007). GC-MS provides a full structure overview along with determination of molecular masses for the unknown compounds (Proestos et al. 2006) based on the associated library. GC-MS has been used for characterization of hemp seed oil (Leizer et al. 2000) and chloroformic fractions of yellow sweet clover (Kovaleva et al. 2009). It was also used for identifying components of flax shives: lignin (Ross and Mazza 2010) and wax (Athukorala et al. 2009). Therefore, GC-MS is a powerful analytical tool for separation and identification of components in a mixture.

Figure 2.3 GC-MS equipment used in this study

2.12 Antimicrobial Efficacy Testing

Phytochemical research has been very active in the area of investigating plant extract for many applications including antimicrobial activity (Setzer and Vogler 2006). It is essential to carry out an antimicrobial efficacy testing against the organisms of interest to identify promising

plant extracts. Selecting an appropriate assay is important for evaluation of antimicrobial activity (Gallant-Behm et al. 2005). Antimicrobial susceptibility tests are performed to find the agents with potential antimicrobial activity against the tested microorganisms (Collins et al. 1995). Antimicrobial susceptibility tests can be performed using diffusion or dilution methods.

Rios et al. (1988) reviewed both methods for testing antimicrobial activity of plant extracts. They advised the use of diffusion methods for testing the polar compounds with high solubility in water. However, diffusion methods are used commonly for screening alkaloids, flavonoids, terpenoids, etc. Diffusion method is only used as a preliminary investigation technique and not as a definitive method. However, dilutions methods (both agar and broth) were suggested for testing non-polar compounds. Due to the low diffusion properties of plant extracts, it is recommended to use dilution methods to ensure their contact with the test organism. Broth dilution is complex due to the solubility concerns but it is better than agar dilution.

2.13 Water sorption

Water is the main constituent of food and biological materials. To explore the applications of a hygroscopic material such as stem, it is essential to understand the moisture uptake and release properties (Zaihan et al. 2009). Presence of water in biological materials determines the structure, and physical and chemical properties (Le Maguer 1987). Relationship of water activity and moisture content provides a way to monitor the water behavior (Le Maguer 1987). Water activity is defined as the ratio of vapor pressure of water in a material to the vapor pressure of pure water at the saturation point at the same temperature (Rahman and Sablani 2009). The term water activity is used in food processing industry whereas equilibrium relative humidity (ERH) is commonly used for solid materials such as ground grains and seeds, feed or dry products, etc. (Rahman and Sablani 2009). The local moisture content acquired by the cell

wall when exposed at a constant RH where the dynamic equilibrium of influx of water molecules into the cell wall equals the outflux of water molecules is known as equilibrium moisture content (EMC) (Hill et al. 2010). Relationship between the EMC and relative humidity in the material represents water sorption isotherm (Bertuzzi et al. 2003).

Understanding the moisture behavior of a material is very important to find their end application. For instance, moisture content of natural fibres has been researched due to their application in composites as reinforcement (Bismarck et al. 2002), textiles, and pulp and paper industry (Eichhorn et al. 2001). Flax and hemp products already in market are being used in high humidity environments. The ability of the fabrics made from natural fibres to swell up and access their cells walls' water sites (hydroxyl groups) is known as hygroscopicity (Xie et al. 2011). Thus, it is necessary to understand the complex properties of water sorption-desorption and how it affects the products (Mustata and Mustata 2013). Good moisture intake of fibres makes the interaction with the hydrophobic matrix of composites a complex matter (Gouanve et al. 2006). Information on moisture content and RH, which is provided by sorption isotherms at the given temperature, is useful to prevent the growth of microbes in the material during storage (Stencl et al. 2010).

2.13.1 Factors responsible for water sorption

The factors responsible for water sorption of hygroscopic material such as stems and natural fibres are similar. Not all constituents present in natural fibres such as cellulose, hemicellulose, and lignin are responsible for sorption attributes. Cellulose has high -OH to C ratio but not all -OH groups are exposed to water (Pott 2004, cited in Lee and Bismarck 2011). Hemicellulose also has a high -OH to C ratio with lignin having the lowest -OH to C ratio, however, both are available to water molecules (Lee and Bismarck 2011). Natural fibres and

wood absorb moisture due to the presence of hydroxyl (OH) groups present in their macromolecules (Hill et al. 2009). Cell walls of lignocellulosic material shrink due to the absence of water when capillary forces act within the cell walls, and the presence of lignin (Hill et al. 2009). High lignin corresponded to larger area between the adsorption and desorption isotherms and an increase in temperature resulted in a smaller hysteresis loop in a study for natural fibres (Hill et al. 2009). Adsorption or desorption isotherms provide us information on water solid interactions and solid porous structure (Arlabosse et al. 2003). Capability of wound dressings to absorb moisture is also an important factor for faster healing (Aramwit et al. 2010).

There has been little reported on sorption isotherms of stems. However, sorption isotherm of natural fibres follows an International Union of Pure and Applied Chemistry (IUPAC) Type II isotherm which can be disintegrated into 3 regions (Lee and Bismarck 2011): region I (0-15% RH) represents "monolayer adsorption of the molecules onto the cell wall", region II (15-70% RH) represents a multilayer formation of water molecules in the transient microcapillary network, and region III (above 70% RH) represents capillary condensation of the water molecules. From the above description, it is clear that adsorption (surface phenomenon) takes place at low RHs whereas the absorption (intracellular phenomenon) takes place at high RH values (Lee and Bismarck 2011).

2.13.2 Methods for determining sorption characteristics

There are many methods used for determination of water sorption characteristics: colligative properties methods such as vapor pressure measurement, freezing point measurement, and boiling point measurement; gravimetric methods such as methods with discontinuous registration of mass changes and methods with continuous registration of mass changes (static chamber, dynamic systems, and evacuated system); and hygrometric systems (Rahman and

Sablani 2009). The gravimetric methods have a few advantages over the other two methods: (a) determination of exact dry weight of the sample, (b) less temperature alteration between sample and its surroundings, and (c) ability to record change in weight with respect to water vapor pressure (Rahman and Sablani 2009).

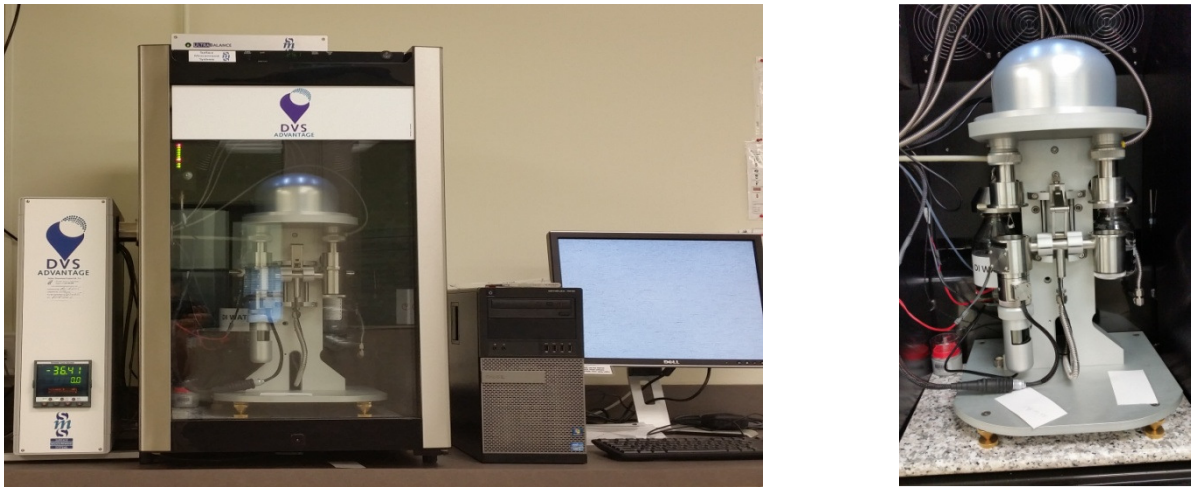
2.13.2.1 Static gravimetric method

Among the aforementioned methods, static and dynamic gravimetric methods are commonly employed. The static method uses different saturated salt solutions in desiccators or sorbostats or glass jars to maintain different RH values (Rahman and Sablani, 2009). By subjecting the sample to different RHs, the moisture content is determined with respect to the change in weight gained over time. This traditional method has been used for many years for obtaining water sorption isotherms of edible starch based films (Bertuzzi et al. 2003), soya beans (Aviara et al. 2004), amaranthus stems (Stencl et al. 2010), etc. However, this method is time consuming and the material can take days or even weeks to reach the equilibrium (Arlabosse et al. 2013) which can lead to the growth of bacteria (Rahman and Sablani 2009). Another downside is the change in atmosphere inside the desiccators when the samples are weighed over a certain time. However, this method is still in practice in academic labs and is slowly being taken over in industry by the dynamic system of measurement of sorption characteristics.

2.13.2.2 Dynamic gravimetric method

Dynamic vapour sorption (DVS) apparatus maintains the temperature and RH in a chamber where the sample is held in a microbalance with an ability to measure the change in mass of 1 in 10 million parts (Fig. 2.4, SMS 2015). Ratio of saturated and dry carrier gas (nitrogen) is controlled with mass flow and vapor concentration monitoring. A known concentration of water vapor flows over the sample and change in mass during sorption and

desorption is measured (SMS 2015). These dynamic flow conditions and a smaller sample size enable the equilibrium conditions faster.



a b

Figure 2.4 (a) DVS unit used in this study and (b) RH chamber

DVS method has been used for determination of sorption isotherms of oil palm trunk and rubberwood (Zaihan et al. 2011), modified wood (Xie et al. 2010), developing and mature cotton fibres with changes related to maturity and crystallinity (Ceylan et al. 2012; Ceylan et al. 2014), stems of lemon balm (Argyropoulos et al. 2012), natural and other fibres (Xie et al. 2011), flours from hard and soft wheat (Roman-Gutierrez et al. 2002), freeze-dried amorphous sucrose (Yu et al. 2008), Sitka spruce (Hill et al. 2010), medium rice varieties (Bingol et al. 2012), and to characterize the rate of drying using cotton fabric in hard water and surfactants (Donnarumma et al. 2014). It has also been used to determine water vapor adsorbed and absorbed from human Type 1 collagen and pigskin dermis, which were found to be consistent with the Raman spectroscopy results (Zhang et al. 2011).

2.13.1.3 Comparison between the two methods

DVS is better than the static gravimetric methods because of: (a) using smaller sample size which leads to a faster equilibrium, (b) using the same sample for the entire experimental

run, (c) the ability to produce isotherms for the entire range of RH values, and (d) no disturbance to the sample in humidity controlled chamber throughout its run (Rahman and Sablani 2009). Both methods assume an equilibrium restriction on the samples but the saturated salt method has a severe condition for material to reach equilibrium. DVS criterion restriction is much higher than the salt saturated method, which means that we can obtain the sorption data within a few days with DVS as compared to a few weeks for the salt saturated method (Arlabosse et al. 2003). A study conducted by Arlabosse et al. (2003) showed comparable results for saturated salt method and DVS as long as the apparent MC within the material was high.

The literature studies present the medicinal background of four plants and their stems. However, there has been little attention paid to investigating antimicrobial activity of the stems. Due to the presence of secondary metabolites in stems, it is possible that stems possess properties such as antimicrobial, antioxidant, anti-inflammatory, etc. To put this theory into perspective, GC-MS analysis of the extracts of plant stems was carried to explore antimicrobial compounds. Water absorptivity of the powdered stems was also determined.

3.0 Separation of Fibre and Shives from Decorticated Flax

3.1 Abstract

Decorticated flax contains significant amount of shive content, which limits applications of flax fibre. Separation of shives from the fibre is essential to improve the quality of flax fibre. The intention of this study was to use pneumatic method for separation of flax fibre and shives. For pneumatic method to be successful, investigation of terminal velocities of individual fibre and shive particles was undertaken. The physical dimensions of the particles such as width, length, and mass were measured. The terminal velocities were measured using a wind tunnel. To improve fibre quality, sorting method was also tested in this study for separation of short and long fibre (longer fibre has higher market value). In sorting tests, a general fibre sorter was used and the treatments were two grades of decorticated flax: Grade 1 and Grade 2. Their initial fibre purities were 51% and 15%, respectively. The outputs of the sorter were analyzed to determine the machine yield and fibre purity. The results of the pneumatic tests showed that the length of fibre particles did not influence the terminal velocities of fibre. For shives, the increase in mass and width showed an increasing trend in terminal velocity. The ranges of terminal velocities for shive and fibre particles were 1.13 to 4.09 m/s and 0.51 to 1.07 m/s, respectively, which were significantly different. The sorting tests improved the fibre purity of Grade 1 to 80% and Grade 2 to 66%, which were a significant improvement when compared to the initial purities. This study demonstrated the potential of the pneumatic and sorting methods for improving fibre quality.

Keywords: Separation, flax, fibre, shive, yield, purity, pneumatic, terminal velocity, sorter

3.2 Introduction

Flax is a fibre crop containing up to 41% fibre content in combined-harvest flax stalk

(Pallesen 1996). Flax fibre is used in various sectors such as biomedical, automobile, aerospace, construction, and composite industries. Flax fibre is extracted through mechanical processing known as decortication. In decortication, flax stalk (bales) is fed into a hammer mill, roll crusher, or other types of machines to obtain the fibre (the outer layer of flax stem). However, the output product from those machines contains an undesirable amount of shives (the inner core of flax stem). Percentage of fibre in the output is termed as fibre purity. Fibre with higher purity has more applications. In a study using hammer mill decortication, Munder et al. (2004) reported the output with low flax fibre purity from 14% to 30%, meaning that 70% to 86% of the material was shives. In addition to fibre purity, fibre length is another important aspect, which affects the value and application of the fibre. In summary, it is necessary to separate shives from decorticated flax to improve the fibre purity, and to separate long and short fibre to increase market value of the fibre.

The major challenge to separate fibre and shives, or to separate long and short fibre, is fibre entanglement and shives entangled within fibres. Entangled fibres exist as clumps and some shives are trapped within those clumps. The traditional sieving or screening equipment for separation of particles with different screen sizes did not work well for such entangled material, even with vibratory screeners (Sadek and Chen 2014). The clumps remained on top of the sieve during and post-screening. Therefore, screening was not an effective cleaning method for decorticated flax. A study conducted by Akin et al. (2004) utilized equipment readily used for cleaning different cotton cultivars for cleaning flax fibre and seed flax straw. The study used 9-roller calendar, top shaker, scutching wheel against unretted, dew-retted, and enzyme-retted samples and concluded their ineffectiveness against unretted flax. These types of equipment are typically used to improve the cotton fibre and yarn quality (Li et al. 2012).

Several alternate methods, such as carding, sorting, and picking have been studied for lint cleaning and animal fibre cleaning. Panigrahi et al. (2012) used Tumbler, a Picker/Opener, Sorter/Dehairer, and Carder to clean flax fibre to use them as reinforcement in bio-composites. Two cycles of following sequence of machines were used: Tumbler, Opener, Cutter, Sorter, and Carder. Shives and other waste were removed by the speed and rotation difference of the rollers on the machines. No fibre purity was reported in this study. These machines processed the fibre through the action of disentangling, stripping, and clamping. However, they were designed for cleaning long animal fibre. Therefore, their effectiveness in terms of fibre purity for flax should be investigated.

For removing hemp cores from hemp fibre, Parvin et al. (2013) tested several methods, including floatation method, carding, and pneumatic method. Floatation method using water worked reasonably well to separate hemp fibre and cores. However, its drawback is that fibre needs to be dried afterwards. The carding method was not very effective, although it improved the purity of hemp fibre. In addition, carding is more suitable for long fibre, however, flax fibre is typically short. The pneumatic method could be potentially used to separate a mixture of hemp fibre and shives if the fibre and shives were not entangled together (Parvin et al. 2013).

Pneumatic method has been used in various other applications such as; separation of coffee cherries and beans, which is useful for improving coffee production (Júnior et al. 2007); and separation of shell and kernel particles of walnuts (Nahal et al. 2013). There is no literature for the implementation of pneumatic method for separation of flax fibre and shives. Pneumatic method uses airflow to separate different particles based on the differences in terminal velocities (Júnior et al. 2007; Gupta et al. 2007; Kılıçkan and Güner 2006). Designing of well functioning pneumatic equipment depends on the information of terminal velocity of the material to be

handled (Gupta et al. 2007). Therefore, determination of terminal velocities of individual fibre and shive particles is essential for the implementation of pneumatic method for separation of decorticated flax mixture.

In summary, decorticated flax has low fibre purity, and hence post-decortication separation of shives from fibre is essential to improve the fibre purity and meet the growing demand of fibre industries. One of the great challenges of this study is the entanglement of fibre. Among the fibre cleaning methods, sorting and pneumatic methods showed potential for cleaning decorticated flax. The main objective of this study was to separate shives from fibre to improve flax fibre purity. The specific objectives were to: (1) measure the terminal velocities of individual flax fibre and shive particles, and (2) investigate the fibre purity and yield resulting from a Sorter for two different grades of decorticated flax.

3.3 Materials and Methods

3.3.1 Materials

Flax samples were obtained from a hammer mill decortication facility in Canada. The sample contained mixtures of fibre and shives with various particle sizes (Fig. 3.1). The samples had fibre lumps with some shive particles trapped in the lumps. This study used two grades of decorticated flax: Grade 1 and Grade 2. The main difference between the two grades was that Grade 1 contained more long fibre and less shives, and thus, it had higher market value than Grade 2.

