An- Najah National University

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“Drying and Phytochemical test for Mullein herb”

This project was submitted in partial fulfillment of the requirements for the degree of Bachelor in Chemical Engineering

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Abstract

Mullein is one member of the Scrophulariaceae family. Mullein or Verbascum is found in few places in Palestine, but it is not known well in folk medicine in Palestine.

It has been used medicinally since ancient times in Europe; the popularity of common mullein has been increasing commercially for the past few years. Today, the dried leaves and flowers swallow capsules, alcohol extracts and the flower oil of this plant can easily be found in health stores in the West. The use of common mullein extracts in folk medicine begun recently to be supported by an increasing number of research studies. The leaves, flowers, and whole aerial parts of Verbascum plants have been widely used for the treatment of respiratory and inflammatory disorders.

The objectives of the project were to introduce people to the importance of the plant and its medicinal uses, two drying experiments at different temperatures were conducted, a phytochemical screening of the methanolic extracts was performed and the DPPH experiments are started but not completed for well-known reasons.

Qualitative analysis has carried out for the ethanolic herb extract. It was tested for the presence of phytochemical constituents like alkaloids, tannins, flavonoids, carbohydrates, phenols, and saponin. These scientific findings strengthen the thoughts that Mullein is really medicinal plant.

DPPH experiments have been started but not completed due to a disease outbreak. Besides that drying experimental data conducted by Dr. Husni Odeh at ambient temperature and at 55-60°C are analyzed by our group. The drying rate equations and drying characteristic curve for Mullein herb are derived and presented. The drying rate R is increased from 0.09 to 2.3 (kg water/(h.m^2) by increasing the temperature of about 40°C. So that R55-60=25 R20-22. The drying characteristic curve of Mullein has the same shape and tendency to other medical herbs presented in the literature.

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Chapter One: Introduction

1.1Background

Medicinal plants play a crucial role in industries and health care programs, in both developing and developed countries (Ghasemi and Lorigooini, 2016). This is mainly due to the population believes that herbal drugs, in comparison to conventional drugs, are with low or no adverse effects (Jamshidi-Kia, 2018).

The utilization of medicinal herbs in pharmaceutical industries is of particular importance (Froutan, 2011). The recognition of the medicinal species is of a particular position in making use of the medicinal herbs. Medicinal herb species constitute a considerable part of Palestine flora. Among these plants, the literature on mullein leaf plant is scarce.

Mullein grows wild on stony ground, in a wasteland, woodland, clearings, and roadsides, which is a genus of about 360 species of flowering plants. Verbascum has been named differently in various regions of the world, harebell, mullein leaf, and velvet plant.

Scrophulariaceae family with 200 genera and 2500 species is one of the largest plant families (Turker and Gurel, 2005). This family scattered in a great many of the world, because of their vast areas of biological activities such as antimicrobial, anti-oxidant, anti-mutagenic, hepatoprotective, healing, and anti-inflammatory particularly in cold and temperate regions. Antioxidants act as a protective agent for human health and reduce the risk of chronic diseases such as cancer and heart disease. Fruits, vegetables, herbs, and other materials such as vitamins C and E are sources of antioxidants (Leaves, 2014).

The plants from the genus Verbascum are rich in flavonoids, provides, saponins, and polysaccharides (Klimek, 1996). The mullein plant species contain ingredients such as provides, Phenylethanoid Glycosides, monoterpene glycosides, neolignan glycosides, flavonoids, steroids, spermine alkaloids, phenolic acids, and fatty acids (Tatli and Akdemir, 2004). In other studies, the existence of various phytochemical ingredients such as mucilage, carotenoids (Sotoodeh, 2016) as well as provide glycosides, phenyl etanoid (Armatu, 2011), and ascorbic acid terpenoids (Riaz, 2013).

We have traced the presence of mullein in Palestine, especially in the north governate in West Bank. This plant is characterized by its growth on the side of the roads.

Figure 1: Mullein Plant (altagardener, 2008).

1.2 Introduction to drying of herbs

Requirements of worldwide progress in the cultivation of medicinal and aromatic herbs are necessary for their processing and safety storage. The need for high-quality herb raw material is increasing. This phenomenon is proven by a number of cultivation results and registration procedures, concerning medicinal and aromatic plant cultivars, recently reported from different countries. In pharmacy, plant raw materials are important sources of new medicines and their substitutes.

Natural medicines of plant origin have a wider therapeutic spectrum, milder action, and less frequent side effects compared with synthetic substances. According to the data of the World Health Organization, about 70000 plant species are currently used for medicinal purposes; about 1000 species are used in the European pharmaceutical industry (Bernáth, 2002).

Preservation of production is a very important problem to be solved by producers of these products. One of the ways of preservation of products is drying. Medicinal plants can be dried in a number of ways: in the open air (shaded from direct sunlight); placed in thin layers on drying frames, wire-screened rooms, or buildings; by the dielectric source as microwave; or infrared devices (Čiplienė, 2015). When possible, temperature and humidity should be controlled to avoid damage to the active chemical constituents. The method and temperature used for drying may have a considerable impact on the quality of the resulting medicinal plant materials.

Chemical changes are the most important in the post-harvest of medicinal herbs that can be influenced by drying. Moreover, drying can promote changes in product appearance (color) and smell, modifying the final quality.

The drying process is characterized by the existence of transport mechanisms such as surface diffusion, pure diffusion, capillary flow, evaporation, thermo-diffusion, etc. Many studies were done to process medical plant drying by small heated air.

The most ancient form of drying is open **sun drying**. This leaves no space for control over the process. Hence **air-drying** came into existence. This method can preserve the

product for a longer time, but unfortunately, the quality of the air-dried product reduces to a much greater extent when compared to the original sample (Khraisheh, 2000).

Compared with other drying techniques; **microwave drying** offers opportunities as uniform energy and high thermal conductivity to the inner sides of the material, space utilization, sanitation, energy-saving, precise process control, and high quality of the finished product. It also reduces the drying time (Ratti, 2001).

Vacuum drying is the best method for the removal of water from the herb samples compared to other methods (Feng and Tang, 1998). Vacuum drying is a drying technique that is used for the drying of various products; retaining color and vitamin content (Maskan, 2000) Better product quality can be retained by high-degree vacuum treatment (Doymaz, 2004). The key benefits of vacuum drying include lower process temperatures, less energy usage, and hence greater energy efficiency, improved drying rates, and in some cases, less shrinkage of the product, although the cost of the process is high (Togrul and Pehlivan, 2002).

Another method called **freeze-drying** has become an increasingly important preservation technology for high heat- sensitive and delicate biological products. Freeze drying is a method of dehydrating materials by sublimation under vacuum. This process is known for its ability to sustain product quality during low-temperature drying with a minimum loss of flavor, aroma, and valuable components as well as negligible shrinkage, etc. (Ozdemir and Devres, 1999).

Drying processes effect (partially or totally) the quality of the product. Various changes in physical, chemical, and/or biological characteristics of samples occur. These changes alter the physical aspect such as color and structure. Undesirable reactions cause degradation of aroma compounds and nutritional substances (Carroll and Churchill, 1986). These changes cause a reduction in product quality (Chirifeand and Buera, 1995). The loss of volatiles in herbs during drying depends mainly on drying conditions and the biological characteristics of the herb. Some volatile compounds evaporate during drying, while others are partially retained. Some compounds arise as oxidation products during drying. The choice of the right drying technique directly affects product quality.

1.3 Project statement

In recent days, there are many diseases and health problems that need to be treated, a major part of people resorts to the use of chemically synthesized medicines. To most of their problems, they find the answer in using medicinal natural herbs.

The main idea of the project was to carry out DPPH tests. The emphasis of the presence of anti-cancer phytochemicals is done by conductions of DPPH tests. In parallel to this, qualitative phytochemical tests for the presence of different chemical families are carried out. The drying characterization of this plant is presented as well.

1.4 Objective

The objectives of the Mullein project were, first, to produce a medicinal extract of leaves and flowers of Mullein, These extracts have a strong healing effect on the respiratory and pulmonary system. The test of antioxidants and the presence of the active substances will indicate that Mullein has an anti-cancer effect. After that, it is important to provide people with information about this plant. This herb is unknown in Palestine folk medicine. Any scientific finding will encourage the people to apply it as alternative natural medicine.

