An- Najah National University

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Graduation Project Report 1

Mullein Plant : healing, phytochemicals and antioxidant tests

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Abstract

Verbascum thapsus L. is an important species of the genus and of Scrophulariaceae Is found wild on stony ground, in wasteland, woodland, clearings and roadsides. It grows as a wild plant in the Palestinian mountains and hills, especially in the western and southern areas of Nablus and Qalqilya.

Plants have been the basis of medicinal treatment since prehistoric times, and herbal medicine or herbal medicine is still widely practiced today. Modern medicine makes use of many compounds derived from plants as an essential raw material in the pharmaceutical industry. These plants were used in folk medicine, as mullein flowers were used to treat sore throat, cough, and colds. Other uses include asthma, diarrhea, colic, gastrointestinal bleeding, migraines, joint pain, and gout.

In this project our main goal is to study the chemical composition and raw material of mullein flower, by using a phytochemical composition test. The GC-MS analyzer remains one of the most powerful, flexible, and widely **used** tools for analyzing chemical mixtures in in plant extract. Which has been achieved at this stage.

The next step will be to determine the existence the anti-oxidant substances in the plant. The antioxidant (DPPH) experiments will be conducted in the next semester and another anti-cancer test will also be performed to learn more about Mullein plants.

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Chapter One: Introduction

1.1: Background

Verbascum thapsus L. is an important species of the genus of Scrophulariaceae It Is found wild on stony ground, in wasteland, woodland, clearings and roadsides.

In its first year of growth, the common mullein, with its large leaves that can grow up to 1 foot long (30.5 cm), forms a large basal rosette. In disturbed landscapes, such as along roadsides, railroad tracks and once-cultivated fields, the plants grow well. In alkaline soil, common mullein also grows particularly well. The common mullein can develop a large, erect flower stalk in its second year of growth that can grow from 2 to 8 feet (0.6 to 2.4 meters) tall. There are wooly, branched hairs covering this rigid stalk. Alternating leaves, smaller than the rosette leaves, attach directly to the stem in such a pattern as to direct rainwater down the stem to the plant's roots. (Buscher,2005)

Verbascum thapsus common name mullein is a herb in folk medicine with a long history of usage. With growing interest in herbs and preferring the 'greener' lifestyle, the commercial popularity of this plant has increased over the past few years.

It is a popular herb found throughout Europe, in temperate Asia, in North America, and well-reputed because of its medicinal properties. This herb includes numerous chemical components, including saponins, glycosides of iridoids and phenylethanoids, flavonoids, vitamin C, and minerals. It is known for the care of various human and animal food disorders in diverse cultures worldwide.

It is known for the care of various human and animal food disorders in diverse cultures worldwide. A variety of pharmacological activities have been attributed to this herb, such as anti-inflammatory , antioxidant, anticancer, antimicrobial, antiviral, antihepatotoxic and anti-hyperlipidemic activity. The plant is also used to treat tuberculosis, ear pain and bronchitis. (Turker and Gurel,2005)

In Palestine, particularly in Salfit, Tulkarm, Qalqilya governorate and west of Nablus, we have discovered the existence of mullein. This plant is characterized on the side of the roads by its growth.

The project supervisor, over a period of three years, works on the reproduction and production of this plant - and now large quantity of these plants is available in his farm. As their availability, especially in the three summer months, provided us the necessary raw materials such as dried leaves and flowers for examination throughout the days of the project.

Plants have been the basis of medicinal treatment since prehistoric times, and herbal medicine or herbal medicine is still widely practiced today. Modern medicine makes use of many compounds derived from plants as an essential raw material in the pharmaceutical industry. These plants were used in folk medicine, as mullein flowers were used to treat sore throat, cough, and colds. Other uses include asthma, diarrhea, colic, gastrointestinal bleeding, migraines, joint pain, and gout.

The chemical composition of mullein has not been studied enough, and only a few data are available on the presence of biological active substances in it, so it is necessary to conduct detailed studies of the raw materials of this plant. Verbascum has been named differently in various regions of the world, Orange Mullein, Woolen, Woolly Mullein, harebell, Rag Paper, and velvet plant.

Main chemical constituents present in flowers of V. thapsus are flavonoids, phenolic acids, fatty acids, triterpene saponins, iridoid, and phenylethanoid glycosides and polysaccharides (Kumar, Singh, Kaul, & Ahuja, 2005). Accordingly, these plant structures can be used to treat many disorders such as, spasmodic coughs, asthma, inflammation, different cancer types and, stroke and heart diseases (Riaz, Zia- Ul- Haq, & Jaafar, 2013). An extensive array of ethnomedicinal activities of V. thapsus in terms of antioxidant, antimicrobial, antiviral, anticancer, antihyperlipidemic, cytotoxic, and wound healing activities have recently reviewed by Riaz et al. (2013).

Based on the experiences of a supervisor on dry cough patients, the extracts of this plant helped treat many people who used it. Its juice has been used in the manufacture of creams and has proven effective in treating wound infections.