Figure 3.1 Flax fibre sample (Grade 2) showing a mixture of fibre and shives

3.3.2 Pneumatic Experiment

3.3.2.1 Description of Equipment

A wind tunnel was used to determine the terminal velocities of flax fibre and shive particles to explore if shive particles could be pneumatically separated from fibres to improve the fibre purity. Parvin et al. (2013) designed the wind tunnel used for this experiment for separation of hemp fibre and cores. The apparatus consisted of a supporting wooden frame, a vertical wind tunnel, an air blower (Fasco Distributing Co., Type U85, Cassville, MO), a flow straightener and, a wire mesh screen (Fig. 3.2). The transparent wind tunnel, which was 910 mm high and 139 mm in diameter, allowed visible inspection of the pneumatic behavior of particles. The air blower provided vertical airflow upwards from the bottom of the tube. The air blower was connected to a variable transformer (General Electric volt-pac 9T92A86, USA), which allowed for changing the speed of the air during a test. For the test, a fibre or shive particle was placed on the mesh screen inside the tube. Several mesh screen sizes were tested before the tests to accommodate various particle sizes. A 52-µm aluminum wire mesh was selected because this mesh size allowed testing of wide range of fibre and shive particles. A 19-mm thick honeycomb flow straightener with a round opening size of 3 mm was used to provide uniform airflow to the

sample placed on the wire mesh screen.

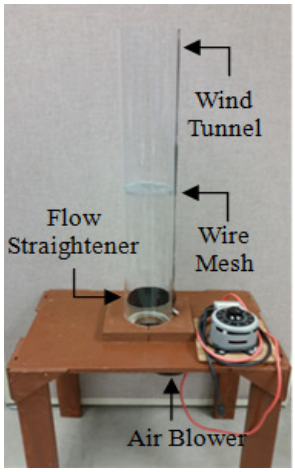
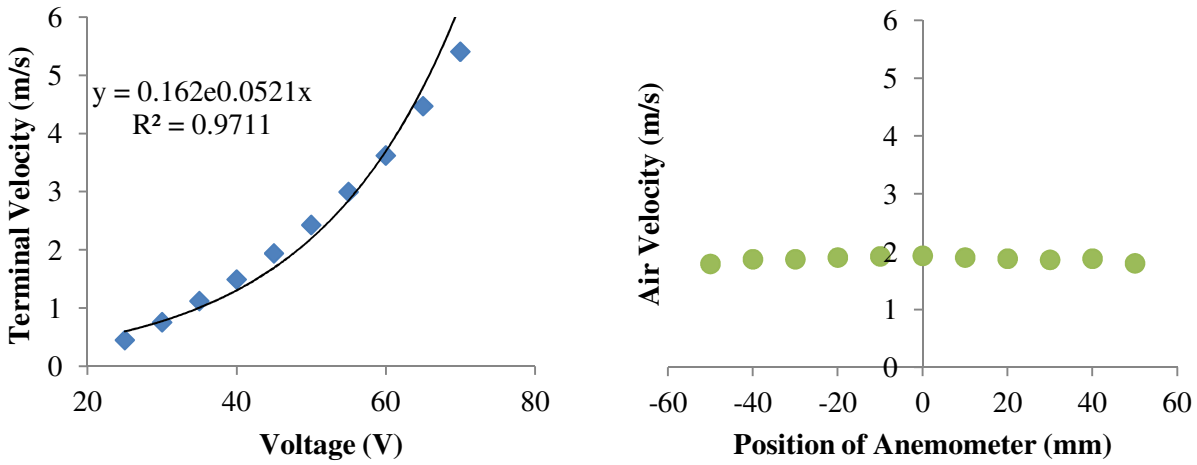


Figure 3.2 Apparatus for Pneumatic Method

3.3.2.2 Calibration of Air Velocity

The air blower attached to the wind tunnel supplied air at different voltages as adjusted through the variable transformer. A calibration experiment was performed using an anemometer (Bacharach-florite 800, PA) to obtain a relationship between the air velocity and the applied voltage. The air velocity was measured at the top of the transparent tube in the center of the tube cross-section at 5V intervals over the range of 25 to 70V. The results showed that the input voltage and air velocity had an exponential relationship with a high correlation value of 0.97 (Fig. 3.3a).

The uniformity of air velocity across the cross-section of the transparent tube was also tested. The air velocity was measured using the same anemometer at the top of the tube at 11 locations across the diameter of the tube. The results (Fig. 3.3b) showed that the velocity was constant along the diameter of the transparent tube. It proved that the flow straightener was effective in providing uniform airflow across the tube cross-section. Therefore, a fibre or shive particle experienced uniform airflow during the experiment.



a b

Figure 3.3 (a) Calibration of air velocity with input voltage of the blower, and (b) Air velocity distribution across the diameter of the wind tunnel at 45V

3.3.2.3 Experiment

The aforementioned wind tunnel was used to measure the terminal velocity of individual fibre and shive particles. Individual fibre and shive particles with various sizes (Figs. 3.4a, b) were randomly picked from the mixture shown in Fig. 3.1. Tests were performed for 25 fibres and 25 shive particles in triplicates. Thus, 150 tests (25 fibre particles x 3 + 25 shive particles x 3) were performed.

a b

Figure 3.4 Samples used for the pneumatic experiment: (a) Fibre and (b) Shive

3.3.2.4 Measurements

3.3.2.4.1 Sample Characteristics

Shives in the sample were characterized by length, width, projected area, and mass. The

length and width of shives were measured using a digital vernier caliper, and the mass was measured using an analytical scale (Cole-Parmer Symmetry, PA). Fibre samples were less variable in shape and diameter. In addition, the fibres were extremely light (< 10^{-5} m), and mass was not measured. The fibres were only characterized by length using the digital vernier caliper.

3.3.2.4.2 Terminal Velocity

In a test, a sample (fibre or shive particle) was carefully placed on the center of the wire mesh screen. Next, the air velocity was slowly increased until the sample was about to leave the wire mesh surface. At this point, the air velocity was recorded as the terminal velocity of that particle. This measurement was repeated three times for the sample and the average value of three data points was used for data analysis.

3.3.3 Mechanical Separation Experiment

3.3.3.1 Description of Equipment

A Sorter (Belfast Mini Mills, PEI, Canada) was used to separate long and short fibre of the flax samples shown in Fig. 3.1. The Sorter comprised of a conveyor belt (Fig. 3.5a), two feed-in rollers (Fig. 3.5b), and an assembly of three processing rollers (Fig. 3.5c). Flax sample was laid out on the vibratory conveyor (117" long and 48" wide), which transported the sample to the feed-in rollers. The feed-in rollers took the sample into the machine assembly and aligned the fibres. The samples were next transferred to the processing rollers: licker-in (rotating at 340 rpm), swift (rotating at 470 rpm), and the doffer (rotating at 5.5 rpm). The Sorter gently separated long fibres and short fibres and removed some unwanted contaminated particles, such as fine fibres and shive particles. Long fibres were combed off from the doffer. Long fibre and short fibre streams were collected separately.

a b c

Figure 3.5 Sorter: (a) Entire unit; (b) Feed-in rollers; and (c) Processing rollers

3.3.3.2 Experimental Design and Procedure

Sorting tests were conducted with two grades of decorticated flax: Grade 1 and Grade 2, with three replicates. Before a test, fine particles in the mixture were removed using a Tumbler (Belfast Mini Mills, PEI, Canada) having 25 mm screen mesh. To obtain more uniform feed for the Sorter, a Picker (Belfast Mini Mills, PEI, Canada) was used to open the fibre lumps. In a sorting test, the sample (approximately 920 grams) was carefully laid out by hand on the conveyor belt of the Sorter (Fig. 3.5a) for a continuous and uniform input to the in-feed rollers. The outputs of the Sorter from the short fibre stream were collected in the cage-like structure (Fig. 3.6a) and the long fibre stream in the bin at the end of the Sorter (Fig. 3.6b). The outputs from the long and short fibre streams were collected separately and labeled for later analysis. The rollers of the Sorter were cleaned out after each test.

a b

Figure 3.6 Sorter output collection bins for: (a) Short fibre stream and (b) Long fibre stream

3.3.3.3 Measurements

3.3.3.3.1 Machine Yield

Machine yield included long fibre yield and short fibre yield. The yield was the ratio of the mass of the output to the initial mass of the sample fed into the Sorter.

$$Y = \frac{m_O}{m_I} \tag{1}$$

where Y = machine yield (%),
m_O = mass of output (g), and
m_I = mass of input (g).

3.3.3.3.2 Fibre Purity

To obtain representative samples for fibre purity analysis, the entire output collected from each stream was mixed thoroughly and laid out in a container. Sub-samples were taken from various locations in the container. These sub-samples were mixed and laid out in a container again. Sub-sub-samples were collected again from the container. This process was continued until approximately 5 g of sample was obtained. Next, this final sample was manually separated into fibre (Fig. 3.7a) and shives (Fig. 3.7b) to determine the fibre purity. There have been no other reliable methods available for fibre purity analysis in the literature. The fibre purity for the original sample was also determined. The purity of a sample was defined as the ratio of mass of fibre to the total mass of the sample.

$$P = \frac{m_f}{m_t} \tag{2}$$

where P = fibre purity,
m_f = mass of fibre, and
m_t = total mass of the sample

a b

Figure 3.7 Manually separated (a) Fibres and (b) Shives

3.3.4 Statistical Analysis

Statistical Analysis Software (SAS) version 9.3 was used to carry out statistical analysis. The Analysis of Variance (ANOVA) was used to compare the terminal velocity differences and Scheffe's method was used to compare the treatment means for the data from the mechanical separation experiment. The level of significance was chosen as 5%.

3.4 Results and Discussion

3.4.1 Properties from Pneumatic Experiment

3.4.1.1 Sample Characteristics

Various characteristics of the individual fibre and shive particles used for pneumatic experiment were measured. The length of the fibres varied from 17.4 to 100.5 mm, whereas a smaller length range from 7.6 to 35.2 mm was recorded for the shives. The width of shive particles ranged from 0.32 to 3.07 mm giving a wider range of the projected areas of the shives (17.2 to 73.7 mm^2). The wide ranges of the sample characteristics showed the random selection of these particles for the tests, which represented the true nature of decorticated flax.

3.4.1.2 Terminal Velocity of Shives Particles

Data did not show any particular trend for the effect of projected area on the terminal velocity of shives. Thus, the related data were not included. The effects of the other sample characteristics are presented. There was a high range and variance of terminal velocity values due to the variable width of the shives. With an increase in width of shive particles, there was a general increase in terminal velocity (Fig. 3.8). The width of shive particles ranging from 0.46 to 3.07 mm showed terminal velocity ranging from 1.13 to 4.09 m/s. A linear regression line between the terminal velocity and particle width had a correlation coefficient of 0.70, meaning that 70% of the data could be described by the linear relationship. This was considered a good trend, given the highly variable nature of shive particles.

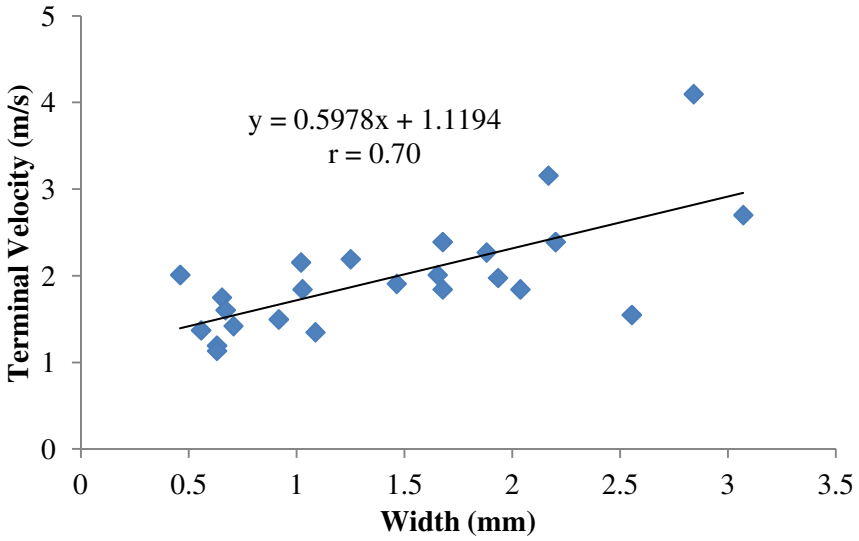


Figure 3.8 Terminal velocities of shives with different widths

Most shive particles weighed less than 0.026 g, although the heaviest shive particle weighed 0.081 g (Fig. 3.9). In spite of the uneven distribution of shive mass, a linear trend was seen for its relationship to terminal velocity. The linear equation had a correlation coefficient of 0.82. The slope of the linear equation was much higher when compared to the equation in Fig. 3.8. This indicated that the effect of shive mass on the terminal velocity was more pronounced

than the effect of shive width.

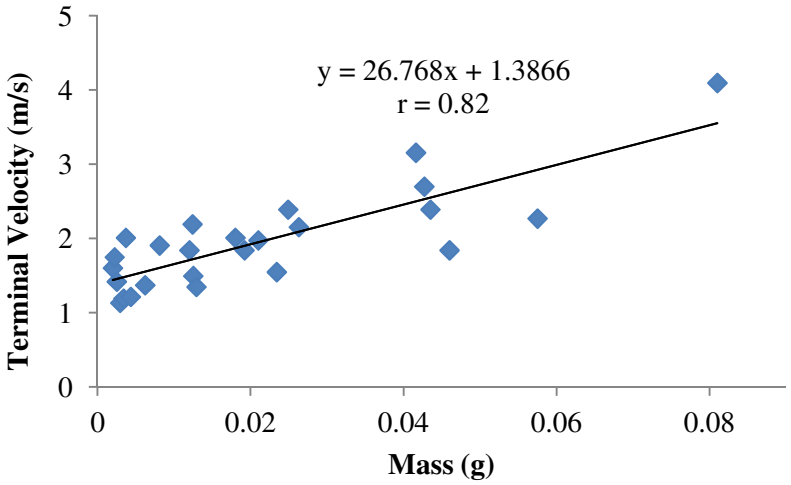


Figure 3.9 Terminal velocities of shives with different masses

3.4.1.3 Terminal Velocity of Fibre and its Comparison with Shives

Both fibre and shive particles were compared with respect to different particle lengths. Thus, the data were plotted together to show the effect of length and to compare differences between the terminal velocities of fibre and shive particles (Fig. 3.10). There was no or little change in terminal velocity over the entire range of fibre length as shown by the trend line, which is almost parallel to the x-axis. For shives, there was an increasing trend line, although the terminal velocities were highly scattered. When comparing the two particles, the terminal velocities of all shive particles were higher than fibre particles, as shown in Fig. 3.10. The ANOVA results demonstrated that terminal velocities of fibre and shive particles were statistically different ($p < 0.05$).

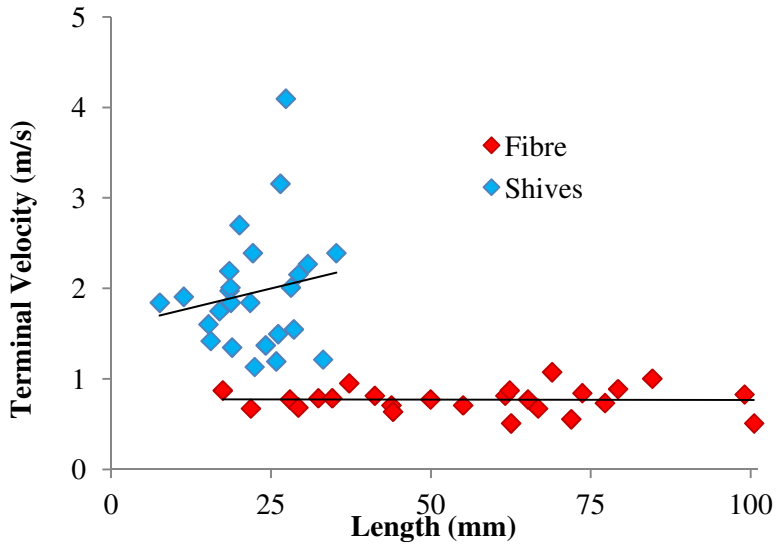


Figure 3.10 Comparison of length of fibre and shives

Parvin et al. (2013) reported that the terminal velocity ranges for hemp fibre and core particles were 0.56 - 1.36 m/s and 1.28 - 3.52 m/s, respectively. These values are comparable with the terminal velocities of flax fibre and shives found in this study. The terminal velocity ranges of hemp fibres and cores overlapped. However, the terminal velocity of flax fibre and shives ranging from 0.51 to 1.07 m/s and 1.13 to 4.09 m/s, respectively, had no overlap. The differences in the terminal velocities between fibre and shives prove the potential of pneumatic method to separate them. This result can ultimately help the industry to obtain fibres with higher purity. The highly pure fibre would create a great prospect for the already existing market.

3.4.2 Yield and Fibre Purity from the Sorter

The initial fibre purity for Grade 1 and Grade 2 before sorting was 51% and 15%, respectively. After passing through the Sorter sequence, the purity of the sample was improved due to the combing action of the rollers. Overall, the output in the long fibre stream had higher fibre purity than that in the short fibre stream. This could be observed visually in Fig. 3.11. Regardless of sample grade, short fibre stream had higher yield than long fibre stream. During the sorting process, some small particles fell through the vibratory conveyor, and some fell

through the rollers on to the floor. Some fibre and shives were also stuck on the rollers. These losses were a small percentage of the total mass input and they were not reported. Data were analyzed separately for the long and short fibre streams and the results are presented below.

a b

Figure 3.11 Sorter output for (a) Short fibre stream and (b) Long fibre stream

3.4.2.1 Long Fibre Stream

Figure 3.12a shows the average purity and machine yield for the long fibre stream. The error bars indicate the ranges for all the three replicates. The average fibre purity for Grade 1 and Grade 2 was 79% and 66%, respectively, which was significantly different ($p<0.05$). For Grade 1, the purity increased from the initial purity of 51% to 79%. The fibre purity for Grade 2 also improved significantly from its initial purity of 15% to 66%. Although unretted flax was used for the same pieces of equipment in this study, the machine yield for Grade 1 were comparable with a study conducted by Panigrahi et al (2012) which used retted flax. The machine yield of the long fibre stream for Grade 1 is significantly higher than Grade 2 but both values were very low.