Analytically, it was important to make qualitative tests for phytochemicals in plants. These phytochemicals have strong healing effects. To use this medicine at any time drying and grinding is a pre-request issue. Therefore, the knowledge and finding out the drying characteristic curves and equations at different temperatures is important.

1.5 Significance of the work

Despite the widespread use of mullein in Asian and European countries because of its medical benefits, but it is still unknown in Palestine, The outcomes of this work will familiarize this plant because of its medicinal benefits and it is available.

Mullein has many therapeutic and aesthetic properties as it is used to treat wounds, eczema, and burns and also contains compounds and elements that act as antioxidants and anti-inflammatory and antimicrobial.

We can produce many products from this plant such as ear drops syrup ointment and medicine pills.

The conduction of qualitative phytochemical tests and drying characterization of the herb upgrade this work from traditional work to scientific engineering one.

1.6 Organization of the Report

This report consists of eleven main chapters, Chapter One introduces the Mullein herb and clarifies the problems that will tend to be solved. Chapter Two shows the main constraints and standards in this study. Chapter Three shows the previous researches and studies published on this subject and included a literature review of the topics related to Mullein herb, general information about it, and medicinal values. Chapter Four includes a brief methodology of our work, summarizes what has been done. Chapter Five includes experimental work. Chapter Six shows the results and analysis. Chapters Seven and Eight are discussion and conclusion. Chapter Nine is a recommendation. Finally, Chapters Ten and Eleven are the references and appendices that contain pictures.

Chapter Two: Constraints, Standards

There are many challenges encountered during the study and during the experimental work. Study difficulties include choosing the safest and most appropriate extraction method within standard conditions at a temperature of less than 60°C. Choosing proper extraction solvent is another challenge, methanol, for example, is a toxic primary alcohol that causes vomiting, blindness, and can be fatal in a small amount. Although it has been reported that methanol extraction has the highest oxidation resistance among all solvents used, there should be awareness in dealing with this type of extraction.

The outbreak of COVID 19 on the first days of March is one of the challenges encountered, that halted the experimentation works in our labs' university. This directed the work towards the theoretical characterization of drying experiments provided by the supervisor of the project.

The lack of chemicals needed to continue phytochemical constituent's tests such as alkaloids, tannins, flavonoids, carbohydrates, phenols, and saponins is another limitation in this project.

Chapter Three: Literature review

3.1 Mullein General Uses

There are many uses for mullein, and it is not only the leaves but also the seeds and flowers that can aid in your herbal healing. for instance: Prepare tea from dried leaves, extract the essential oil from these leaves, mix crushed leaves into a smoking blend, The leaves and flowers can be made into a tincture (Muhammad Riaza, 2013).

Figure2: Mullein Tea (altagardener, 2008).

3.2 Mullein medical uses

There are many impressive benefits to using mullein leaves, particularly for respiratory ailments, cardiovascular health, and various infections, such as Bursitis, ear infection, disinfectant, respiratory health, chest infections, Wounds, Inflammation, and Stomach upset (Turker and Camper 2002).

In the United States, common mullein extracts used in folk medicine. These include mullein products found in health stores such as dried leaves and flowers, swallow capsules, alcohol extracts, and the flower oil of the mullein plant. These study shows the antibiotic activity of common mullein leaves and demonstrated that a methanol extract of leaves had antibacterial activity against Escherichia coli, Mycobacteria phlei, and Staphylococcus aureus methicillin-resistant (Turker and Gurel, 2005).

The leaf of the mullein plant has antibacterial properties in both Gram-positive and Gram-negative bacteria such as Klebsiella pneumonia, E. coli, Staphylococcus aureus. The leaves have large medical value so it is available as tea, extract, oil, powder, capsule, and elixir.

The syrup was prepared by the extracts from the species mullein plant in Iran's Golden Plant Company, also, an herbal tea made of Mullein leaves is effective on the improvement of ulcers and the leaf powder of the plant is sprinkled on the wound for its curative traits (Jamshidi, 2019). Also, the dried and natural forms of the leaf or flower are used to make creams (Ghoshal, 2020).

Mullein has been used in clinical trials only as a topical cream for reducing the episiotomy pain in primiparous women (Taleb, 2016)

Applications topical ointments obtained from the extract of V. inulifolium [0.5% and 1% (w / w)] cause a marked acceleration of wound healing in wound models, non-diabetic skin and diabetics incisional and excisional skin wound (Ozay, 2019).

Mullein oil is extracted from the leaves of the plant. The oil is used as a treatment for ear pain, eczema and some other skin conditions (Ghoshal, 2020).

Experiments were carried out on children ages 5 to 18 with an ear infection. They were given antibiotics or herbal drops with or without a local anesthetic. Herbal drops have been found to reduce pain. It is less expensive than antibiotics and does not have any side effects (Ghoshal, 2020).

Figure3: Mullein Flowers (altagardener, 2008).

Figure4: Mullein extract for lungs (altagardener, 2008).

3.3 Drying of herbs

The researchers investigated the influence of some process parameters (temperature, sample thickness, layer thickness, airflow rate, etc.). The effect of the used airflow and drying air temperature on the drying kinetics was studied in (Čiplienė, 2015).

Drying is the most common and fundamental method for post-harvest preservation of medicinal plants. Natural drying can be considered only for drying of small quantities. In the case of mass production, the use of technical drying applications is indispensable. For the preservation of active ingredients of plant material low drying temperature is recommended (less than 60°C). It means a long drying duration. Drying represents 30-50 % of total costs in medicinal plant productions. The energy demand for drying represents is a significant cost factor. It is largely due to the high moisture content of the leaves and flowers, to be dried. Different parts of the plant and their drying aspects were considered in (Jelgava and Aboltins, 2016).

For indoor drying, the duration of drying, drying temperature, humidity, and other conditions should be determined on the basis of the plant part concerned (root, leaf, stem, bark, flower, etc.) and volatile natural constituents, such as essential oils.

Plenty of literature deals with the drying mechanism of different various popular medicinal herbs.

The different drying protocols such as air drying, microwave drying, sun drying, combined convective and microwave drying, infrared drying, freeze-drying, etc. are done taking into account many important characteristics such as final moisture content, color, drying time, volatiles, etc.

3.4 Extraction

Extraction is the first step in any study of medicinal plants and the most influential factors affecting the extraction effectiveness process are temperature, solvents, pressure, and time of extraction. (Zhang, 2018). The process of extracting the active substances in mullein was conducted with a solvent methanol (Turker, 2002).

10 gm. dried leaves of V. Thapsus were taken and put in a conical flask (250 ml), then 90 ml of solvent methanol was added. The flasks were covered with aluminum foil and allowed to stand for 3-5 days for extraction. These extracts were filtered through Whatman filter paper no. 1 and evaporated at 40°C using a rotary evaporator. The extracts were collected and weighed (Prakash, 2016).

3.5 Anti-oxidants

Many herbal plants contain antioxidant compounds that protect cells against degenerative effects of Reactive Oxygen Species (ROS) which is a free radical such as singlet oxygen, superoxide, peroxy-, and hydroxy- radicals. The concept of oxidative stress is that, when a balance between ROS production and antioxidant defenses is lost, 'oxidative stress' result which through a series of events deregulate the cellular function and leads to various diseases such as aging, arthritis, asthma, carcinogenesis, diabetes, rheumatism and various neurodegenerative disease.

Antioxidants are substances that neutralize free radicals and their actions. Ascorbic acid is also a part of a normal protecting mechanism. Other non-enzymatic antioxidant includes carotenoids, flavonoids and related polyphenols, alpha-lipoic acid, glutathione (Prior and Schaich, 2005).

3.6 Chemical composition in mullein

The plants from the genus Verbascum are rich in flavonoids, iroides, saponins, and polysaccharides (Kanzaki, 1998). The mullein plant species contain ingredients such as iroides, Phenylethanoid Glycosides, monoterpene glycosides, neolignan glycosides, flavonoids, steroids, spermine, phenolic acids, and fatty acids (Tatli and Akdemir, 2004). In other studies, the existence of various phytochemical ingredients such as mucilage,

carotenoids (Sotoodeh, 2016) as well as iroide glycosides, phenyl etanoid (Armatu, 2011), and ascorbic acid terpenoids (Riaz, 2013) have been reported.