Among the most important components present in plant tissues of common mullein, a very low attention was paid on its composed polysaccharides and functional potentials. It has been proven that the mucilaginous polysaccharides of mullein leaves significantly decreased the cholesterol and triglyceride levels (Aboutabl, Goneid, Soliman, & Selim, 1999). Amount of water- soluble mucilage polysaccharides of V. thapsus flowers was reported about 3% which after their hydrolysis can be converted to arabinose, galactose, and uronic acid (Warashina, Miyase, & Ueno, 1992). However, content of antioxidant polysaccharides has been more estimated. Use of an efficient solvent along an ideal extraction technique can result in the improved extraction yield of these biomacromolecular components (Skenderidis, Petrotos, Giavasis, Hadjichristodoulou, & Tsakalof, 2016).

1.2: Project statement

The aim of the project is to study and identify Mullein leaves and flowers. This is done through the investigation of its medicinal and nutritional benefits through conducting experimental tests. This will help to make Mullein widely known for Palestinian people.

The decoction of this plant can be used directly to heal many respiratory system problems. More sophisticated analytical tests will be carried such as determination of phytochemicals and determination the antioxidant in the plant.

1.3: Objective

1- The main goal is to study the chemical composition and raw material of mullein flower, by using a phytochemical composition test of GC-MS device and by conducting an antioxidant (DPPH) test.

2- Provide people with information about this very valuable flower.

3- Transfer active ingredients into effective products in the form of any simple methods of taking medication.

4- Develop a medicinal product derived from the Mullein flower to cure the respiratory tract as the flowers of Mullein have expectorant and demulcent properties used by herbalists to treat respiratory problems such as bronchitis, dry cough, whooping cough, tuberculosis, asthma and hoarseness.

1.4: Significance of the work

Despite the prevalence of mullein and its therapeutic uses in Europe and in USA, Zuni people in Mexico use the plant in poultices of powdered root applied to sores, rashes and skin infections, The German Commission E describes uses of the plant for respiratory infections, Japan and northern Europe.

The decoction of the Palestinian Mullein plant is used directly to heal many respiratory system problems.

Mullein has many aesthetic and therapeutic properties, as Mullein oil is extracted from the flower or leaves of the plant. The oil is used as a remedy for earaches, eczema, and some other skin conditions. And also contains compounds and elements that act as antioxidants and anti-inflammatory and antimicrobial.

Many products can be produced from this flower such as oil, syrup, ointment and medicine pills. This study will provide properties of mullein flower and its healing effect by made antioxidant tests and GC/MS analysis.

1.5: Organization of the Report

The report is classified into several chapters: the first chapter, where the properties and information about mullein plant, the aim and steps of this study. Uses of mullein and some of the tests that we will be done on this flower in the second chapter. The third chapter about the previous studies on Mullein plant. This is followed by the pre-experiments. Results conclusion is presented in separate chapter. The future work is presented at the final part of the project .

1.6: Standards, Limitations and Constrains

The scientific analysis carried in this project is subject to international protocols and standards. We mean here the process of conducting the DPPH test, the GC-Ms analysis.

The spread of the Coronavirus has limited our ability to work in laboratories or even face-to-face meetings. So it is not possible to do all the experiments we want

1.7: source of data

All the information was supplied by the library database and electronic search (Google Scholar, ScienceDirect, PubMed, ResearchGate, etc.).

Chapter Two: Literature review

2.1: Mullein General Uses

Mullein flower extract is used as dye to change hair to golden color, mullein Leaves are used to insulating shoes to safe the feet from cold. Also mullein was used as a poison for fish because the seeds contain several compounds saponins, glycosides, coumarin and rotenone that are toxic to fish (Grieve, 1981). The stems are used as wicks for candles, the leaves are used topically to soften and protect the skin, and it may be grown use as plant for decoration. (Haughton, 1978).

2.2: Mullein medical Uses

Mullein has been used as an analgesic, anti-inflammatory, antiseptic, spasmolytic, astringent, diuretic, emollient, expectorant piles, bruises and frostbites. It used for burns and earaches and are also a good treatment for wounds, tumors, and ulcers(Riaz , Zia & Jaafar, 2013)

Mullein is used as mucolytic, expectorant, diuretic, and lenitive agents in the traditional, it used in the treatment of respiratory diseases such as tuberculosis, bronchitis, dry coughs, and asthma. It is valuable sources of various pharmaceutically active substances that have long been used due to antioxidant, antimicrobial, antibacterial, and anti-inflammatory activities (Nofouzi, 2016).

Many years ago, for the first time, Dioscorides recommended this plant for lung diseases; Leaves were smoked to try to treat lung ailments. Also used to treat athlete's foot, eliminate the irritating hairs, Oil of mullein flowers was used against catarrhs, colic, earaches, frostbite, it used for the treatment of warts, boils, carbuncles, hemorrhoids, and chilblains, The plant has been used in an attempt to treat croup, sunburn and other skin irritations. (Riaz , Zia & Jaafar, 2013)

Flower infusion in olive oil is used as earache drops with good bactericidal properties.