3.4.2.2 Short Fibre Stream

Figure 3.12b shows the average purity and machine yield of the short fibre stream of Sorter. The fibre purity for short fibre stream for Grade 1 and Grade 2 were 47.2% and 26.2%, respectively (Fig. 3.12b). A relatively high fibre purity value of Grade 1 here also attests to the presence of high amount of short fibre and shives in the initial sample. This also explains the low

machine yield for long fibre stream (Fig. 3.12a). However, fibre purity was relatively low for Grade 2 because Grade 2 sample initially contained 15% fibre by weight. This value was improved in Sorter waste (short fibre stream) and it contained approximately 26% fibre by weight (Fig. 3.12b).

The machine yield of the short fibre stream was 71.4% and 86.9% for Grade 1 and Grade 2, respectively (Fig. 3.12b). The mechanical separation using the Sorter generated a larger amount of output in the short fibre stream including the shives and leftover waste, which led to a low yield in the long fibre stream (Fig. 3.12a). This is again due to the presence of shorter fibre and a lot of small shive particles in the initial sample which allowed some of the small shive particles to pass the equipment before the Sorter as output and hence, reducing the final yield of the long fibre stream. However, the fibre purity of approximately 80% was recorded for the long fibre stream of Grade 1, which was a significant improvement based on the reports in the literature (Anthony 2002; Akin et al. 2004; Parvin et al. 2013).

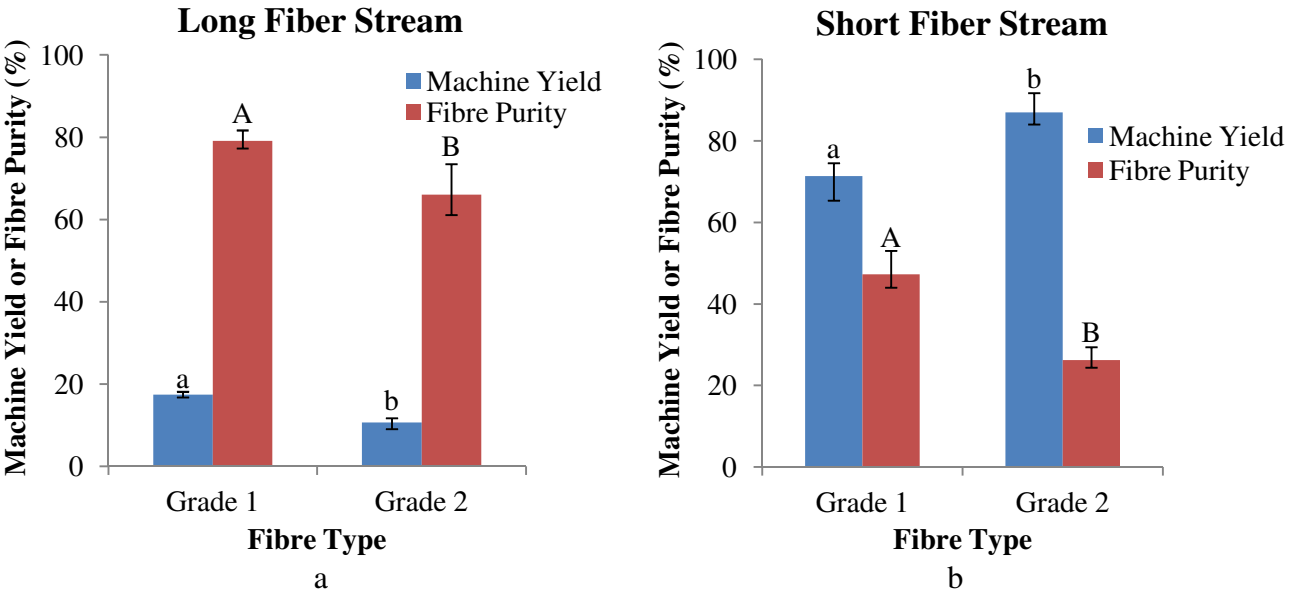


Figure 3.12 Machine Yield and Fibre Purity for two fibre streams (values labeled with different lower case letters or upper case letters were significantly different at $p < 0.05$)

3.5 Conclusion

Decorticated flax contains shives, short fibre, and long fibre. To improve fibre purity and obtain long fibres, tests of separating flax fibre and shives, and sorting short and long flax fibre were carried out using the pneumatic and sorting methods, respectively. The terminal velocity of shives varied with the length, width, and mass. The terminal velocity of fibre did not vary with length. The terminal velocity of flax fibres and shives ranged from 0.51 to 1.07 m/s and 1.13 to 4.09 m/s, respectively, which were significantly different, regardless of particle characteristics (length, width, and mass). Therefore, the pneumatic method can be potentially used to separate flax fibre and shives. The Sorter can separate long flax fibre from short flax fibre with reasonably high fibre purity (79%) for long fibre. However, the yield of the long fibre output was low (17%). This study showed the potential of using the pneumatic method to separate flax fibre and shives, and sorting method to obtain longer fibre and improve fibre purity.

3.6 Acknowledgements

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4.0 Properties of hexane extracts from canola, hemp, flax, and sweet clover plants

4.1 Abstract

With microorganisms becoming resistant to synthesized antibiotics, examination of plant parts to discover new antimicrobials is ever increasing. Since medieval times all portions of plants have been used medicinally. Plant tissues, including stems, possess secondary metabolites (SMs), including phenolics and terpenoids, which have known antimicrobial properties. The purpose of this study was to show the presence of antimicrobial compounds in stem extracts of canola, flax, hemp, and sweet clover; and study the sorption-desorption behavior of their powdered stem material. GC-MS analysis of all extracts showed the presence of many SMs, including fatty acids, terpenoids, steroids, and sterols, etc., many of which have shown antimicrobial activity against a broad spectrum of organisms according to literature. Water sorption isotherms of stems showed a typical IUPAC Type II sigmoid curve similar to natural fibres. EMC of canola and sweet clover was significantly higher than flax and hemp at 95% RH ($p<0.05$). However, EMC of all stems was higher than the fibre saturation point of wood (27%) (Kymäläinen and Pasila 2000). The preliminary investigation via GC-MS showed promising results with the presence of many antimicrobial compounds and water absorptivity results of hygroscopic stems is a starting point to find new applications for stems.

4.2 Introduction

Parts of many plants such as flax and hemp have been used as a medicine since 900 BC (Kuddus et al. 2013). However, the use of these plants in biomedical applications has been limited. Lately, researchers have begun exploring applications of flax fibre in biomedical

engineering, for example in wound dressing (Czemplik et al. 2011) and surgical meshes (Michel et al. 2014). However, natural fibres are mostly composed of cellulose and hemicellulose (Mustata and Mustata 2012), which are not renowned for their antimicrobial properties. However, the secondary metabolites such as phenolics, terpenoids, and fatty acids, etc. possess antimicrobial properties (Bakkali et al. 2008), which are present in many parts of the plant, namely; seeds, leaves, and fruits, etc.

Different parts of canola, flax, hemp, and sweet clover plants have also been investigated. For instance, proteins and peptides of canola have shown antimicrobial activity (Aachary et al. 2015); presence of antimicrobial cannabinoids in hemp (Appendino et al. 2008); and use of aerial parts of yellow sweet clover for treatment of skin wounds, allergies, and pyoderma (Mamedov et al. 2005). Therefore, stems of each plant were considered instead of just the fibres, and it was hypothesized that comparable antimicrobial activity would be present in the stems. The long-term goal of this research was to explore the potential biomedical application of plant stems such as potential natural antimicrobials, wound dressing component, tissue engineering scaffolds, biomedical implants, etc. The short-term goal was to look for potential antimicrobial compounds in stem extracts of hemp, flax, canola, and sweet clover.

Prior to testing of properties, pretreatment of the samples, such as extraction is required. The best method for extraction from solid materials is Soxhlet extraction. The sample is placed in a container which is regularly provided with condensed solvent. When the condensed solvent fills up the container, the solute is drained down into the distillation flask along with the extractives (De Castro and Priego-Capote 2010). This cycle takes place until all the extractives are collected. Soxhlet extraction has been used for plant extracts by many researchers (e.g. Vadlapudi 2012; Ocheng et al. 2014; Sahoo et al. 2012). Hexane was used to extract

phytochemicals in this study to focus on the non-polar fractions of plant stems.

The sample is subjected to a number of fractionation techniques such as gel permeation chromatography (GPC), which separates the components in a mixture based on molecular size differences and improves the effectiveness of the purification process (Reeve and Crozier 1976). Many chromatographic techniques such as high performance liquid chromatography (HPLC) and gas chromatography (GC) have been developed for separation of components of a mixture. Most of these techniques have well developed protocols for identification of certain types of compounds. Another separation technique - Gas chromatography (GC) is typically coupled with Mass spectrometry (MS), a technique for detection of components, collectively known as GC-MS, which reduces the probability of error in detection of unknown components in a mixture (Sahil et al. 2011). Volatile mixtures are easily analyzed using GC-MS, which provides a full structure overview along with determination of molecular masses for the unknown compounds (Proestos et al. 2006) based on the associated library. Therefore, GC-MS is a powerful analytical tool for separation and identification of components in a mixture. GC-MS was used in this study to look for antimicrobial compounds in the stem extracts of the aforementioned plants.

The applications of a hygroscopic material such as plant stems can be fully explored if its physical and mechanical behavior is fully understood (Zaihan et al. 2009). Therefore, sorption characteristics should be studied prior to experimenting on a new material. The moisture content (MC) of samples can be determined non-destructively using static and dynamic gravimetric methods. The static method uses different saturated salt solutions in desiccators, sorbostats or glass jars, to maintain different RH values (Rahman and Sablani 2009). Kymäläinen and Pasila (2000) used a static gravimetric method to determine the sorption characteristics of hemp and flax straw fractions. However, the static methods still exist, but dynamic sorption methods are

more prominent in industry as it provides continuous determination of MC with a user-defined range of RH values (Argyropoulos et al. 2012). Dynamic vapor sorption (DVS) Advantage equipment maintains the temperature and RH in a chamber where the sample is held in a microbalance that has the ability to measure the change in mass to 1 in 10 million parts (SMS 2015).

DVS has been used for determination of sorption isotherms of oil palm trunk and rubberwood (Zaihan et al. 2011), modified wood (Xie et al. 2010), and natural fibres (Hill et al. 2009; Xie et al. 2011). Moisture sorption of hemp and flax stalks was determined using dynamic gravimetric method at a few RH values by Nilsson et al. (2005) and non-linear regression isotherm models were fitted accordingly. Canola kernels were subjected to successive adsorption-desorption cycles at 40°C in the RH range of 10-70% by Yang and Cenkowski (1993). No sorption-desorption studies for canola and sweet clover stems have been reported.

It is hypothesized that presence of terpenoids, essential oils, fatty acids, and other secondary plant metabolites in plant stem will contribute towards antimicrobial properties. Testing the plants for the aforementioned properties will be a stepping-stone for proving their applicability in biomedical applications, which can provide a potential source for a natural antimicrobial agent. The objective of this study was to investigate stem extracts of flax, hemp, canola, and sweet clover for antimicrobial compounds through GC-MS analysis of their extracts, and determine water sorption characteristics of their powdered stem material.

4.3 Materials and methods

4.3.1 Plant Material

Linseed flax plants (Sorrel type) were provided by Composites Innovation Center (CIC) based in Winnipeg, Manitoba. Baker Farms based in Dauphin, Manitoba, provided hemp plants

(Alyssa type). Canola stalks (Red River 1861 type) were provided by Glenlea Research Center, University of Manitoba, and sweet clover plants (wild type) were obtained from a park in Winnipeg, MB. All plants were hand-harvested in the summer of 2014 (Fig. 4.1). Leaves, sub-branches, seeds, etc. were removed from all plants and only stems were used in this study. The main branch was cut into small sections (Figs. 4.2a-d) and henceforth, the stems were washed twice with tap water (Mahesh and Satish 2008), and once with reverse osmosis water to remove dirt and other small particulates and air-dried for 15 days (Ikhane et al. 2015). The moisture content of the air-dried samples was determined by placing them in the oven for two hours at 135°C (AACC International Method 1999).

a b c d

Figure 4.1 Hand-harvested plants: (a) Canola, (b) Flax, (c) Hemp, and (d) Sweet Clover

a b c d

Figure 4.2 Plant stems cut in small sections prior to grinding: (a) Canola, (b) Flax, (c) Hemp, and (d) Sweet Clover

4.3.1.1 Preparation of Plant Stem Extracts

The air-dried stem samples were ground using Stein Laboratory Mill and manually sieved to a mesh size 18 (1 mm) (Anderson 2004). The powdered stem material was used to determine water sorption characteristics. The extract prepared from powdered stem was investigated for antimicrobials via GC-MS. Hexane was used as a solvent as per previous work in using a traditional Soxhlet apparatus to prepare stem extracts (Kotan et al. 2014; Ozel and Kaymaz 2004; Vadlapudi 2012). N-hexane was purchased from Sigma Aldrich. This research utilized an automated Soxhlet extraction unit, specifically a FOSS Soxtec 2050, to extract non-polar phytochemicals. The crude extracts were left in the fume hood to evaporate the solvent and henceforth sealed in a glass container and refrigerated at 4°C (Anderson 2004).

4.3.1.2 Fractionating the extracts

Extracts were fractionated by GPC that separated extract components based on size-exclusion with heavy molecules filtering first. In brief, one gram of plant extract was dissolved into 10 mL of a mobile phase solvent system (1:1 dichloromethane: hexane) and filtered through a 1 µm Teflon filter. The extract was then transferred to the GPC column and fractionated as follows: F0 as the first 100 ml; F1 through F10 as 10 ml each, collected between 100 - 200 ml; and F11 as the next 50 ml, collected between 200 - 250 ml. Fractions were then reduced in volume to 0.5 mL and stored at 4°C prior to GC-MS analyses.

4.3.2 Experimental Design

All experiments were completely randomized and the treatments consisted of flax, hemp, canola, and sweet clover stems with four replicates each for water sorption tests.

4.3.3 Measurements

4.3.3.1 GC-MS Analysis

Fractions from GPC were injected into a GC-MS system consisting of a Trace 1310 Thermo scientific gas chromatograph coupled with a TSQ 8000 Evo Triple Quadrupole mass spectrometer operating in Electron Impact mode with the source temperature set to 230°C. Data were acquired at a mass range of m/z 50 - 1100. A capillary column ZB-5MS (30 m in length; 0.25 mm i.d.; 0.25 µm in film thickness) was used for chromatographic separation using helium as a carrier gas at 1 ml/min flow rate and a transfer line temperature of 280°C. One µl injections were performed by a TriPLUS RSH autosampler; the injector was set to 250°C and injections were made in the splitless mode. The stepped oven temperature program began at 80°C with a holding time of 0.5 min, and increased at the rate of 15°C/min until 130°C and again increased at the rate of 25°C/min until 300°C with a holding time of 12 min. Some of the extracts were diluted and re-injected as required.

Chromatographic separation of the extracts' components showed up as separate peaks in the chromatogram. The total ion chromatogram (TIC) for mass spectrum (50-1100 amu) for each peak was selected separately and compared with spectrum of known compounds in NIST 2014 Mass Spectral Library. The library software provided possible compound ID along with probability of match between sample spectra and library. The tentative compound IDs with a probability of 80% or greater were selected as the basis for further analysis.

4.3.3.2 Determination of water sorption isotherms

Water sorption isotherms of the powdered stems of canola, flax, hemp, and sweet clover were determined using a DVS Advantage apparatus (Surface Measurement Systems Ltd.,

London, UK) as described in Hill et al. (2009). Nitrogen gas was used as a carrier. The sample weighing 8 mg was placed in the sample pan. The sample size was selected as per the training of the equipment and a test run prior to the experiments. After the pan was stable, relative humidity (RH) was kept at 0% for 15 minutes and was suddenly increased to 90% to remove the static built up within the chamber and a moisture curve was obtained. The RH value fell back to its default value after exceeding 90%. A "drying curve" was also produced with RH initially at 90% for 15 minutes and suddenly falling to 0% until the sample weight was stable (Hill et al. 2009).

An experimental run started at 0% with a step increment of 5% till 95% for sorption and then decreased from 95 to 0% at the same rate for desorption. Constant RH was maintained until a change in mass with time (dm/dt) was less than 0.005% per minute over duration of 10 minutes, which is in line with other studies (Hill et al. 2009; Xie et al. 2010). A similar condition has produced moisture content within 0.1% of the equilibrium moisture content for the sample at a prolonged exposure (Hill et al. 2009). Normal room temperature (25°C) was selected for determination of water sorption. All the samples were tested in quadruplicates.

4.3.3.3 Statistical analysis

Statistical Analysis Software (SAS) version 9.3 was used to carry out statistical analysis. The Analysis of Variance (ANOVA) and Scheffe's method of comparison of treatment means was used for hysteresis values, sorption and desorption values, and duration at each 5% step change in RH. The level of significance was chosen as 5%.