3.7 Chemical relations of compounds and biological effect

Antioxidant activities are due to the existence of various ingredients including phenolic constituents (Kumar, 2010). The ability for controlling the phenols is because of the presence of hydroxyl groups in the molecules of these ingredients. These constituents effectively act as hydrogen donors and are considered strong antioxidants.

Flavonoids, inter alia the phenolic compounds, have the highest antioxidant activity (Turker and Gurel, 2005). Anti-inflammatory and antimicrobial activities are due to the existence of phenolic compounds and flavonoids and Phenylethanoid (Asgary, 2016).

Saponins are biologically active compounds that act as a defensive agents against pathogens and herbivores (Francis, 2002). Studies have shown that these compounds possess antifungal effects, as well.Saponins are highly toxic to the fungi (Wang, 1998).

Alkaloids possess physiological properties such as anti-inflammatory, Alkaloids possess physiological properties such as anti-inflammatory, pain-relieving, and antimicrobial effects. Also, some of the alkaloids are influential on the respiratory system and bronchitis (Turker and Gurel, 2005).

3.8 Toxicity

There are no data on genotoxicity, carcinogenicity, and reproductive, and developmental mullein flower (European Medical Agency, 2009). In (Kalinina, 2017) study, activity and toxicity of some V. nigrum extracts and fractions reported. The V. nigrum extracts demonstrated a low toxicity profile. No effect on mouse behavioral responses and no mortality cases were observed during the 72 h period after the administration of the doses up to 5000 mg/kg (Kalinina, 2014).

In recent days, there are many diseases and health problems that need to be treated, a major part of people resorts to the use of chemically synthesized medicines. To most of their problems, they find the answer in using medicinal natural herbs.

The main idea of the project was to carry out DPPH tests. The emphasizing of the presence of anti-cancer phytochemicals is done by conductions of DPPH tests. In parallel to this, qualitative phytochemical tests for the presence of different chemical families are carried out. The drying characterization of this plant is presented as well.

Chapter Four: Methodology

4.1 Research methodology

In this engineering project the experimental work focused on different areas: drying of Mullein leaves and analytical characterization, extraction of plant with proper solvent, conduction of DPPH test, and qualitative tests for phytochemicals.

4.2Drying of Mullein Leaves

- **Drying the leaves:** The fresh leaves were collected, then washed under running tap water, and then dried periodically on a perforated textile to allow air to reach it from all sides. The drying process was done as quickly as possible to prevent the growth of molds, it is done in an area protected from light to prevent the loss of nutrition values and vitamins and protected from dust and pests to prevent contamination.
- **Milling the dried leaves into powder**: Dry mullein leaves can be ground using a milling machine.
- **Solid-liquid extraction:** Ground leaves were placed in a conical flask (250 ml) with the addition of ethanol solvent, then covered and retained in a shaker for 72 hrs.for the shaking purposes to make an extraction of mullein effective ingredients, as shown in figure X1.
- **Suction Filtration** was used to separate the solution of ethanol and mullein extracts from solid leaves. Then, this solute was stored in a dark glass bottle at a dark place.
- **Separation** of mullein extracts from ethanol solvent was occurred using a rotary evaporator.
- **Rotary evaporator:** The solution was put in a rotate flask to determine the concentration of extracts in the solution, as shown in Figure X2.

4.3 Phytochemical analysis

The plant extract ethanol was tested for the absence or presence of phytochemical constituents like alkaloids, tannins, Phlobatannins, flavonoids, carbohydrates, phenols, saponin, cardiac glycosides, proteins, glycosides, and terpenoids, and will be detailed in Chapter 5.

Chapter Five: Experimental Work

5.1 Drying herbs

5.1.1Materials and methods

The laboratory measurements were carried out at the Laboratory of the chemical engineering department. The drying experiments are carried out on the available fresh leaves of Mullein. The fresh leaves are collected and weighed. Then the measured material samples were placed on plastic perforated sheets of that used in Aluminum window frames.

Two drying experiments are carried out at different temperatures one is at ambient temperature on 20-22ºC. The other drying experiment is carried out at 55-60ºC.

Drying at ambient temperature: during the experiment, the average ambient temperature was around 20- 22 ºC. The dimensions of drying samples are presented at next Table 1. The average relative humidity in the Nablus area is about 60%.

The start parameters of dried herbs are as the following:

Table 1: Parameter of dried mullein leaves.

Temperature °C	Start weight (g)	Drying surface area (cm^2)	Average dimension of drying plate (cm)
20-22	500	900	30x30x3
55-60	200	177	D=15cm , height 2-3 cm

The air temperature and humidity were measured by thermometer and humidity sensor. The moisture content was identified by gravimetric measurement (on balance) in regular time intervals. The samples were weighed on the digital laboratory balance with maximum load weight 500 g and with resolution 0.01 g.

The total drying time was adapted to the need for determination of the final moisture content.

Experimental data of mullein at 20-22^{o}C, shown in table 2.

Table 2: Experimental data of mullein at 22^{o}C.

Time (h)	Weight (g)
0	500
10	403
24	375
30	207
40	142
50	105
60	90
70	80
80	75
90	72
100	74

Drying of Mullein at 55-60ºC

Here the sample is placed on a circular tray on a pan; the diameter of the tray is 15cm, with177cm^2 surface area. The heat is provided by hair drier that placed and hanged on about 25-30cm from a sample. The weight is continuously and directly measured. The temperature is measured by a digital thermometer. The start weight is taken 200g of fresh herb (Marigold flower, Mullein leaves, and Common lavender).

Experimental data of mullein at (55-60)ºC, shown in table 3.

Table 3: Experimental data of mullein at (55-60)ºC.

Time (min)	Weight(g)
0	200
10	163
20	116
30	75
40	55
50	46
60	42
70	38
80	36
90	33
100	32
120	29
140	29.5

5.2 Ethanol extraction of Mullein Leaves

Dried and ground Mullein leaves are extracted with ethanol to prepare ethanolic extract to be used in DPPH experiments and in phytochemical tests as well. The drying and extraction experiments are presented below in detail.

5.2.1 Pre-experiment preparation

At this stage, the sample was prepared as follows:

1. Mullein leaves raw material was collected and dried for 5 days with continuous reciprocating to avoid rotting of raw material.
2. Dried leaves were ground using the milling machine located within the laboratory of operating units in the Faculty of Engineering / Chemical Engineering Department.
3. The mullein bag, plastic was prepared to maintain the sample resulting from grinding and it was equal to 500 grams.

5.2.2 Ethanol extraction

1. 10 grams of dried, ground mullein leaves was collected for extraction.
2. The sample was put in the conical flask (250 ml).
3. 90 ml of 99.9 wt% ethanol solution was added to the sample and covered to avoid Ethanol evaporation.
4. Mixing of ethanol solution and solid material was accomplished for 72 hours, at 40^{o}C and 100 rpm by shaking water bath.
5. Suction Filtration was used to separate the solution of ethanol and mullein extract from solid petals. After that, the solution was stored in a dark glass bottle in a dark place.

5.3 Phytochemical analysis

The ethanolic herb extract was tested for the presence of phytochemicals as follow:

- **Alkaloids**

For the detection of alkaloids, a few drops of Wagner's reagent (Potassium iodide) were added to 2 ml of ethanol extract. The formation of reddish-brown precipitate showed the presence of alkaloids.

- **Tannins**

Ferric chloride test was used for the detection of tannins.

Ferric chloride ($FeCl_3$) solution was mixed with ethanol extract. The formation of blue-green coloration indicated the presence of tannins.

- **Phlobatannins**

In test tubes, the extract was taken, 3ml distilled water was added, and shaken for a few minutes then 1% aqueous hydrochloride (HCl) was added and boiled on water both. The presence of phlorotannins isindicated by the formation of red color.

- **Flavonoids**

For flavonoids detection, ethanol extract was treated with sodium hydroxide (NaOH) solution. red precipitation formation of indicating the presence of flavonoids.