4.4 Results and Discussion

4.4.1 General Properties

Table 1 lists the initial moisture content of the stems of the four plants and soxhlet

extraction yields. It should be noted that the initial yield after extraction of 50 g of plant material was higher (approximately 3%) for all plants but hemp. However, with time, the unit did not yield high amounts of extracts and the yields were significantly lower which made the final yield very low (Table 4.1). The drawback with using Soxtec 2050 was its inability to use large sample sizes to obtain large amount of extract at once. Recommended sample sizes if the fat content is 0-10%, 10-25%, and over 25% were 2-3, 1-2, and 0.5-1 grams, respectively (Anderson 2004). Therefore, sample sizes of 2 to 6 g were used at once to extract a few milligrams of extract.

Table 4.1 Results of Soxhlet extraction yield and initial moisture content

Plant Stems	**Material weight (g)**	**Extract (g)**	**Yield (%)**	**Initial Moisture content$^{d.b.}$ (%)**
Canola	249	4.903	1.969	4.945
Flax	261	5.429	2.080	3.696
Hemp	110	1.732	1.575	3.348
Sweet Clover	204	4.105	2.012	4.094

4.4.2 Confirmation of Antimicrobials using GC-MS

The quantification of crude extracts was difficult due to the presence of many compounds in the mixture. Therefore, the extracts were fractionated using GPC before running them in GC-MS. The sensitivity and resolving power of capillary GC-MS makes detection of unambiguous mixtures suitable (Proestos et al. 2006). GC-MS identified many compounds from the hexane extracts of four stems and molecular weight, retention time, chemical structure, and mass spectrum were recorded based on the library database.

Twenty-six compounds were found with a probability of 80% or greater. Tables 4.2-4.4

list their names, their class, the plant, and the GPC fraction source. Their spectra in comparison to the spectra of the extracts are shown in the appendix. Fatty acids (Table 4.2) and terpenoids (Table 4.3) were the principal members found with five and six compounds, respectively. The presence of both long chain and short chain fatty acids in all plants is well known and they all show broad spectrum antimicrobial activity (Nair et al. 2005). At least one of the fatty acids was present in each of the extracts. However, octanoic acid was present in both hemp and sweet clover (Table 4.2).

Terpenoids are a class of secondary metabolites, which were mostly present in flax and sweet clover extracts (Table 4.3), and they have shown moderate activity against microorganisms. Table 4.4 lists all the other class of compounds. Among those, sterols and steroids were the two most prominent classes with three compounds each, both of which had shown good antimicrobial activity. Sterols were found in many fractions of flax. However, steroids (γ-Sitosterol and Stigmasterol) were found in multiple fractions of plant extracts. Overall, sweet clover extract had the most number of the compounds followed by flax and hemp. Canola had the least amount of compounds over the 80% mark. However, the less number of compounds does not necessarily mean less antimicrobial activity. The antimicrobial efficacy will depend on the concentration of the compounds in the extract and the effect of these compounds against a certain bacteria in synergy. The compounds present in plastics (PAHs) were also found over the 80% mark. This is due to the use of plastic micropipette tips during the soxhlet extraction while reconstituting the extracts in hexane for transferring into glass vials. These compounds were not included in the tables below.

There are many compounds listed below which have antimicrobial activity against a wide spectrum of bacteria and others, which were found in an extract or an essential oil of a

medicinal plant. Overall, this list of compounds shows that there is huge potential for the four extracts to have antimicrobial activity against a wide spectrum of microorganisms.

Table 4.2 Long chain and small chain fatty acids from GC-MS (C - Canola, F - Flax, H- Hemp, and S - Sweet Clover; F0 - F11 are the 12 GPC fractions. For instance, C-F0,5 means fractions F0 and F5 had the respective compound in canola extract)

Compound name	Plant - Fraction No.	Reference	Antimicrobial activity
N-Hexadecanoic acid	C-F0	Pu et al. 2010	Yes
Pentadecanoic acid	S-F2	Pu et al. 2010	Yes
Tetradecanoic acid	F-F9	Agoramoorthy et al. 2007	Yes
Octadecanoic acid	F-F2, 7	Pu et al. 2010	Yes
Octanoic Acid	H-F4; S-F3	Nair et al. 2005	Yes

Table 4.3 Terpenoids and their derivatives from GC-MS

Compound name	Plant - Fraction No.	Reference	Antimicrobial activity
Ursolic Aldehyde	S-F6	Yimdjo et al. 2004; Pretto et al. 2004	Potential
Friedelan-3-one	H-F4		
Betulinaldehyde	S-F6	Chung et al. 2011	Yes, in synergy
Uvaol	S-F6	Horiuchi et al. 2007	Not significant
Chol-8-en-24-al, 3-(acetyloxy)-4,4,1,4-trimethyl-,(3β, 5α)-	H-F4		
2-Pentadecanone, 6,10,14-trimethyl-	H-F4; F-F2	Yayli et al. 2005	Potentially, in an essential oil

Table 4.4 Other compounds from GC-MS analyses

Class of compounds	Compound name	Plant - Fraction No.	Reference	Antimicrobial activity
Sterol lipid	Ergostanol	H-F4; F-F3,7		
Sterol lipid	9,19-Cyclolanost-24-en-3-ol,(3β)	F-F7,11	Penduka et al. 2014	Potentially in synergism
Sterol derivative	4-Campestene-3-one	F-F7; H-F4	Sharma and Paul 2013	Found in medicinal plant
Steroid	γ-Sitosterol	S-F7,11; F-F3,5,9; H-F1,4,11; C-F0,0,2	Tamokou et al. 2011; Niño et al. 2006	Yes, in a mixture
Steroid	Stigmasterol	S-F2,2; F-F3; H-F11	Tamokou et al. 2011; Niño et al. 2006	Yes, in a mixture
Steroid	γ-Sitostenone	S-F3,6; F-F2,7	Ara et al. 2009	Average, with β-sitostenone
Pyrroles	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-	S-F8		
Piperidinone	4-piperidinone, 2,2,6,6-tetramethyl-	C-F11		
Ketone	15-Nonacosanone	H-F0,11; C-F2,5	Hifnawy et al. 2013	Potentially, in an essential oil
Halogenated compound	2-Chlorocyclohexanol	S-F0	Rocha et al. 2011	In an extract which showed antifungal
Flavonoid	6H-benzofuro[3,2-c][1]benzopyran, 3,9-dimethoxy	S-F11	Magalhães et al. 2007	
Fatty acid ester	Dodecanoic acid	S-F0	Kabara et al. 1972	Yes
Benzoic acid ester	Benzyl benzoate	H-F8; C-F6	Ali et al. 2002; Farjam et al. 2012	Yes, in an essential oil
Benzofurans	Loliolide	F-F11	Ragasa et al. 2005	Moderate
	Cyclooctasiloxane, hexadecamethyl-	F-F7; S-F0	Hossain et al. 2013	Found in a medicinal plant

4.4.3 Sorption dynamics of stems

The change in mass (%) represented as moisture content to a step change in RH from 0% to 95% produced an asymptotic curve when plotted against time (Fig. 4.3). Each curve at a step RH change represents the moisture content approaching the local EMC value at a given RH (Hill et al. 2009). The moisture content increases with the sorption process and decreases with desorption process when a new target RH is acquired. This depends on the dm/dt value of 0.005% min^{-1} for an interval of 10 minutes after which the RH value is preset to the next value in sequence. Canola samples took 53 hours to reach EMC, which was significantly more ($p<0.05$) than flax, hemp, and sweet clover, which all took approximately 45 hours for the entire sorption-desorption run. The time profile may depend on geometrical morphology, chemical composition of the stems, and sample size (Xie et al. 2010). The total mass was set at 8 mg based on a trial run and training of the DVS equipment. However, there was approximately 6% difference between the initial sample sizes of replicates for the same plant stem. This is due to the limitation of the weighing balance with a readability of 0.1 mg. It is to be noted that Fig. 4.3 represents the time profile of only one of the specimens for each stem. It can be speculated that different time profiles for replicates within each sample is a representative of different parts of the plants (stem and fibres) in those specimens.

The time taken for each interval for sorption and desorption at step change of 5% RH was determined using Matlab (Fig. 4.4). The x-axis represents 5% intervals of RH starting from 0% and increasing up to 95% and then decreasing to 5% at the same rate. As RH increases, stems take more time to swell up, especially, after the interval 65-70% RH for sorption, and release moisture after the interval 55-50% RH for desorption. Canola samples took the most time for the interval 90-95% RH that was greater than the other samples (Fig. 4.4). This interval took the

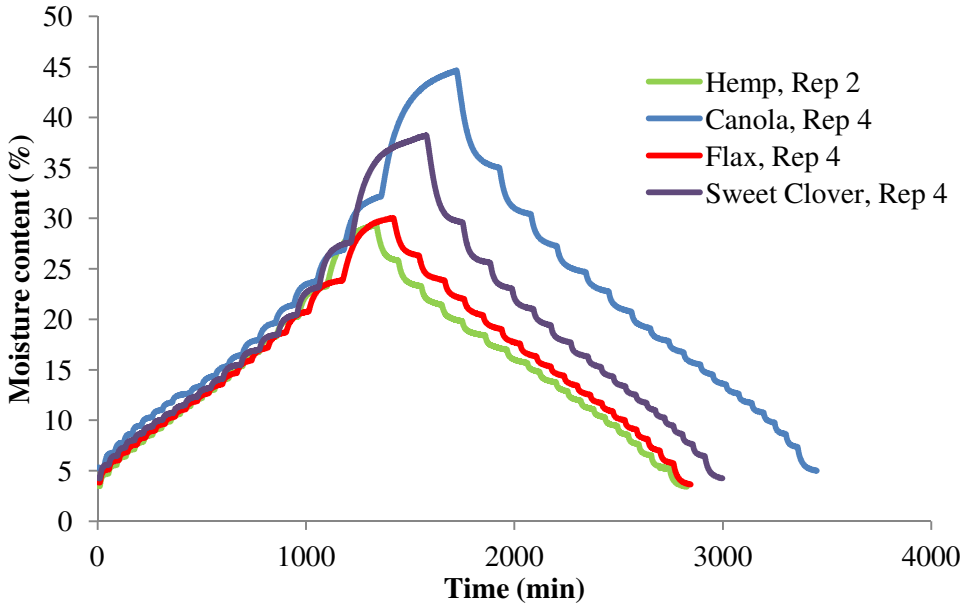

Figure 4.3 Moisture content change with respect to the step changes in RH over time

most time because the stems already possessed too much moisture at 90% RH, which made it difficult to find the available OH groups and micro-capillary pores. It is also interesting to note that there is an increase in time when the stems have to give up the leftover moisture for the desorption cycle during the interval 5-0%, which attests to the hygroscopicity of the stems.

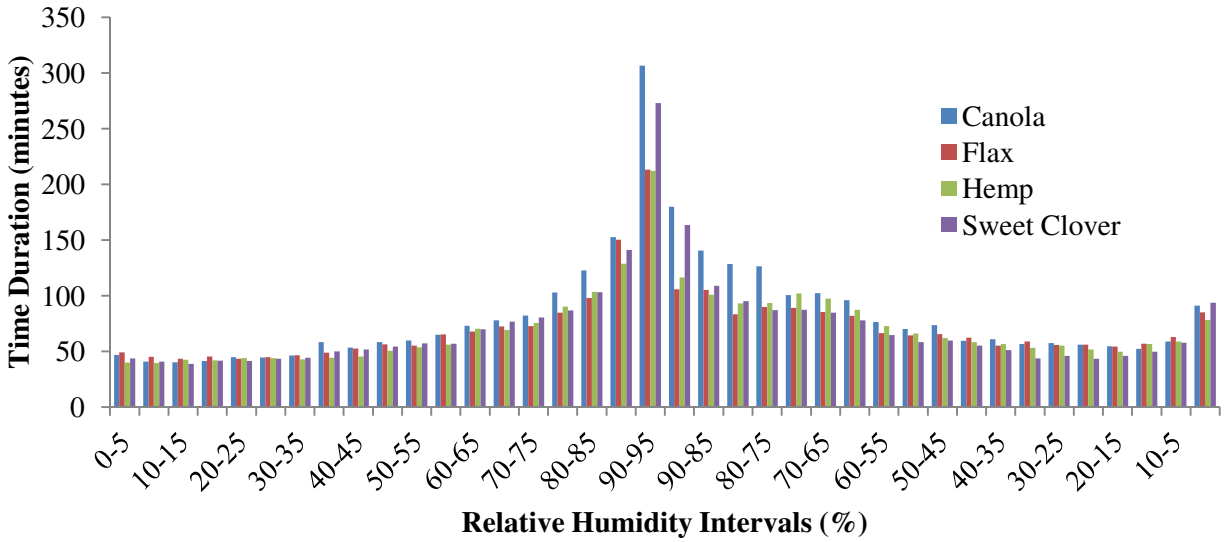

Figure 4.4 Time taken for a 5% step change in RH

4.4.4 Comparison of sorption-desorption behavior

There were clear differences in sorption and desorption behavior as well as the EMCs of the stems (Fig. 4.5). Canola exhibited the highest EMC of 40.6% at the 95% RH, followed by sweet clover at 36.0%, which were both significantly different from hemp and flax stems at 28.1% and 29.2%, respectively ($p<0.05$) (Fig. 4.6). The moisture content for the sorption cycle from 0% to 25% was significantly different for all stems. Canola values were significantly different from at least one of the other stems throughout the isotherm loop ($p<0.05$, Fig. 4.5). All the stems exceeded the fibre saturation of wood (27%) (Kymäläinen and Pasila 2000) at 95% RH. Moisture content is a measure of the OH group availability in the material (Xie et al. 2011; Hill et al. 2009), chemical and morphological structure of the plants and pectins (Kymäläinen and Pasila 2000), and the presence of lignin (Hill et al. 2009). Presence of lignin results in less OH units and may result in lower hygroscopicity for unretted straws (Kymäläinen and Pasila 2000). However, Hill et al. (2009) speculated that lignin can intake more moisture within the cell wall because they saw an increase in moisture trend for lignin-rich fibres in their study of natural fibres. Pectin can take more moisture from air, and, therefore, the unretted stalks would acquire more moisture (Kymäläinen and Pasila 2000). Moisture content of different types of wood is different due to the chemical composition of cellulose, hemicellulose, and lignin (Zaihan et al. 2009) and this variability of chemical constituents depends on the variety, maturity of stalks, and the method of analysis (Salmon-Minotte and Franck 2005).

It was found that all the stems showed an International Union of Pure and Applied Chemistry (IUPAC) Type II sigmoid shape of the isotherms with the capillary condensation region from 70% RH onwards showing a rapid increase in moisture content intake (Fig. 4.5), which is typical for natural fibres (Lee and Bismarck 2011). The small standard error bars

throughout the isotherm curves represents the reproducibility of the DVS equipment.

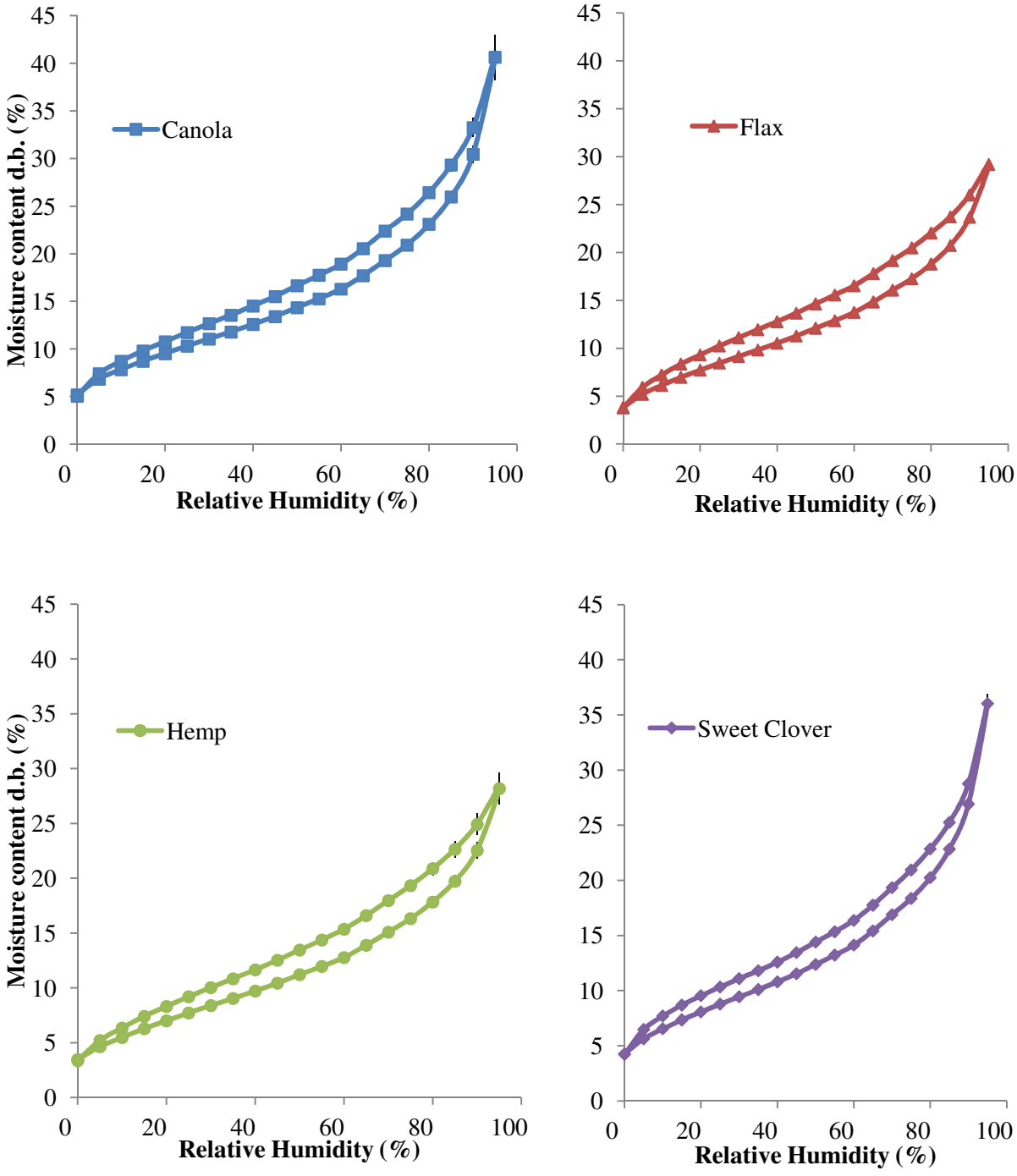

Figure 4.5 Sorption Isotherms for all four stems

Sorption isotherms of biological specimens are highly variable due to many factors such as: time of growth of crop, location, soil conditions, equipment choice, and handling methods

(Wolf et al. 1985). Few sorption studies of hemp and flax straws have revealed comparable data. Kymäläinen and Pasila (2000) studied the EMC of unretted and retted varieties of hemp and flax fractions (fibre, coarse shives, and fine shives) at 20°C at three RH values of 15, 76, and 97%. The data comparison is shown below (Table 4.5). It can be seen that the data obtained within this study is lower than Kymäläinen and Pasila (2000) due to a variety of reasons mentioned initially.