- **Carbohydrates**

For the detection of carbohydrates, 0.5 ml of extract was treated with 0.5 ml of Benedict's regent. The solution was heated for 2 minutes on a water bath. By the formation of reddish-brown precipitate the presence of carbohydrate was confirmed.

- **Phenols**

For phenol detection, 2 ml of ferric chloride ($FeCl_3$) solution was added to 2 ml of ethanol extract in a test tube. Formations of deep bluish green solution showed the presence of phenol.

- **Saponins**

For the detection of saponin, in a test tube, 5 ml of ethanol extract was shaken vigorously. The formation of froth indicated the presence of saponins.

- **Cardiac Glycosides**

For cardiac glycosides detection, 2 ml of extract solution was shaken with 2 ml of glacial acetic acid than added few drops of concentrated sulphuric acid (H_2SO_4) and iron trichloride ($FeCl_3$). The formation of a brown ring indicated the presence of glycosides.

- **Proteins**

For the detection of protein, 1 mL of ethanol extract were treated with 1mL of concentrated nitric acid (HNO_3) solution. The presence of proteins indicated by the formation of yellow color.

5.4 Antioxidant Test (DPPH)

After the ethanolic Mullein extract was prepared, DPPH and stock solution were prepared in order to take the absorbance value from the UV spectrophotometer. In order to measure antioxidant activity, DPPH free radical scavenging assay was used. This assay measures the free radical scavenging capacity of the investigated extracts. DPPH is a molecule containing a stable free radical. In the presence of an antioxidant which can donate an electron to DPPH, the purple color which is typical for free DPPH radical decays, and the change in absorbency at 517 nm is followed spectrophotometrically. This test could provide information on the ability of a compound to donate a hydrogen atom, on the number of electrons a given molecule can donate, and on the mechanism of antioxidant action. The method was carried out as described by (Brand, 1995).

5.4.1 Preparation of DPPH solution (0.004% w/v)

Initially, 0.004% (w/v) DPPH solution was prepared as follows:

4 mg of DPPH was dissolved in 100 ml of methanol then the solution was covered and keep it in the refrigerator.

5.4.2 Preparation of ascorbic acid(standard solution)

4 mg ascorbic acid was dissolved into 5 ml distilled water, so the concentration of the solution is 50µg/ml. This called stock solution then serial dilution was performed in order to prepare different concentration solution (10µg/ml, 20µg/ml, 30µg/ml, 40 µg/ml, 50µg/ml).

5.4.3 Preparation of stock solution

4 mg of extract was dissolved in 10 ml of methanol in order to prepare 50 µg/ml solution and then serial dilution was performed to prepare the required concentration solution.

5.4.4 Preparation of control

- ✓ 3 ml DPPH solution was used. The blank for this solution is ethanol.
- ✓ After the preparation of reagent the values taken from UV spectrophotometer as procedure shows:

1. 2 ml of a methanol solution of plant extract at different concentrations was taken in a test tube.
2. 3 ml of methanol solution of DPPH was added into the test tube.
3. The solution was incubated at room temperature for 30 min in a dark place to complete the reaction.
4. The absorbance of the solution was measured at 517 nm using a spectrophotometer against blank.
5. A typical blank solution contained reagents expect plant extract or standard solution.
6. The percentage (%) inhibition activity was calculated from the following equation.

$$\% \, I = \left\{\frac{(Ao - A1)}{Ao}\right\} \times 100$$

Where Ao : Is the absorbance of the control

A_1 : Is the absorbance of the extract.

7. Then % inhibition was plotted against concentration and form the graph IC_{50} was calculated.

Chapter Six: Results

6.1 Result of phytochemicals screening for V.thapsus

Phytochemicals have therapeutic effects and capability to prevent diseases and give benefits to human health. It is obvious from the results of qualitative phytochemical screening that V. Thapsus is a rich source of biologically active constituents like alkaloids, carbohydrates, saponins, and might be useful in curing various diseases as shown in Table 4.

Table 4: The results of phytochemical screening tests for Mullein leaves.

Component	**Detector**	**Indicator**
Alkaloids	Potassium iodine	Reddish brown precipitate
Tannins	Ferric chloride	Blue green color formation
Phlobatannins	Distilled water + 1% aqueous hydro chloride (HCl)	Red color formation
Flavonoids	Sodium hydroxide (NaOH)	Red precipitation
Carbohydrates	Benedict's regent	Reddish brown precipitate
Phenols	Ferric chloride ($FeCl_3$)	Deep bluish green solution formation
Saponins	Just shaken vigorously	Formation of froth
Proteins	Concentrated nitric acid (HNO_3)	Yellow color formation

Our phytochemical investigation showed the presence of saponins, iridoids, and flavonoids, which may be responsible for the moderate antimicrobial and noteworthy antioxidant activities. And this phytochemical group is a good indication of the presence of antioxidants in the plant, and support with scientific evidence of healing potential.

6.2 Result of DPPH Test

DPPH was prepared with methanol and after it was prepared it was encapsulated with tin foil and was kept in a cool condition. Then the solution was prepared using the extracted solution and methanol, 1 ml of the prepared solution was taken, and also added 3 ml of DPPH solution to each measured sample and mark up each solution with 99% methanol until 10 ml.

After absorbance values are taken, the relationship between concentration and % SCV is plotted as shown in the following Figure 5 and Table 5.

Table 5: DPPH Test Calculation.

Concentration	Absorbance	%SCV	IC50	Log(Conc.)
0	-	-	123.67	-
10	0.214	0.16		1
20	0.206	0.19		1.30
30	0.188	0.26		1.48
40	0.185	0.27		1.60
50	0.238	0.30		1.69
Blank	0.255			

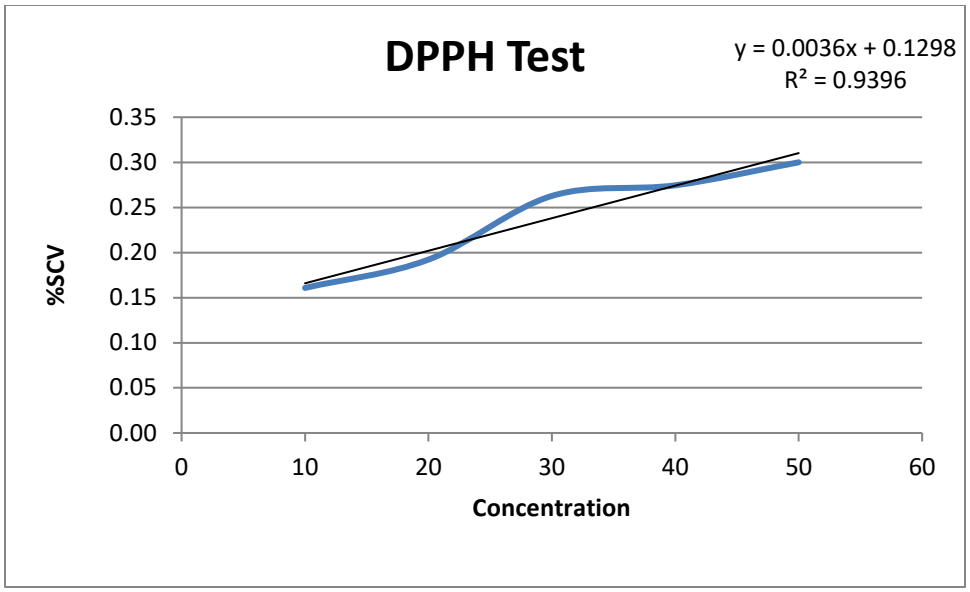

Figure 5: DPPH Test.