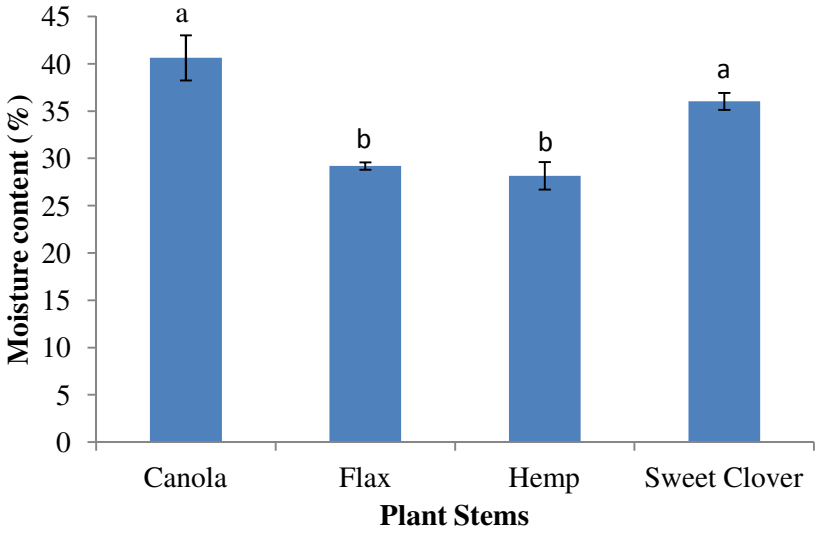

Figure 4.6 EMC of stems at 95% RH (means with the same letters are not significantly different)

Another study by Nilsson et al. (2005) predicted the EMC values of both unretted and frost-retted flax and hemp straws. The predicted values of unretted flax straw (Viola) using Modified Halsey model at RH 75% and 95% were 15% and 31%. The values found at the same RH in this study were 17.2% and 29.2% (Fig. 4.5). The predicted values of unretted hemp straw using Modified Oswin model at 75% and 95% RH were 12.5% and 25%. The values at the same RH in this study were 16.3% and 28.1% (Fig. 4.5). Canola kernels were subjected to successive adsorption-desorption cycles at 40°C in the RH range of 10-70% by Yang and Cenkowski (1993). For the first cycle, the moisture content at 65% RH was 9.5 %, which was 17.7% in this study at 25°C. The difference could be due to the temperature of sorption tests.

Table 4.5 Data comparison with study conducted by Kymäläinen and Pasila (2000) (a - values recorded in this study, n/o - not obtained)

Material	**Moisture content (%), d.b.**			
	at 75% Stems[a]	At 76% Coarse shives	at 95% Stems[a]	At 97% Coarse shives
Green Flax	n/o	14.6	n/o	32.6
Green Linseed	17.2	15.5	29.2	36.2
Green Hemp	16.3	15.9	28.1	33.1

Sorption hysteresis for the stems of the four plants was also compared (Fig. 4.7). Xie et al. (2011) found hysteresis in three ways for natural fibres. However, in this study, hysteresis was determined from the difference in the sorption and desorption values at the same RH level. The initial trend with the rise up to 80% RH with different slopes and the drop in values until 90% was the same for all stems. Canola and flax had significantly higher hysteresis values at 80% RH than sweet clover ($p<0.05$, Fig. 4.7). Hill et al. (2009) found larger hysteresis loops for lignin-rich fibres. This hypothesis is ostensibly true in this study with canola showing the highest hysteresis of all stems. However, the lignin content of stems of canola (Enayati et al. 2009), flax (Salmon-Minotte and Franck 2005), and hemp (Merkel et al. 2014) is approximately 20% which also proves that the hysteresis of lignin-rich stems is higher. Moreover, hysteresis depends on surface adsorption or bulk absorption of the material. Bulk absorption corresponds to higher hysteresis values above 70% RH when the vapor is limited by diffusion (SMS 2012).

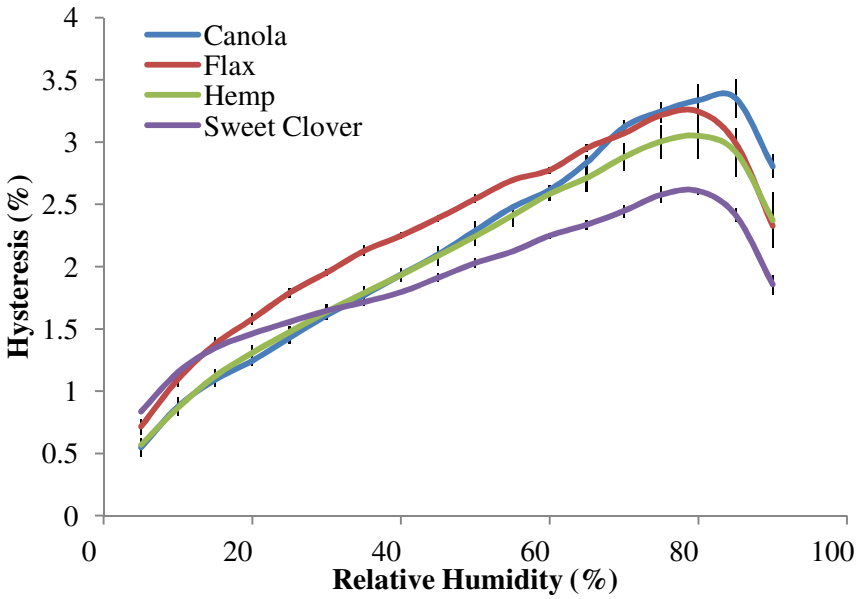

Figure 4.7 EMC Hysteresis of plant stems

4.5 Conclusion

The extracts of stems of four plants were prepared via Soxhlet extraction using hexane. The extracts were fractionated using Gel permeation chromatography. Many potential antimicrobial compounds were henceforth identified from all four-stem extracts via GC-MS analyses. The sorption isotherms, hysteresis, and time profile of the stems was determined and it was found that there were considerable differences between the sorption-desorption behavior of the stems of canola, flax, hemp, and sweet clover. The EMC of canola and sweet clover was higher than flax and hemp.

4.6 Future Work

A lot of future work can be performed from the positive results of GC-MS analyses. The first step would be to test the activity of the extracts against the organisms of interest, which would depend on the future applications. In this regard, the solvent of choice should be reconsidered due to the insolubility of hexane in many universal solvents such as 10% DMSO.

Next, the potential of FTIR for identifying the spectra of molecules could be identified based on the spectra of authentic standards bought from an authorized vendor. FTIR does not have an extensive library of compounds as GC-MS; therefore, this is a challenging task due to the overlap of spectra in the mixture.

Concentration of the antimicrobial compounds present in the extracts can be determined by running the extracts against the authentic compounds in GC-MS. This can be useful to determine the contribution of compounds towards the antimicrobial activity. Lastly, if the concentration of few of the compounds in the extracts is higher and marketable then they can be isolated if the endeavor is commercially viable.

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5. Summary and Future Work

Use of flax fibres in many industry sectors such as automobile, textiles, aerospace, composites, etc. has increased manifold in the 20th century. The industries require long fibre and high fibre purity in the initial mixture. However, decorticated flax fibre available in the market contains many unwanted particulates such as shives, dust, chaff, etc. Therefore, decorticated flax requires further cleaning to meet with the industry requirements.

In this study, cleaning of flax fibre was carried out using a pneumatic method and a sorting method. Terminal velocity of shives varied with the length, width, and mass. However, length of fibre particles had no influence on the terminal velocity. The terminal velocities of individual fibre and shive particles ranged from 0.51 to 1.07 m/s and 1.13 to 4.09 m/s, respectively, which were significantly different. Sorting process improved the long fibre purity for Grade 1 from 51% to approximately 80%; and Grade 2 from 15% to 66%. The sorting equipment met our goal of 80% fibre purity in the end mixture but the yield of the long fibres was low at 17%, which was still comparable to the other cleaning processes used in industry. To conclude, the differences in the terminal velocities of the two particles and high fibre purity of long fibre from the Sorter proves their potential in separating flax fibre and shives.

For future studies, pneumatic and sorting methods can be collectively used for a better degree of separation and cleaning. The Opener/Picker used as a pretreatment before sorting was used to open up entanglements of shives within fibre bundles, which was the main challenge for separation of post-decorticated product. The product from Opener can be subjected to the vertical wind tunnel for separation of fibre and shives by using the terminal velocity differences between the two particles recorded in this project. Next, the separated fibre batch can be run through the Sorter to obtain long fibres to meet the industry requirements. The above sequence opens up

entanglements, separates the fibre and shives, and sorts short and long fibre.

Moreover, the working of pneumatic method for separation of decorticated flax mixture can be investigated by introducing agitation. During the preliminary tests, it was observed that agitation was successful to separate the two particles in the mixture when they were placed in a vertical wind tunnel under the influence of air stream. Implementation of the agitation bar at an appropriate angle and the circular (or other) motion required to separate the two particles would be the key for the separation of entangled shives within the fibre bundles.

The second part of the thesis focused on finding potential antimicrobial compounds from the stem extracts of canola, flax, hemp, and sweet clover. Twenty-six compounds were found via GC-MS analysis of all extracts over the probability hit of 80% using the library database. The most prominent class of compounds amongst them were terpenoids, fatty acids, steroids, sterols, etc., most of which have known antimicrobial activity against a wide spectrum of bacteria according to literature. Water sorption results showed that equilibrium moisture content of both canola (40.6%) and sweet clover (36.0%) was significantly higher than hemp (28.1%) and flax (29.2%) at 95% RH. However, due to many factors such as hydroxyl group accessibility, chemical and morphological structure of the stems including their lignin and pectin content, it was difficult to explain the difference in their sorption behavior and hysteresis. The preliminary results of GC-MS show the potential of the stem extracts as antimicrobial agents and water sorption results provides a promising start for the implementation of the stems in a variety of applications.

Future work for sorption testing would depend on the type of application. For instance, DVS Advantage used in this study for sorption testing is capable of producing isotherms by subjecting the material to many cycles of sorption and desorption in succession to test their

reversible nature. This application is useful for pharmaceutical drugs to see their behavior in these situations.

There can be multitude of studies and experiments that can be performed from the positive results of GC-MS. Primarily, the extracts should be formally tested against an active or wild strain of a certain microorganism. The choice of organism will depend on the application of interest. Secondly, the focus can be turned towards a different (polar) solvent for extraction. Dealing with hexane was very complex due to the insolubility of the stem extracts in the universal solvents such as 10% DMSO and water. Next, with the positive efficacy testing against a certain organism, it would be interesting to know the compounds responsible for the antimicrobial activity. This can be easily determined by running the extracts in GC-MS along with the authentic standards bought from an authorized vendor. This experimental run will provide the concentration of the authentic standard in the extract. Lastly, isolation of the antimicrobial compound of high concentration and production as an antimicrobial agent or drug can be performed if it is cost efficient. This should only be performed after obtaining data about the toxicity of the compounds against human or animal cells, their mechanism of action, effects *in vivo*, and positive and negative effects of their interactions with commonly available antibiotics (Rios and Recio 2005).

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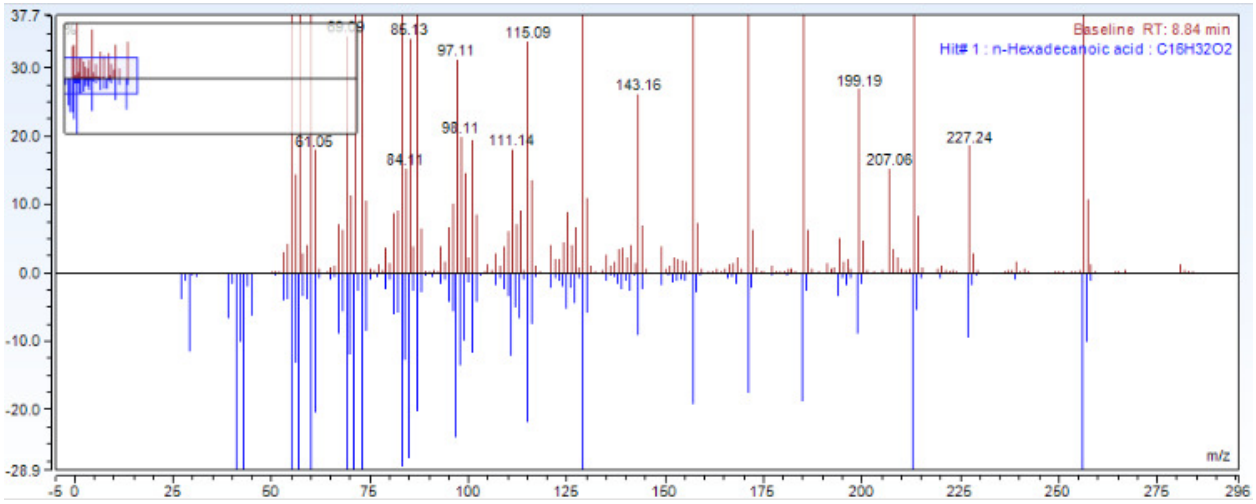
APPENDIX

The spectra of all 26 compounds with the probability of match greater than 80% from GC-MS analysis of extracts is presented along with the retention time and probability of match of the compound found in the GPC fraction according to National Institute of Standards and Technology (NIST) 2014 Spectral Library. The top spectrum in the figures belongs to the respective spectra of the extract and the bottom spectra belong to the compound.

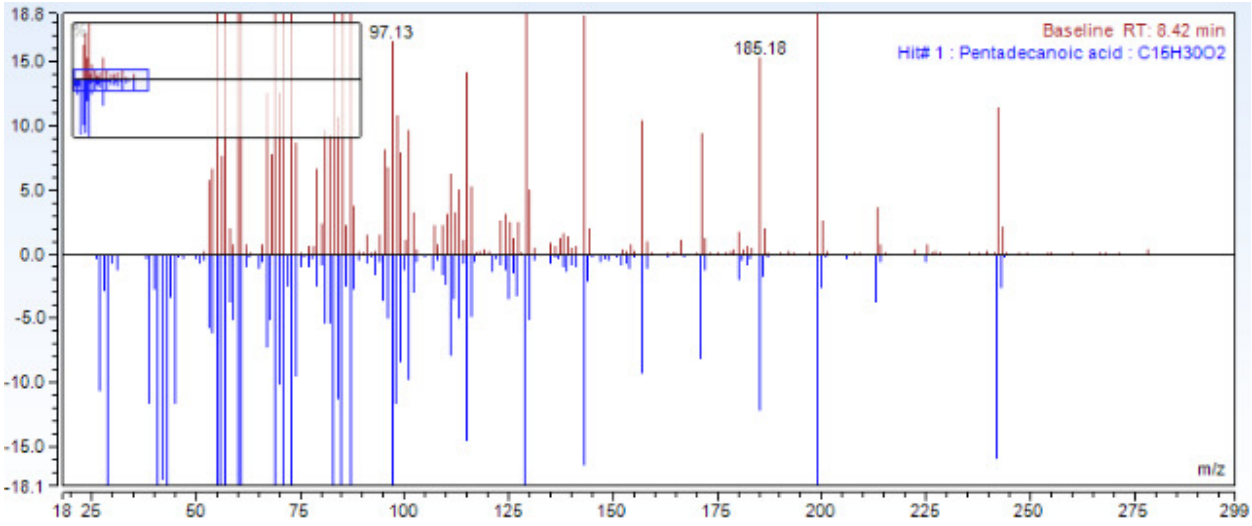
N- Hexadecanoic acid

N- Hexadecanoic acid was found in fraction F0 of canola extract at a retention time of 8.84 minutes and a probability of match of 87.9%.