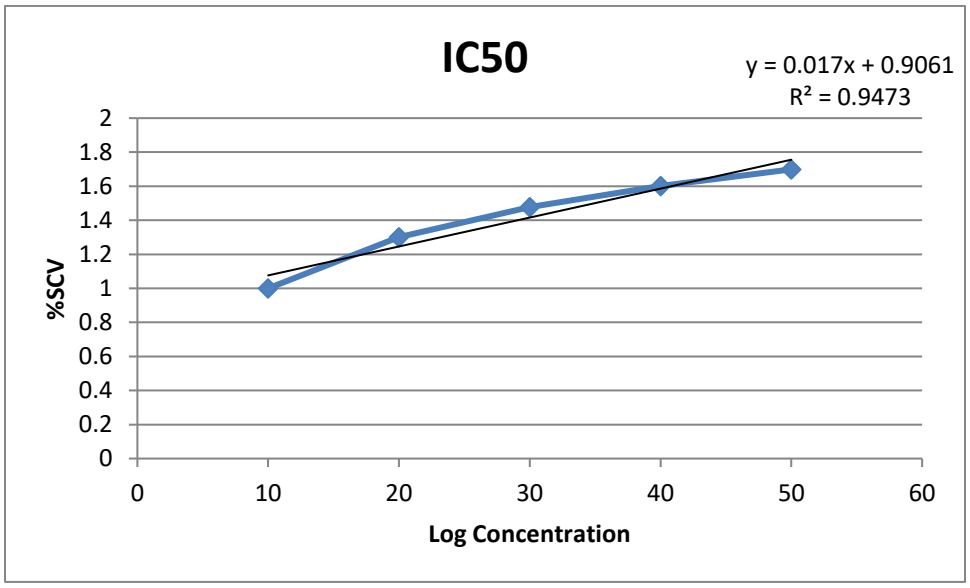

Figure 6: IC50

The methanol extracts of the mullein plant showed a highly effective free radical scavenging in the first DPPH assay. These extracts exhibited a remarkable antioxidant effect at low concentrations.

6.3 Results of Drying

The demonstration of experiment data is shown in different forms as presented in drying literature. This shown in Figures 7 and 8. This presents how given property changes with time.

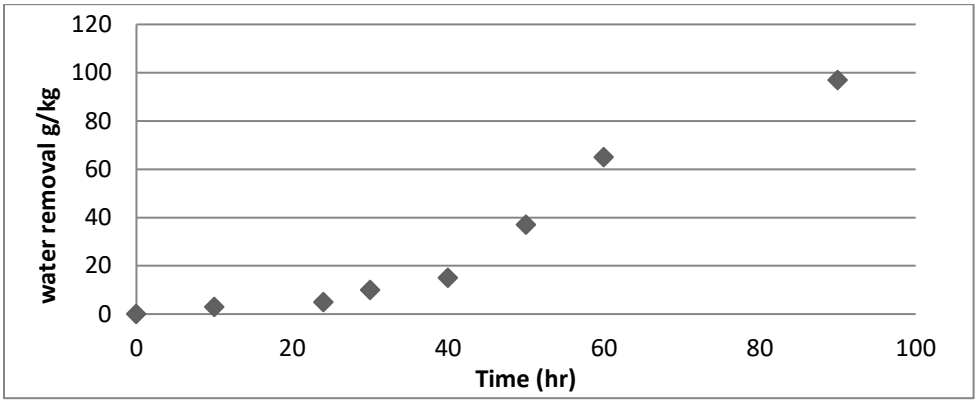

Figure7: Drying Dynamics for Mullein.

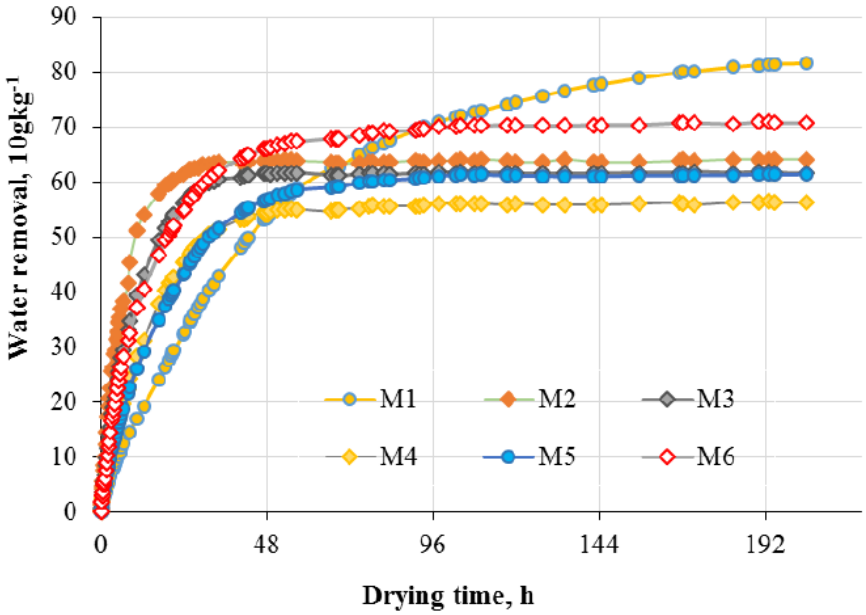

Fig. 4. **Different plant drying dynamics:** M1 – garden marigold; M2 – lemon balm; M3 – origanum; M4 – common agrimony; M5 – common lavender; M6 – common sage

Figure 8: Different Plant Drying Dynamics (Jelgava and Aboltins, 2016).

The tendency of drying curves earned at ambient temperature 20-22ºC is similar to that obtained at 55-60ºC. The only difference is the drying time is reduced for about 2.5-3hr. While the drying time at ambient temperature takes a few days.

The drying rate at 55-60ºC is obviously higher that of ambient temperature at both the constant drying rate period and the falling rate of the drying period. (R at 55-60ºC =2.3kg/hr.m^2 , R at ambient =0.093 kg/hr.m^2).This means that, the drying rate is increased by 25 times with increasing temperature about 40ºC.

The falling rate of drying at 55-60ºCand ambient temperature can be estimated by straight line equation (R=aX+b) for both drying temperature.

- **Sample of calculation for calculation of free moisture X**

Free Water (X):

$$X_{Free\ of\ water} = \frac{W - W_{lowest\ value}}{W_{lowest\ value}}$$

X: Free Water (g_{Water}/g_{dry})

W: Weight of plant

W_1=500 g , $W_{lowest\ value}$=72g

$$X = \frac{500 - 72}{72} = 5.94$$

Table 6: Weight change over time and free water at 22^{o}C.

Mullein		
Time (h)	Weight(g)	X
0	500	5.94
10	403	4.597
24	375	4.208
30	207	1.875
40	142	0.972
50	105	0.458
60	90	0.25
70	80	0.11
80	75	1.042
90	72	0
100	74	0.028

Table 7: Weight change over time and free water (X) at (55-60)^{o}C.

Mullein		
Time (min)	Weight(g)	X
0	200	5.89
10	163	4.62
20	116	3
30	75	1.59
40	55	0.897
50	46	0.586
60	42	0.448
70	38	0.31
80	36	0.24
90	33	0.138
100	32	0.103
120	29	0
140	29.5	0.017

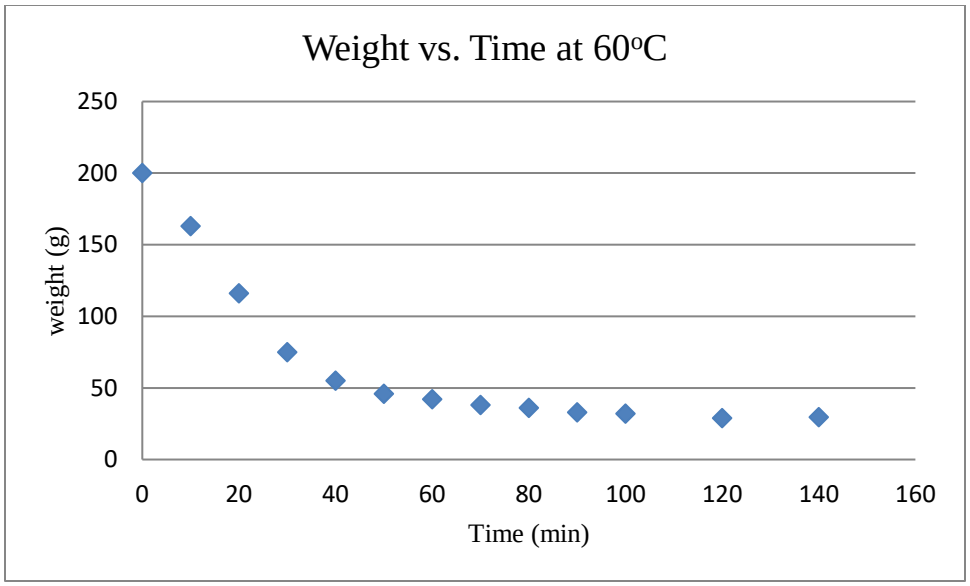

Figure 9: Weight vs. Time at (55-60)°C.