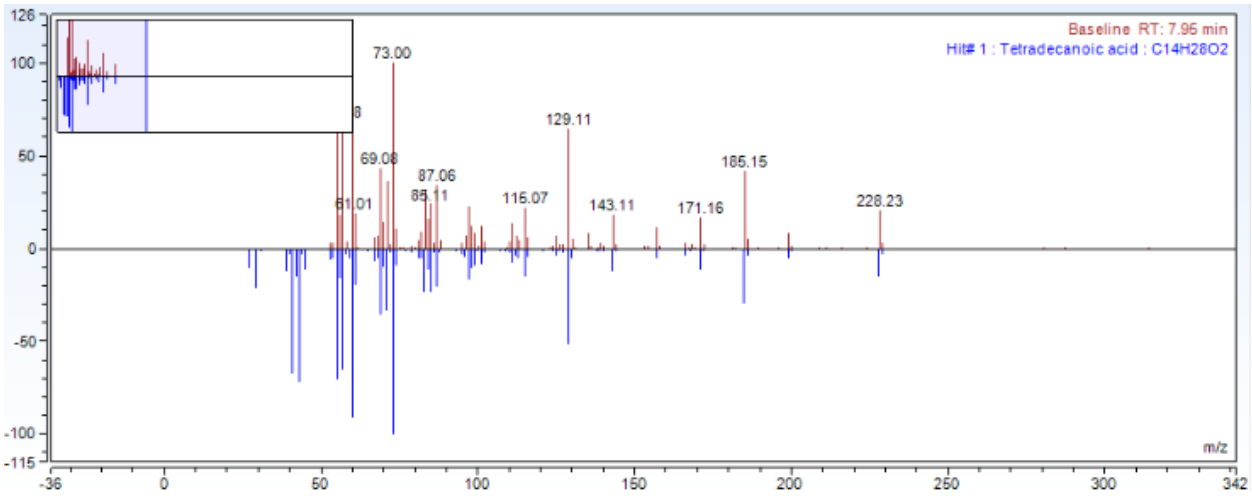
Pentadecanoic acid

Pentadecanoic acid was found in fraction F2 of sweet clover extract at a retention time of 8.42 minutes and a probability of match of 86.4%.



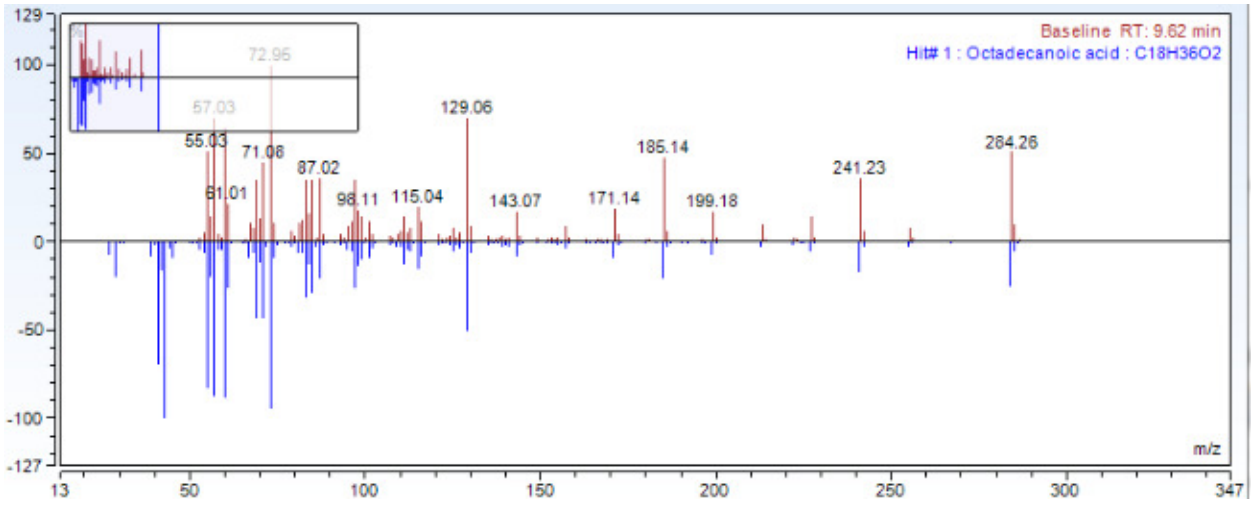
Tetradecanoic acid

Tetradecanoic acid was found in fraction F9 of flax extract at a retention time of 7.96 minutes and a probability of match of 84.1%.



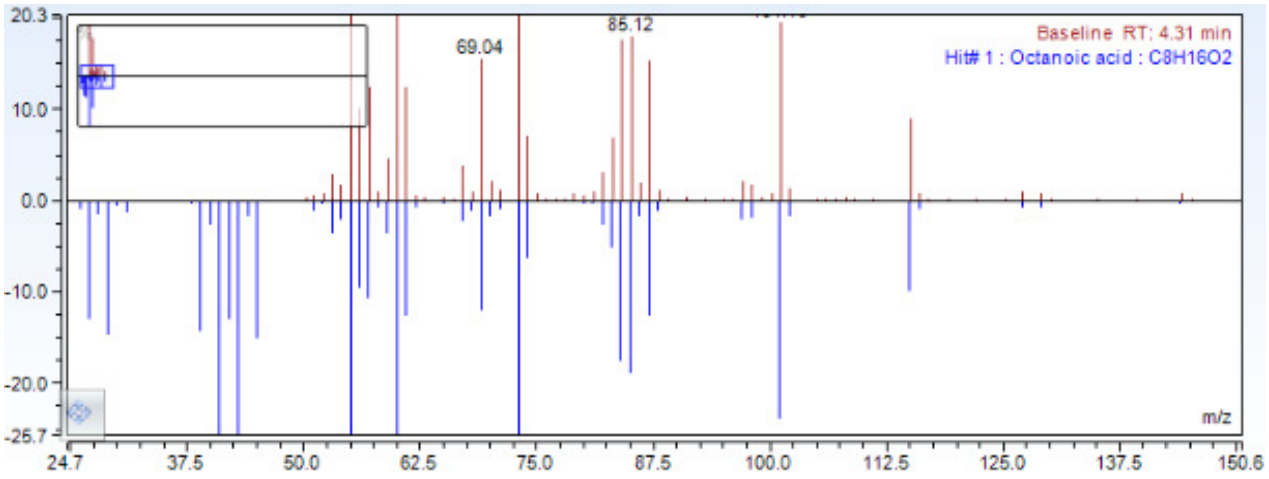
Octadecanoic acid

Octadecanoic acid was found in fractions F2 and F7 of flax extract at the same retention time of 9.62 minutes and a probability of match of 80.6% and 80.9%, respectively. The spectral comparison for fraction F7 is shown below.



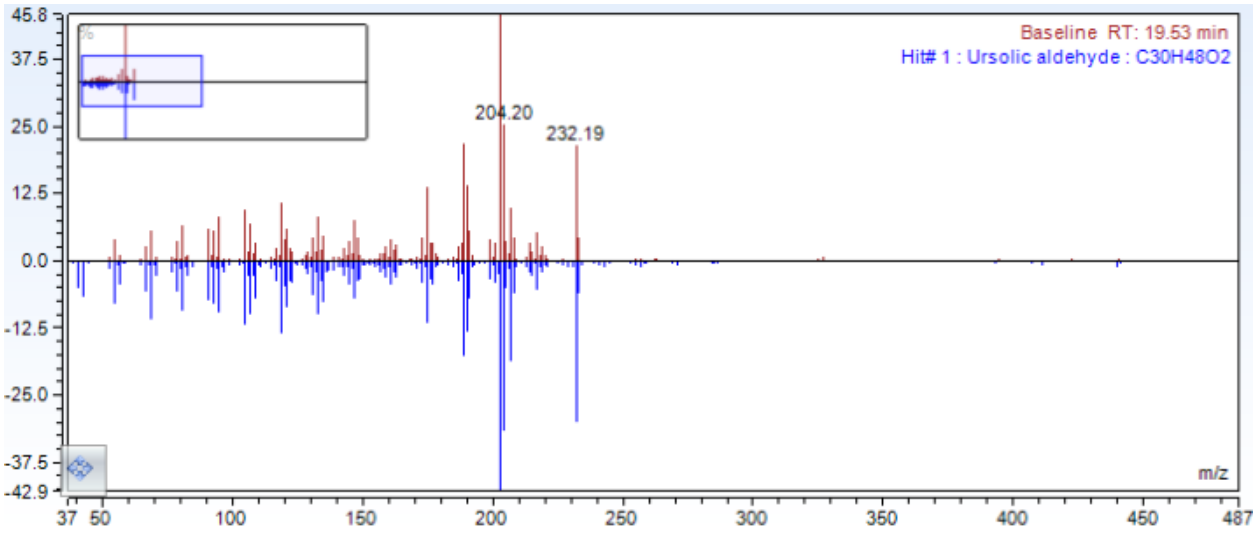
Octanoic acid

Octanoic acid was found in fraction F4 of hemp extract and F3 of sweet clover extract at the retention times of 4.31 and 4.34 minutes, and probability of match of 92.5% and 91.6%, respectively. The spectral comparison for fraction F4 of hemp extract is shown below.



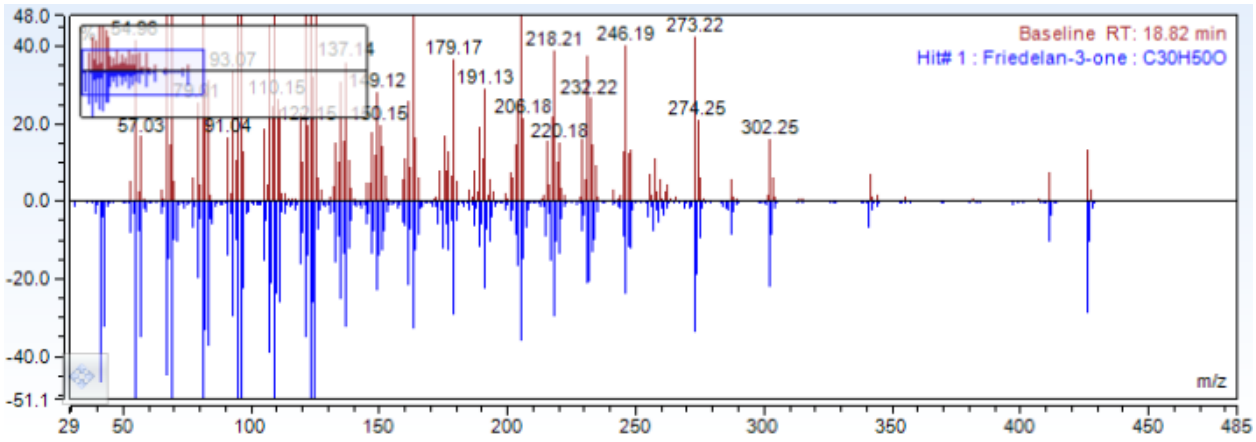
Ursolic Aldehyde

Ursolic Aldehyde was found in fraction F6 of sweet clover extract at a retention time of 19.53 minutes and a probability of match of 90.4%.



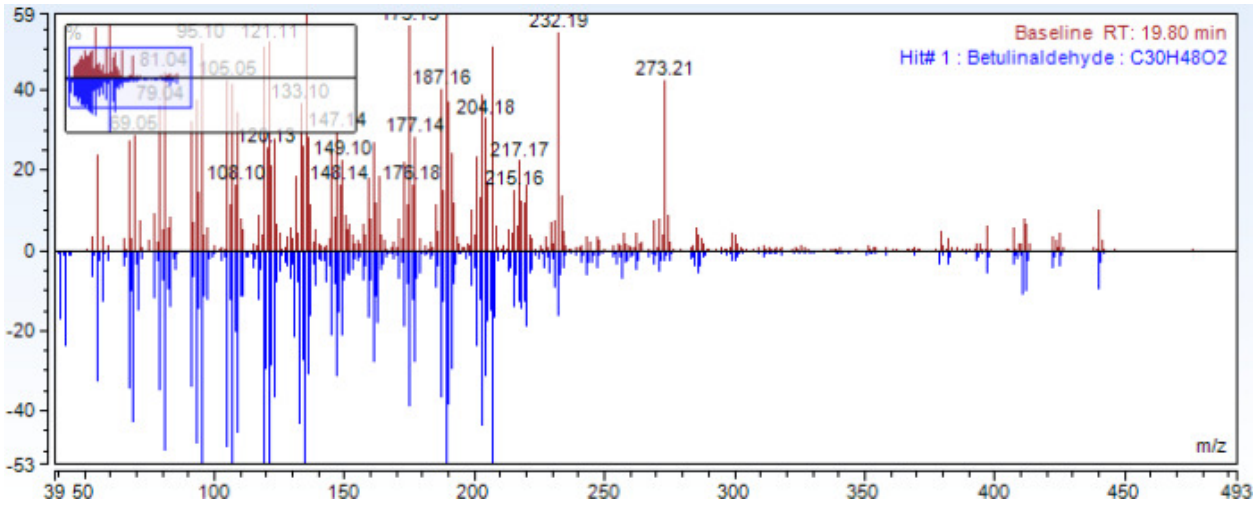
Friedelan-3-one

Friedelan-3-one was found in fraction F4 of hemp extract at a retention time of 18.82 minutes and a probability of match of 88.3%.



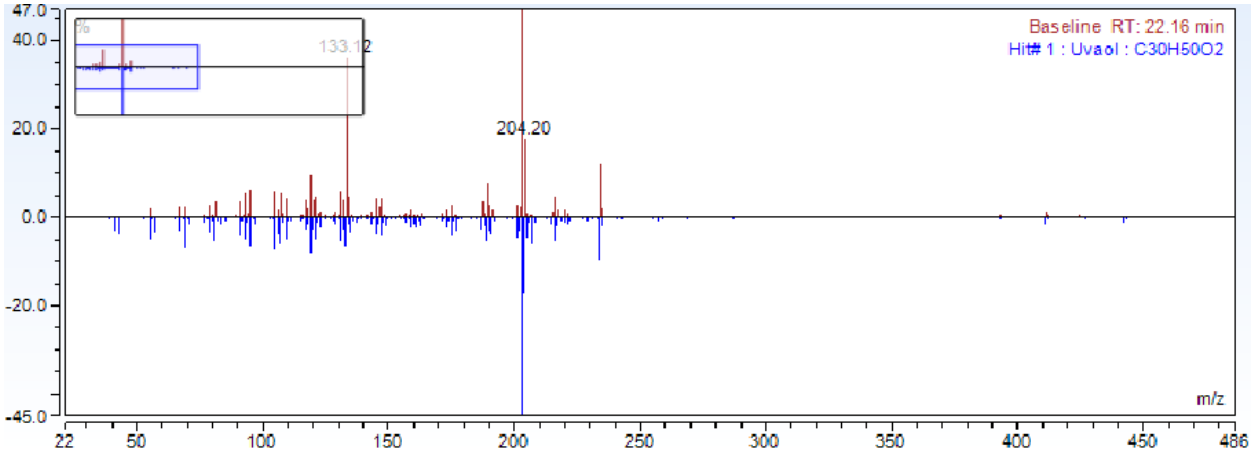
Betulinaldehyde

Betulinaldehyde was found in fraction F6 of sweet clover extract at a retention time of 19.80 minutes and a probability of match of 81.1%.



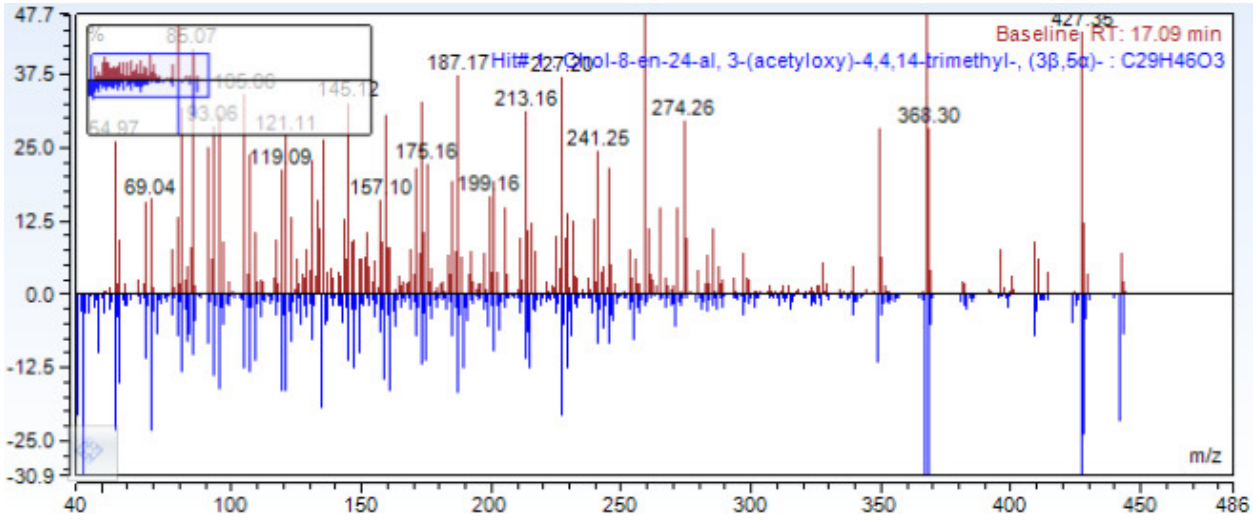
Uvaol

Uvaol was found in fraction F6 of sweet clover extract at a retention time of 22.16 minutes and a probability of match of 86.3%.