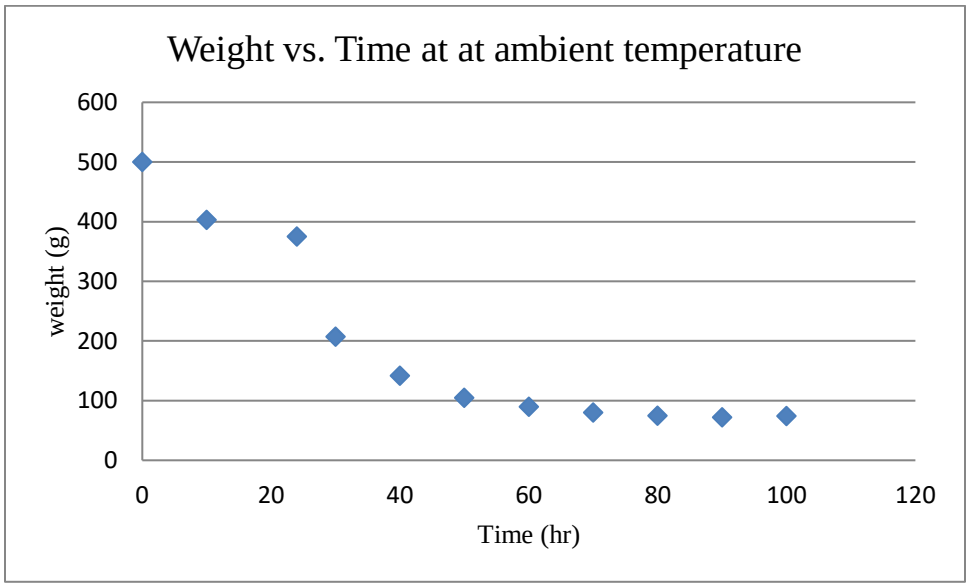

Figure 10: Weight vs. Time at ambient temperature.

Drying rate at ambient temperature

$$R = \frac{L_s}{A} \times \frac{dx}{dt}$$

Where:

R: drying rate in kg/h.m^2.

L_s: kg of dry solid used. , it can be calculated as 72×0.001×0.88 =0.063 kg bone dry solid. Here we assumed that, the herb contains 12% of water at the end of drying.

A: surface area (m^2).Surface area for the 30 × 30cm^2,A =0.3 × 0.3 = 0.09 m^2

$R = 72 \times 0.001 \times \frac{0.88}{0.09} \times \frac{5}{38}$=

R=0.092632 kg/hr.m^2

The below tables (8 and 9) present how free moisture in herb decrease with time at different drying temperatures. While Figure 11 and 13 shows the characteristic curves of drying (X vs. time), the Figures 12 and 14 show the drying rate characteristic curves of leaves of Mullein.

Table 8: Free water at different time at (20-22)^{o}C.

Time(hr.)	X
0	5.94
10	4.597
24	4.208
30	1.875
40	0.972
50	0.458
60	0.25
70	0.111
80	1.0417
90	0
100	0.0278

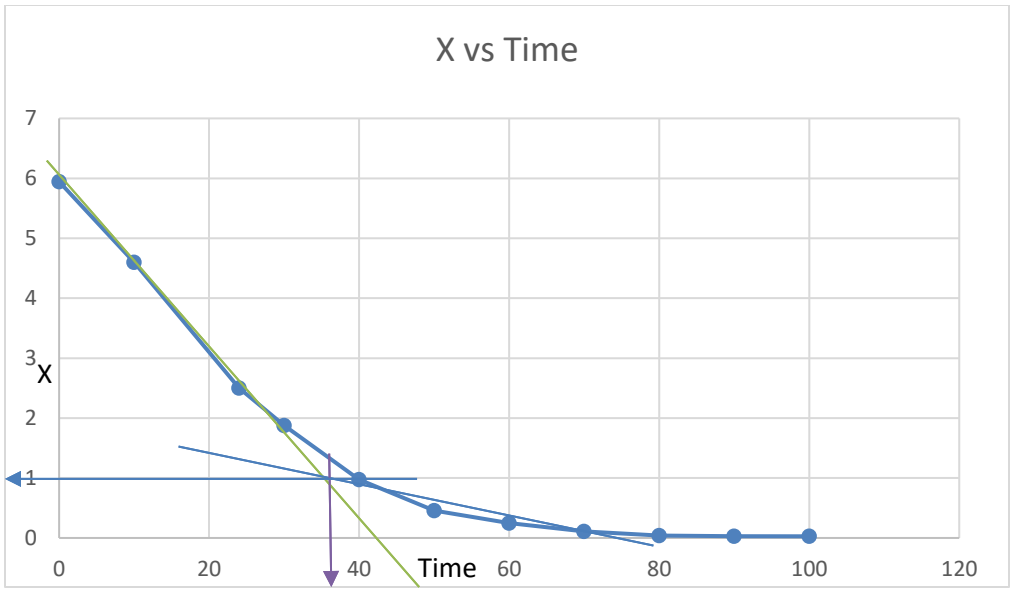

Figure 11: Free water at different time at 60°C.

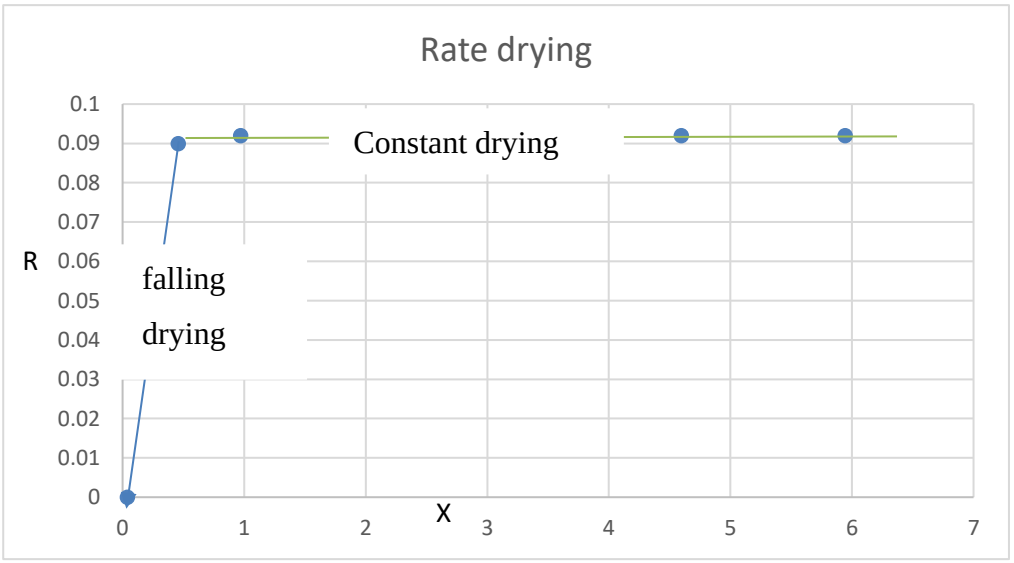

Figure 12: Drying rate vs. free of water at 22°C.

Drying rate at (55-60)C°

Surface area for the 30×30

A $=0.3 \times 0.3 = 0.09\ m^2$

R=2.3kgwater/hr.m^2

Table 9: Free of water at different time at (55-60)C^{o}.