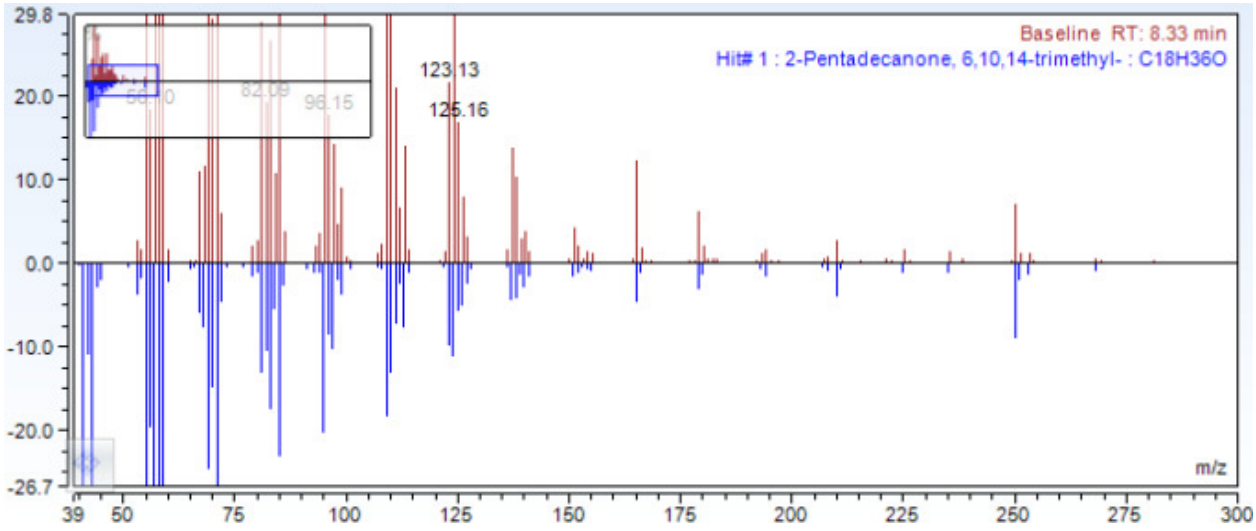
Chol-8-en-24-al, 3-(acetyloxy)-4,4,14-trimethyl-, (3β,5α)-

This compound was found in fraction F4 of hemp extract at a retention time of 17.09 minutes and a probability of match of 85.5%.



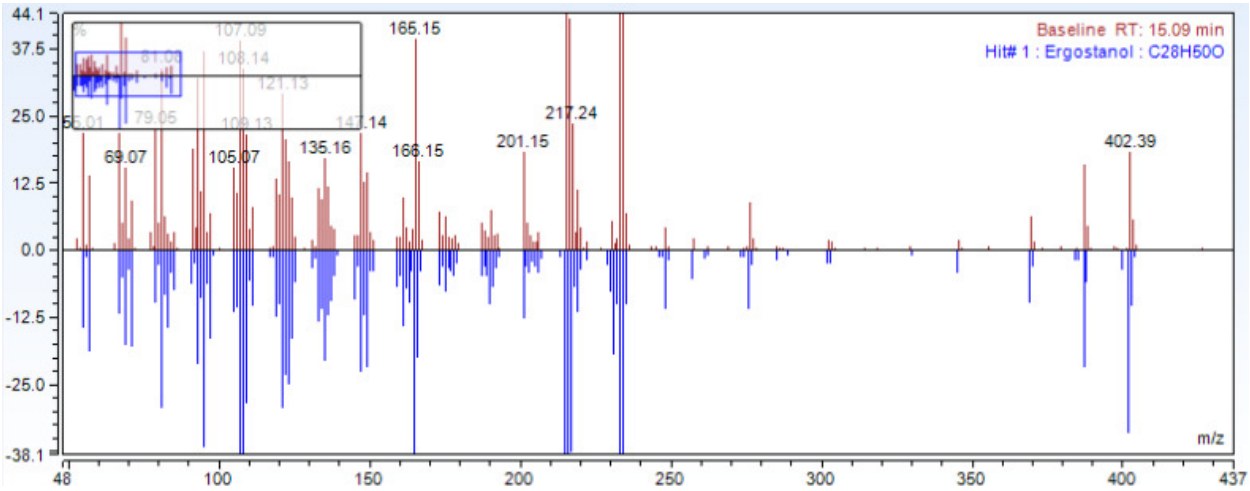
2-Pentadecanone, 6,10,14-trimethyl-

6,10,14-trimethyl-2-Pentadecanone was found in fraction F4 of hemp extract and F2 of flax extract at the retention times of 8.33 and 8.34 minutes, and probability of match of 86.9% and 84.5%, respectively. The spectral comparison for fraction F4 of hemp extract is shown below.



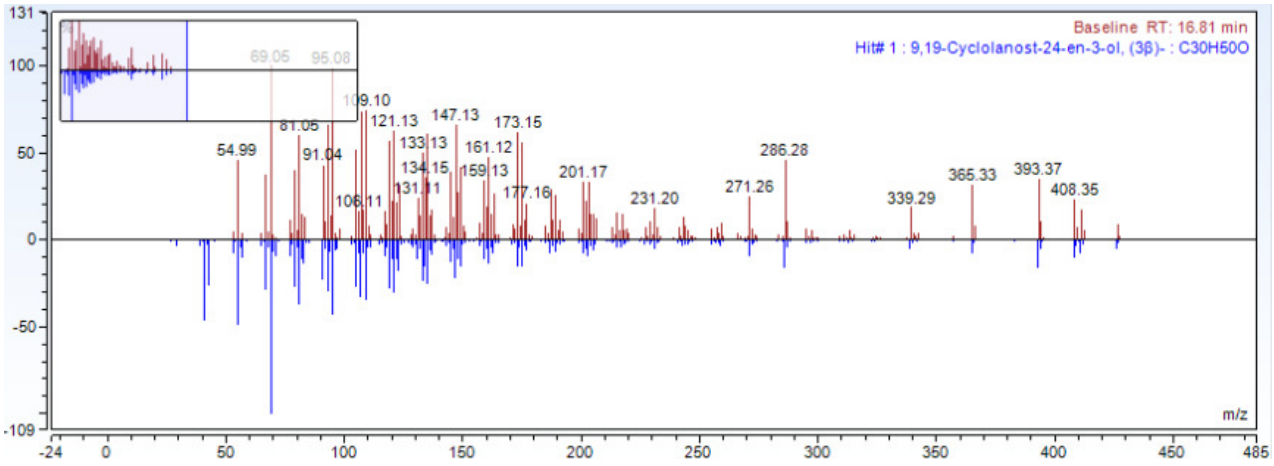
Ergostanol

Ergostanol was found in fraction F4 of hemp extract, and fractions F3 and F7 of flax extract. The spectral comparison for fraction F3 of flax extract at a retention time of 15.09 minute and a probability of hit of 88.2% is shown below.



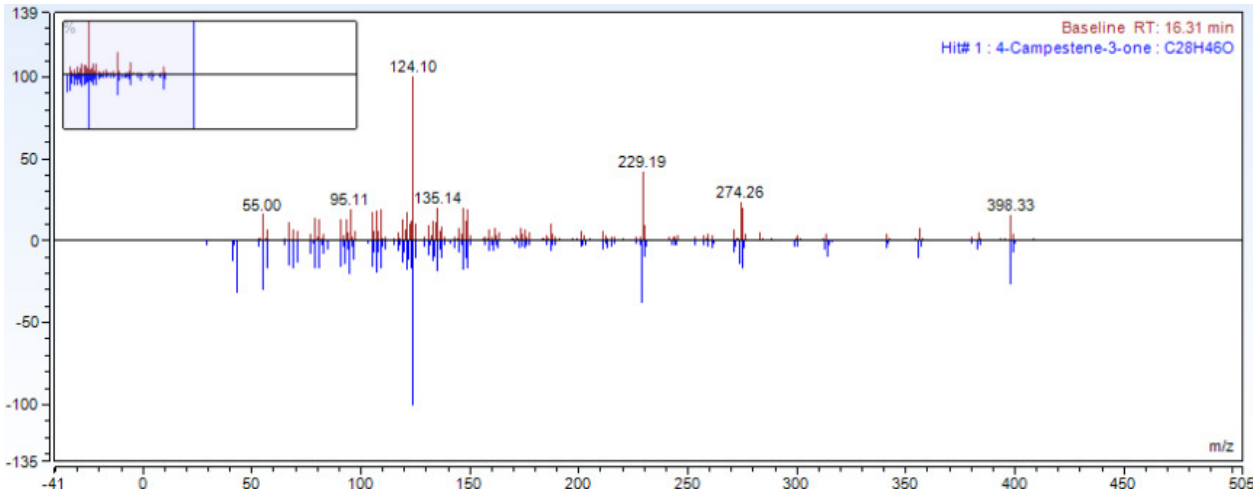
9,19-Cyclolanost-24-en-3-ol,(3β)

This compound was found in fractions F7 and F11 of flax extract at the retention times of 16.81 and 16.9 minutes and probability of hit of 87.2% and 82.8%, respectively. The spectral comparison of fraction F7 is shown below.



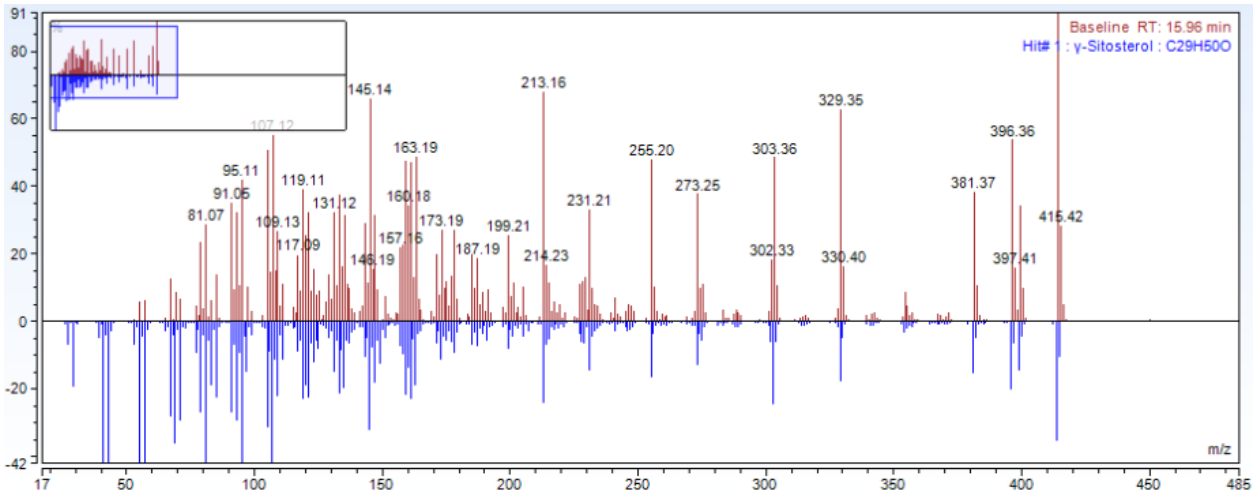
4-Campestene-3-one

4-Campestene-3-one was found in fraction F4 of hemp extract and F7 of flax extract at the retention times of 16.28 and 16.31 minutes, and probability of match of 88.4% and 90.6%, respectively. The spectral comparison for fraction F7 of flax extract is shown below.



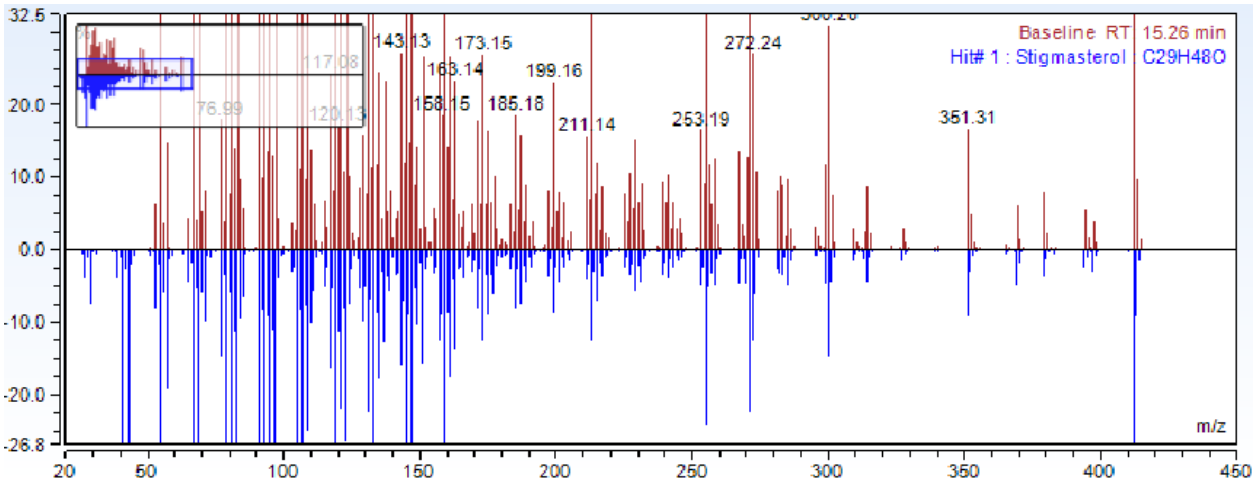
γ-Sitosterol

γ-Sitosterol was found in multiple fractions of all four extracts: F7 and F11 of sweet clover; F3, F5, and F9 of flax; F1, F4, and F11 of hemp; and F0 and F2 of canola. The spectral comparison of fraction F2 of canola at a retention time of 15.96 and a probability of hit of 88.3% is shown below.



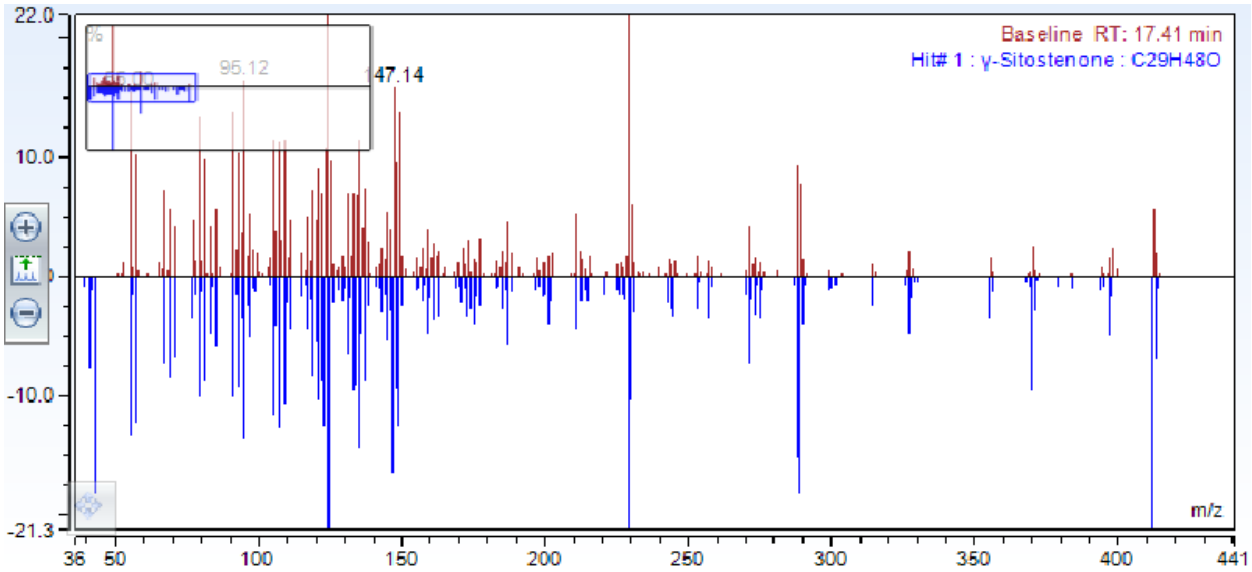
Stigmasterol

Stigmasterol was found in fractions of three extracts: F2 of sweet clover; F3 of flax; and F11 of hemp. The spectral comparison of fraction F2 of sweet clover at a retention time of 15.26 and a probability of hit of 83.0% is shown below.



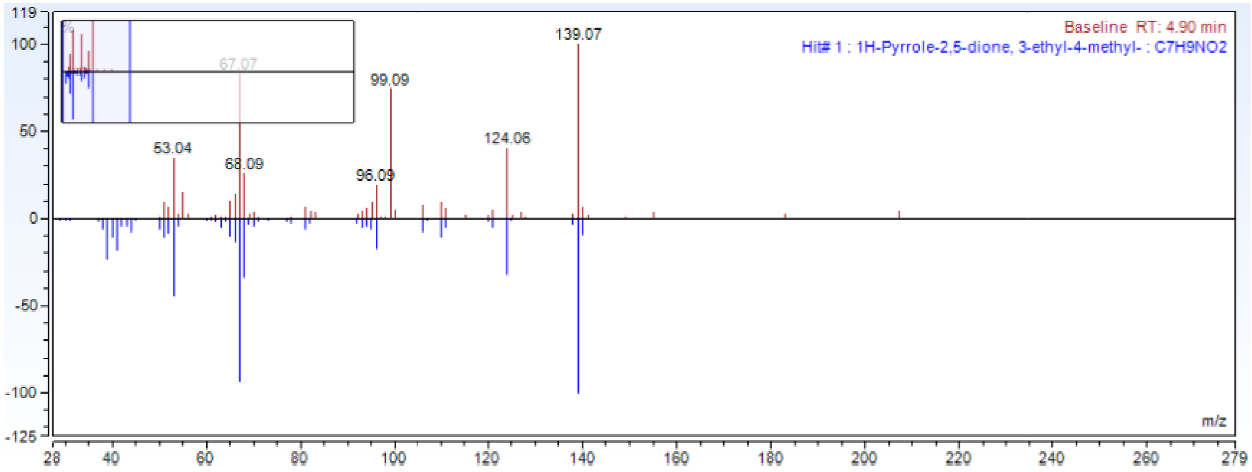
γ-Sitostenone

γ-Sitostenone was found in fractions F3 and F6 of sweet clover and fractions F2 and F7 of flax. The spectral comparison of F3 fraction of sweet clover at a retention time of 17.41 minutes and a probability of hit of 82.3% is shown below.



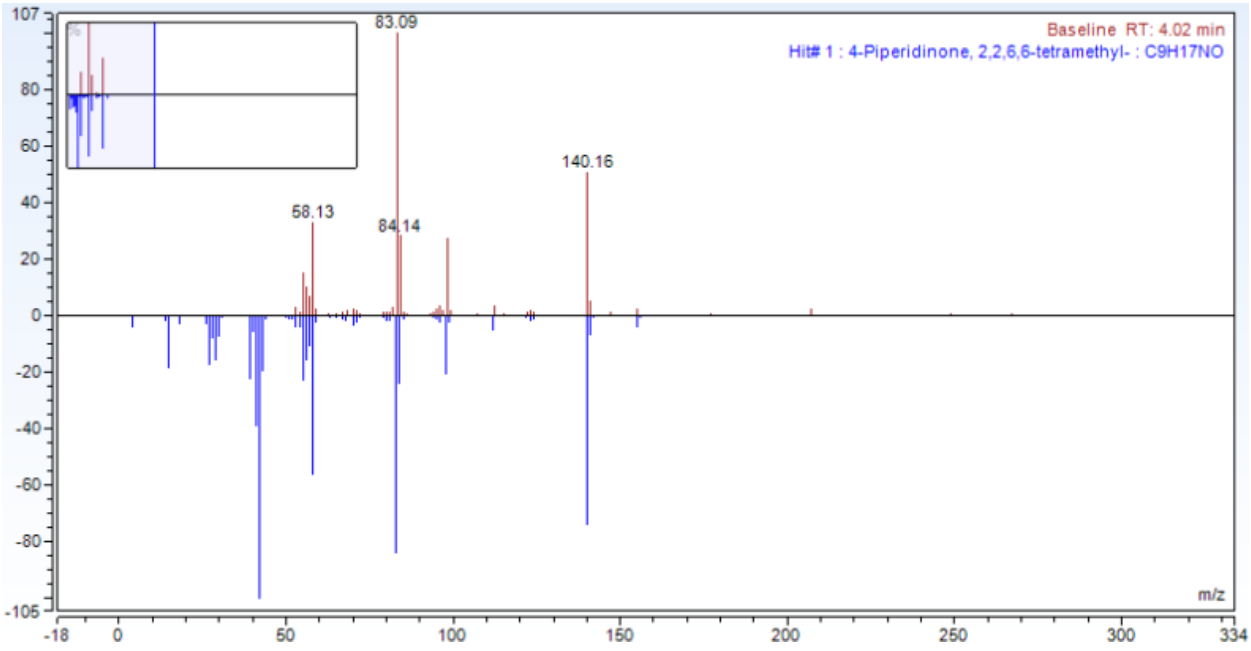
1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl-

This compound was found in fraction F8 of sweet clover extract with a retention time of 4.90 minutes and a probability of match of 89.4%.



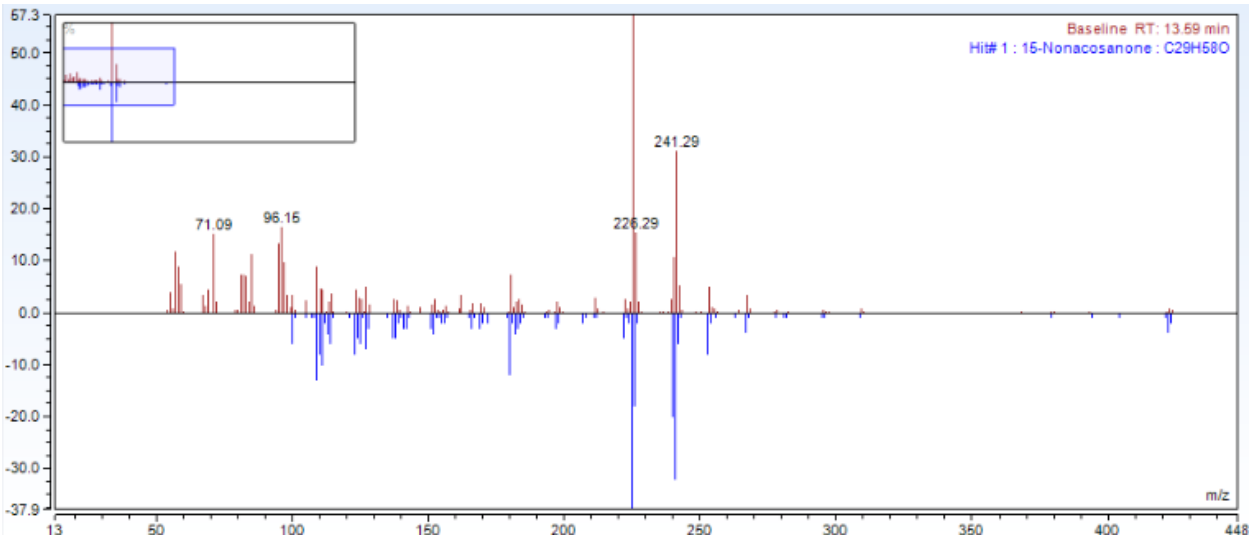
4-piperidinone, 2,2,6,6-tetramethyl-

This compound was found in fraction F11 of canola extract with a retention time of 4.02 minutes and a probability of match of 91.6%.



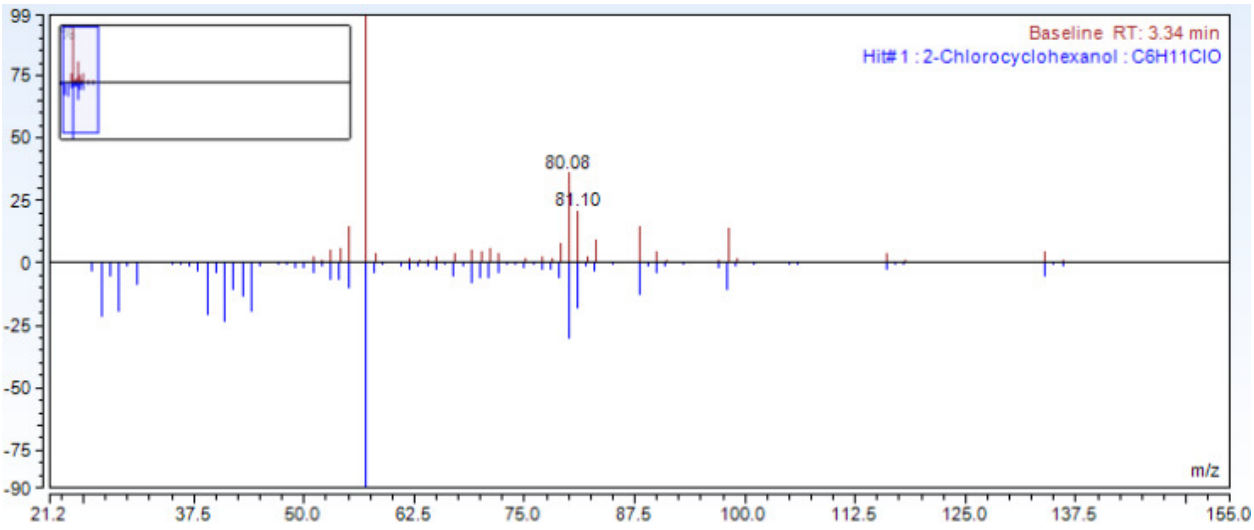
15-Nonacosanone

15-Nonacosanone was found in fractions: F0 and F11 of hemp, and F2 and F5 of canola. The spectral comparison of F2 fraction of canola at retention time of 13.59 minutes and a probability of hit of 87.6% is shown below.



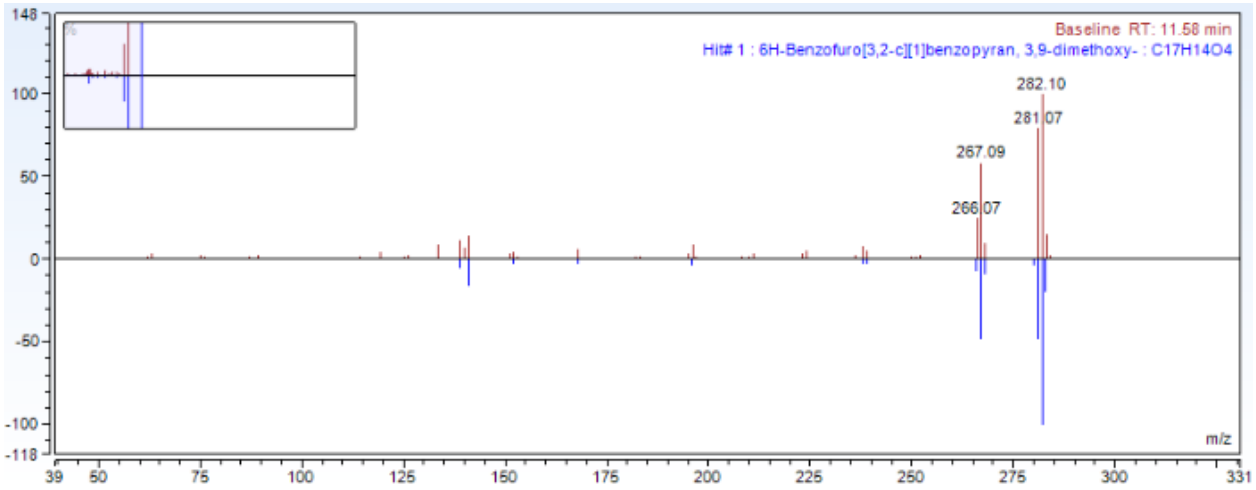
2-Chlorocyclohexanol

2-Chlorocyclohexanol was found in fraction F8 of sweet clover at a retention time of 3.34 minutes and a probability of match of 92.1%.



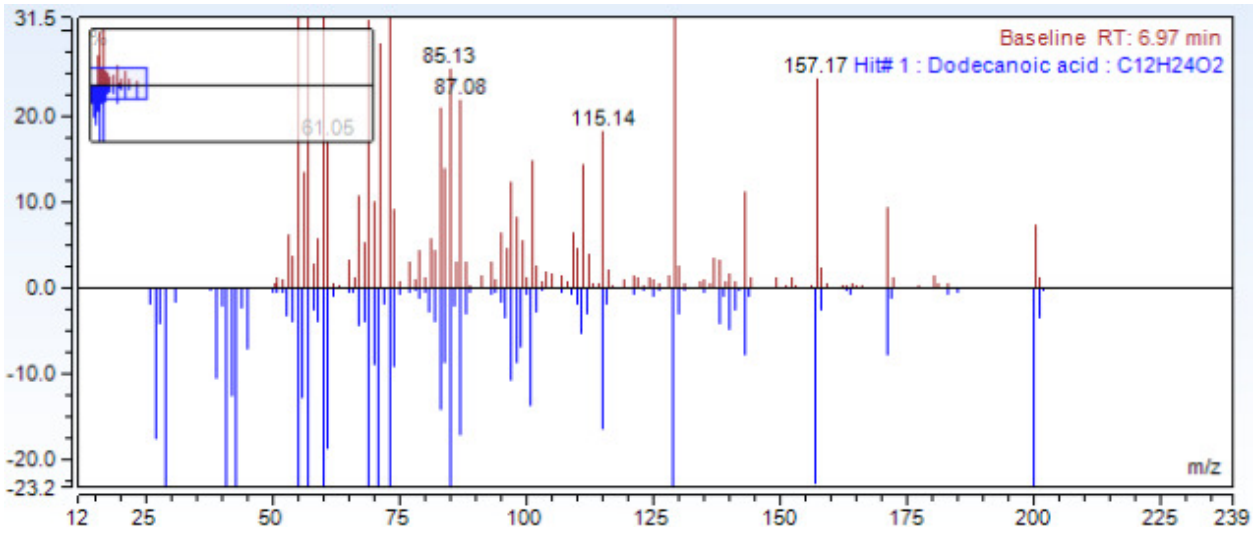
6H-benzofuro[3,2-c][1]benzopyran, 3,9-dimethoxy

This compound was found in fraction F11 of sweet clover extract at a retention time of 11.58 minutes and probability of match of 82.7%.



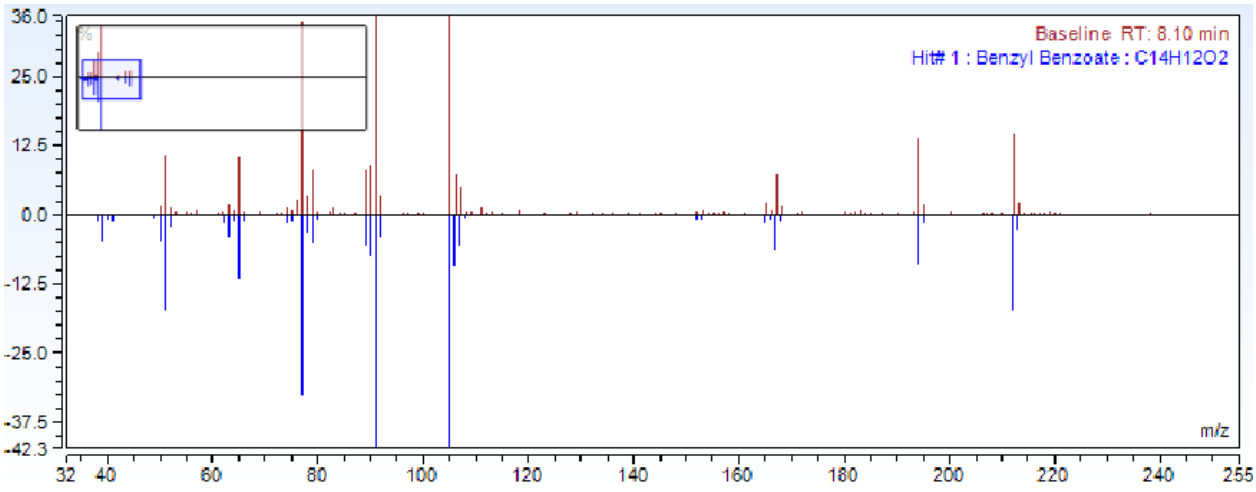
Dodecanoic acid

Dodecanoic acid was found in fraction F0 of sweet clover extract at a retention time of 6.97 minutes and a probability of match of 82.7%.



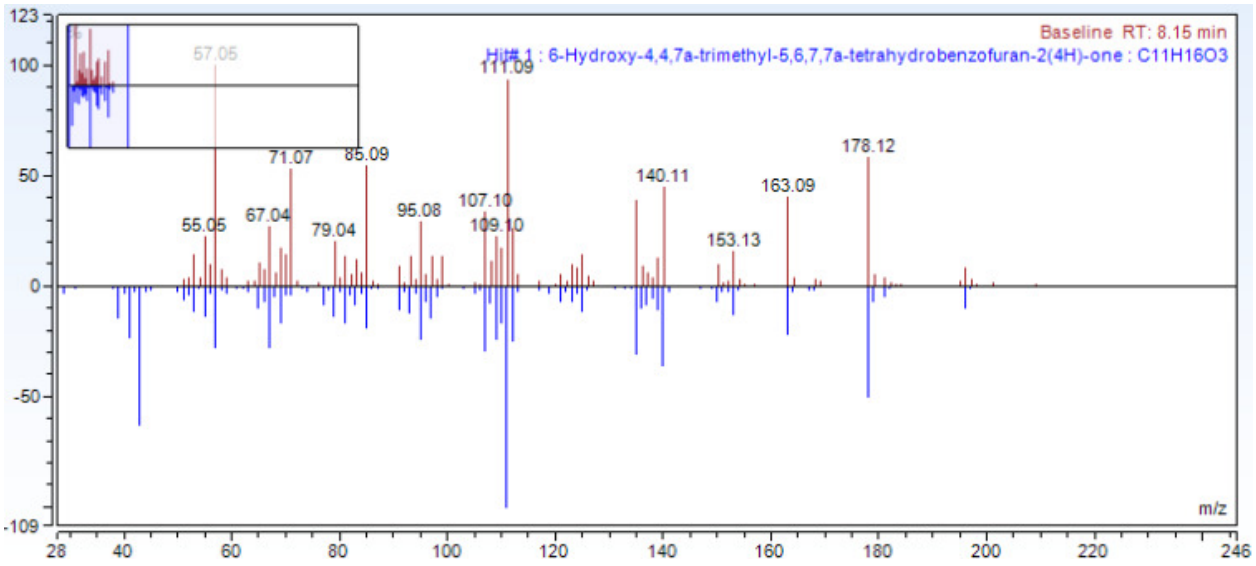
Benzyl benzoate

Benzyl benzoate was found in fraction F8 of hemp and fraction F6 of canola extract at the same retention time of 8.10 minutes, and a probability of match of 87.6% and 94.5%, respectively. The spectral comparison of fraction F8 of hemp is shown below.



6-Hydroxy-4,4,7a-trimethyl-5,6,7,7a-tetrahydrobenzofuran-2(4H)-one

This compound was found in fraction F11 of flax extract at a retention time of 8.15 minutes and a probability of match of 91.8%.



Cyclooctasiloxane, hexadecamethyl-

This compound was found in fractions F7 of flax and F0 of sweet clover at the same retention time of 7.28 minutes and a probability of match of 87.3% and 84.6%, respectively. The spectral comparison of fraction F7 of flax is shown below.