Time (min)	X
0	5.897
10	4.621
20	3
30	1.586
40	0.897
50	0.586
60	0.448
70	0.3103
80	0.2413
90	0.138
100	0.103
120	0
140	0.017

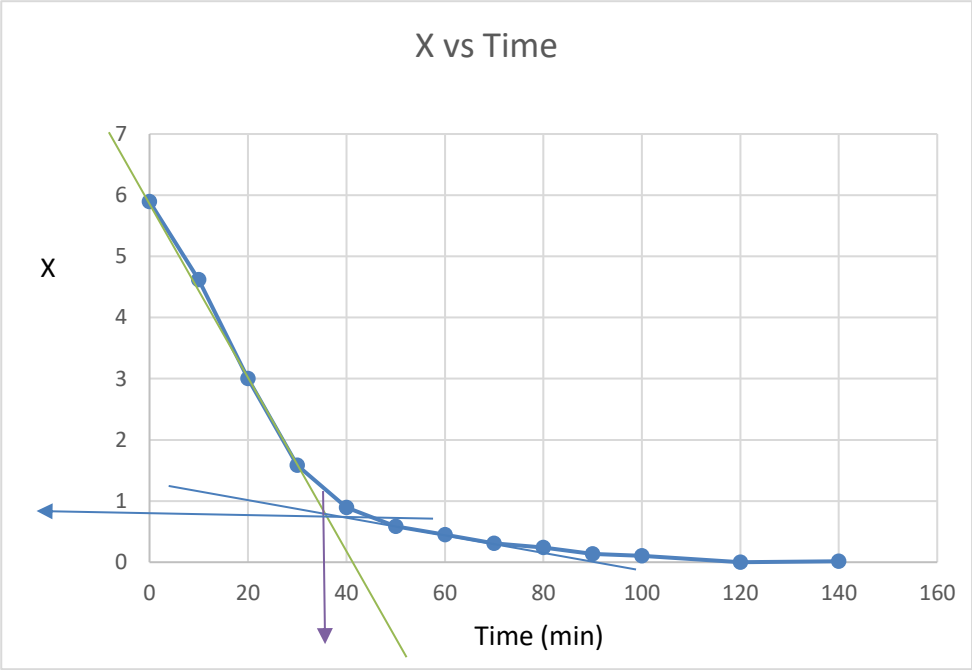

Figure13: Free of water Vs. Time.

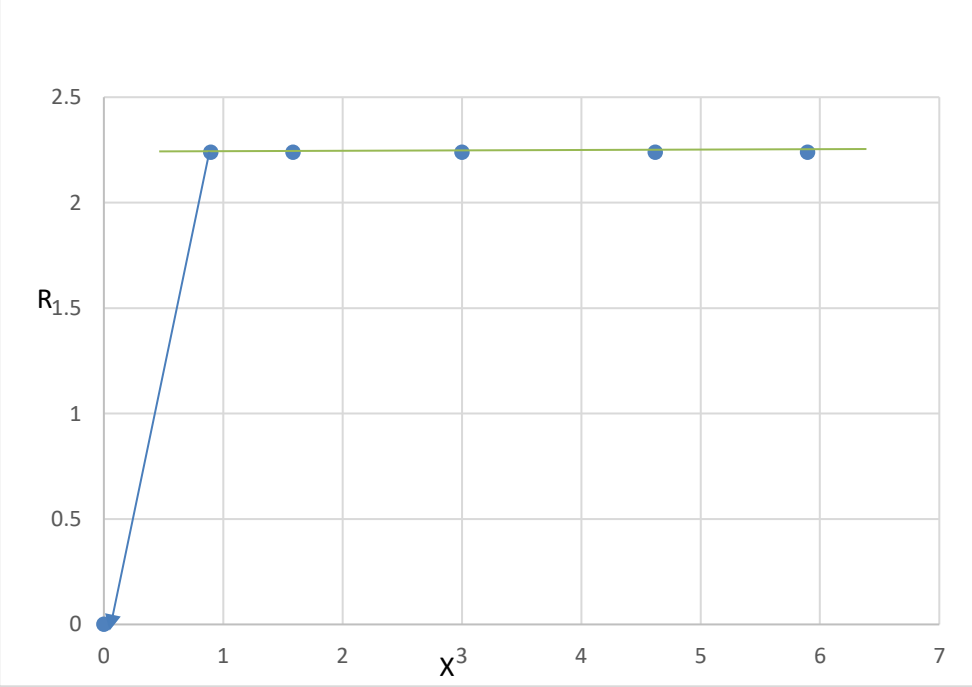

Figure 14: Drying rate vs. free of water at (55-60)^{o}C.

Chapter Seven: Discussion

This project has addressed many of the issues, the first one is the drying process of mullein leaves. This issue is started by collecting the mullein leaves and then washing drying and grinding. This happened under certain conditions for further uses in at extraction and DPPH antioxidant tests.

The extraction process starts with solvent selections which must meet several criteria to obtain high productivity and yield.

- **Type of solvents**

Depending on the Literature survey several types of solvent used and studied on mullein leaves to achieve the safest and the most suitable method of extraction. The most important specifications that should be taken into consideration while choosing the most suitable solvent as following:

- ✓ Non-toxicity.
- ✓ Availability: The solvent should be abundant and available in our country
- ✓ Low cost: It is undesired to have solvents with high costs.
- ✓ Yield: It is defined as the amount of solute extracted by the solvent divided by the mass of the sample. While mullein leaves contain essential Phytochemicals solvent should be effective in the extraction

The screening and scoring method is used to estimate the most suitable solvent to be used in the laboratory for the extraction, as shown in table 9. The positive sign means an advantage or "good", the negative sign means "unsuitable or bad" zero means "same as". The net score for each alternative provides a relative ranking.

Table 9: Comparison of Solvent.

Criterion	Ethanol	Methanol	Chloroform
Toxicity	+	-	-
Flammability	-	-	-
Cost	+	+	-
Availability	+	+	0
Solubility	+	+	+
Sum +	4	3	1
Sum -	1	2	3
Sum 0	0	0	1
Total	3	1	-2

Because chloroform is highly flammable and dangerous to use in such experiments. Therefore, 99.9% by weight of ethanol was chosen for use, providing us with the highest productivity and antioxidant activity.

Antioxidant plays an important role in inhibiting and scavenging free radicals, thus, providing protection to human against infection and degenerative diseases. Now modern research is directed towards “Natural antioxidants” from the herbal plants due to safe therapeutic.

There are various methods for the determination of the antioxidant potential of different biological samples. However, a single method is not suitable for all and there is no shortcut approach to determine antioxidant activity. Amongst all the available methods, the DPPH method has been applied for estimating antioxidant activity in this project.

The method offers the advantages of being rapid, simple, and inexpensive and provides first-hand information on the overall antioxidant capacity of the test system.

At this stage, the extracts were concentrated by evaporating the solvents on a boiling water bath. The dried extract thus obtained was used for the assessment of antioxidant activity through various in vitro models. Preliminary qualitative analysis was carried out to ascertain the presence of flavonoids, tannin, protein, etc.

Noticed that the results of the experiment match the results of the literature, as shown in the table.

Table 10: Comparison of literature and practical experience.

Component (Literature)	**Component (Experimental results)**
Alkaloids	Alkaloids
Tannins	Tannins
Phlobatannins	Phlobatannins
Flavonoids	Flavonoids
Carbohydrates	Carbohydrates
Phenols	Phenols
Saponins	Saponins
Proteins	Proteins

The reduction capability of DPPH radical was determined by the decrease in its absorbance at 5l7 nm, induced by antioxidants. The decrease in absorbance of DPPH radical is caused by antioxidants, because of the reaction between antioxidant molecules and radicals, progresses, which results in the scavenging of the radical by hydrogen

donation. It is visually noticeable as a change in color from purple to yellow. Hence, DPPH is usually used as a substrate to evaluate the antioxidative activity.

However, several preliminary experiments were performed, through which the value of IC50 was calculated, through the following calculations:

$$y = 0.017\ x + 0.906 \qquad \text{(From figure 6)}$$

Can calculate the IC50 as follows:

$$IC50 = \frac{(0.5 - b)}{a}$$

$$IC50 = \frac{(0.5 - 0.906)}{0.017}$$

$$IC50 = 123.67$$

In this project, a first DPPH test was conducted, but global conditions occurred that led to the project stopping at this stage.

For the drying of herbs, the active ingredients of different medicinal plants typically are concentrated in certain parts, e.g., leaf, flower, fruit, bark, or root, and therefore, harvest and drying of plant parts are usually selective. As a result, the dryer design must correspond to the plant parts to be dried. For the drying of flowers and roots, tray covered transparent sun dryers are suggested.

After drying, the herb is crushed (The smaller the particle size is, the higher the drying speed and drying rate is then the leaf particles are removed from the worthless stalks. As the stalks are often 50% of the harvested material and they dry more slowly than the leaf material, this procedure is obviously marked with considerable energy losses.

The aim of drying experiments was to investigate and compare principal drying data obtained by free convections for Mullein herb with that found in the literature. This actually happened by comparing Mullein drying data with other herbs drying data, namely: Marigold, Lavender, Lemon balm, and Sage. The removal of the water's tendency of these plants in time is shown in Figure 15.

Mullein gives the same drying tendency in Figures 7 and 9 if the mirror is placed at the top of fig.9 or 10, figure 14 is obtained. The drying rate at constant and at falling rate periods are found and derived. The drying experiments are carried out at an ambient temperature and at 55-60°C.

The calculation was made per 500 g of material in order to compare the drying dynamics of different types and weights of herbs. The results are shown in Figure 15. Looking at the results it is seen that drying dynamics of all samples are similar except for garden marigold, where longer sample drying is observed. Other examples dry during five days, but Calendula flowers dry up to 6-7 days at the same conditions. The difference between garden marigold and other plant drying can be explained by the fact that flowers of garden marigold are thicker and inside moisture, diffusion affects the drying process.

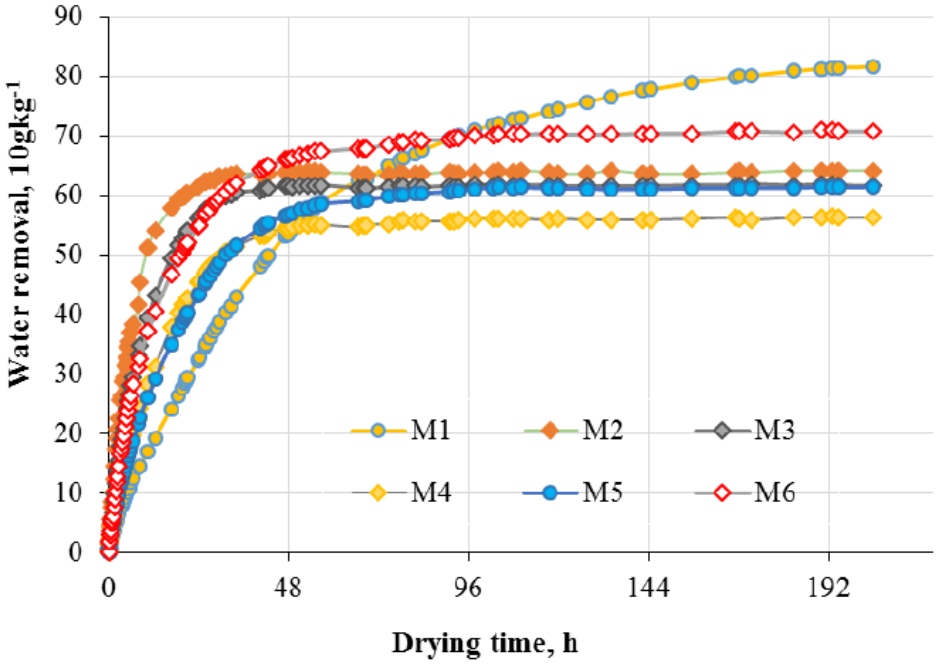

Fig. 4. **Different plant drying dynamics:** M1 – garden marigold; M2 – lemon balm; M3 – origanum; M4 – common agrimony; M5 – common lavender; M6 – common sage

Figure 15: Different Plant Drying Dynamics (Jelgava and Aboltins, 2016).

It has been observed that the value of (Ls/A) is not constant in the two experiments for the drying process, so it is not possible to compare the rate of drying.

➢ The effect of air-drying temperature

Active ingredients

To achieve increased dryer capacity, drying temperature should be chosen as high as possible without reducing the quality of the product.For most of medical herbs temperature must not exceed 60°C. Maximum allowable temperatures depend mainly on the chemical composition of the active ingredients of the medicinal plant species. For glycoside species, a maximum temperature of 100°C is recommended, for mucilage species 65°C and for essential-oil species 35 to 45°C (Maltry, 1975). Due to the high heterogeneity among medicinal plant species, these global recommendations can only serve a rough indication.

Colour

As many medicinal plant species are used as a tea, colour is an essential quality criterion because it is directly apparent to consumers. The pre-drying phase of 3 h is sufficient to prevent colour changes. Based on this knowledge, a conveyor dryer can be controlled following a staged temperature regime to achieve high drying capacity via high temperatures without affecting quality in terms of colour.

Microbial status

Post-harvest processes such as a collection of plant material in the field, transport to the farm, and drying are often suspected to increase microbial contamination of medicinal plants. When the bulk of harvested material is not ventilated, auto-heating due to respiration activity provides favourable conditions for micro-organism growth, in terms of temperature and humidity (Böttcher and Günther, 1995). Drying the material in a conveyor dryer reduced the original microbial count.

Chapter Eight: Conclusion

Plant-derived drugs remain an important resource, especially in developing countries, to combat serious diseases. Approximately 60–80% of the world's population still relies on traditional medicines for the treatment of common illnesses. Traditional remedies have a long-standing history in many locations in Palestine and continue to provide useful and applicable tools for treating ailments.

Nevertheless, little scientific research was done to investigate the plants of Palestine used in herbal medicine.

In the course of our investigations we found that several plants of Palestine possess really interesting biological activities, which could be of interest to all parts of the world.

In this study, mullein herb extracts have been determined for their antibacterial and antifungal activity by means of antioxidant activity using the scavenging activity of the DPPH radical method. These tests are not completed. Furthermore, a phytochemical screening of the methanolic extracts was performed.

The objectives of this project have been achieved. The first goal is to collect the medicinal values of this unknown plant in Palestine. This project provides a therapeutic alternative to cover respiratory and pulmonary diseases. This was achieved by examining the chemical components in mullein leaves. This study attempted to review information about the mullein plant, plant description, and its multipurpose uses.

Different studies reveal that mullein has a direct effect on nutrition and health According to past studies also; it found that mullein can reduce some pulmonary discomforts like bronchitis and pertussis and others. This medicinal herb has very high antioxidant, antibacterial and antimicrobial properties. That has been proven in this project.

Drying is an inevitable and elementary step for further handling of any medicinal herb. One can’t get powder for using the herb as nutrient or medicine without prior drying. Therefore, there is a big need to know experimentally the rate of drying of this plant, this second objective.

Two drying experiments at different temperatures were conducted. For each experiment, drying characteristic curves are presented. Drying rate equations are derived from characteristic curves data. This herb is dried easily. Four-five days are enough for drying at the covered area in spite of low drying temperature. The air velocity is not measured but the wind speed was about 2m/s. When the drying conducted at higher but under safe drying temperature (55-60)°C, less than three hours was enough to dry. This temperature is easily attained under transparent plastic covered places with no need to pay for energy.

The conduction of phytochemical experiments for proving the presence of healing chemicals in this plant is another important finding of this project. Phytochemical constituents' were found methanolic plant extracts like alkaloids, tannins, Phlobatannins, flavonoids, carbohydrates, phenols, saponin, cardiac glycosides, proteins, glycosides, and terpenoids.

In the last years, interest in the antioxidant activity of plant extracts has become larger and very important due to the fact that free radicals e.g. reactive oxygen species (ROS) can be responsible for various diseases, e.g. heart diseases, stroke, arteriosclerosis, and cancer, as well as foraging process. The DPPH experiments are started but at the half road are stopped for well-known reasons.

This was a good opportunity to know all of the above-declared findings of this project.

Chapter Nine: Recommendation

In the project, many challenges faced, therefore some recommendations for the next generations:

- ✓ Provision of the necessary facilities to achieve such projects.
- ✓ This project should be continued to study the DPPH test.
- ✓ Study the active compounds to have more specific products for specific diseases.
- ✓ Making products from different parts of this plant.

Chapter Ten: References

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Appendices

Figure X 1: Mullein Leaves.

Figure X 2: Mullein sample in water bath shaking.

Figure X 3: Mullein sample in Rotary Evaporator.