They use mullein flower in remedy Pulmonary disease, fever and bleeding of lungs and bowel in Pakistan, Inflammatory ailments in respiratory tract and others in Mexico and as cigarettes for asthmatics in Indians (Riaz , Zia & Jaafar, 2013).

2.3 Chemical composition in mullein

Mullein contain Iridoid glycosides, Phenylethanoid glycosides,Terpenes, Flavonoids and carotenoids, Saponins ,Carbohydrates ,Minerals ,Lipids . (Klimek 1996, Kanzaki et al. 1998).

Mullein flower contains approximately 3% water-soluble mucilage polysaccharides, which after hydrolysis yields 47% D-galactose, 25% arabinose, 14% D-glucose, 6% D-xylose, 4% L-rhamnose, 2% D-mannose, 1% L-fucose, and 12.5% uronic acids (arabinogalactans) (Kraus and Franz, 1987; Meyer-Buchtela, 1999; Wichtl and Bisset, 1994); approximately 1.54% flavonoids (apignein, luteolin and their 7-O-glucosides, plus kaempferol and rutin); caffeic acid derivatives including caffeic, ferulic, protocatechuic acids, and verbascoside; iridoid monoterpenes (aucubin, 6-b-xylosylaucubin, methylcatalpol, isocatalpol); triterpene saponins (verbascosaponin) (Klimek, 1996a; Klimek, 1996b; Meyer-Buchtela, 1999; Wichtl and Bisset, 1994); sterols; and 11% invert sugar (fructose + glucose) (Meyer-Buchtela, 1999; Wichtl and Bisset, 1994).

2.4 Chemical relations of compounds and biological effect

Flavonoids and phenolic acids make up one of the most pervasive groups of plant phenolic and they are frequently used in pharmacy and medicine, because of their antioxidant, antibacterial, anticancer, cardio-protective, anti-inflammatory, as well as immune system promoting effects (Ghasemzadeh and Ghasemzadeh, 2011; Tungmunnithum et al., 2018).

Mucilages are carbohydrates featuring high molecular weight. They dissolve in water and they become turgid and voluminous after absorbing water (Sharifnia 2007). Mucilages are applied for relieving from skin discomforts, respiratory system, sore throat problems, intestinal difficulties, and bronchitis treatment (Turker and Gurel 2005). The expectorant, laxative, and softening effects of mullein plant are for the existence of saponin and mucilage (Ghasemi et al. 2015). Saponins are glycosides with high molecular weight and their most important physical attribute is that they produce foams when dissolved in water. These ingredients possess diuretic, laxative, expectorant and anti-cough effects (Sotoodeh et al. 2016). Saponins are biologically active compounds that act as a defensive agent against pathogens and herbivores (Francis et al. 2002).

These compounds have been reported to possess anti-inflammatory effects (Gestetner et al. 1970).

Studies have shown that these compounds possess antifungal effects, as well (Bowyer et al. 1995).

Saponins are highly toxic to the fungi (Wang et al. 1998). It seems that many of this expectorant, antifungal and antimicrobial effects found in Verbascum spp. pertain to the presence of mucilages and saponins. The antimicrobial activity of Verbascum spp. is due to the existence of numerous glycosides and alkaloids (Amirnia et al. 2011). 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). Many of the curative effects reported for the plants belonging to this genus are probably associated with the presence of alkaloids.

2.5 Anti-oxidant

Some researchers suggest that two-thirds of the world's plant species have medicinal value; in particular, many medicinal plants have great antioxidant potential. Antioxidants reduce the oxidative stress in cells and are therefore useful in the treatment of many human diseases, including cancer, cardiovascular diseases and inflammatory diseases. Synthetic antioxidants such as butylated hydroxytoluene (BHT) and butylated hydroxylanisole (BHA) are currently used as food additives, and many plant species have similar antioxidant potentials as these synthetics.

The 1,1-diphenyl-2-picrylhydrazine (DPPH) radical scavenging assay was first described by Blois in 1958 and was later modified slightly by numerous researchers. It is one of the most extensively used antioxidant assays for plant samples. DPPH is a stable free radical that reacts with compounds that can donate a hydrogen atom. This method is based on the scavenging of DPPH through the addition of a radical species or an antioxidant that decolourizes the DPPH solution. The antioxidant activity is then measured by the decrease in absorption at 517 nm. In this method, a 0.1mM solution of DPPH in methanol is prepared, and 4ml of this solution are added to 1ml of the sample solution in methanol at varying concentrations. Thirty minutes later,

the absorbance was measured at 517 nm. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound (Kedare and Singh, 2011).

An important distinction can be made between short- and long-term antioxidant protections. This is related to the reaction kinetics and the rate at which an antioxidant reacts with a specific radical versus the thermodynamics of the reaction and how completely the antioxidant reacts. For instance, disappearance of the DPPH radical followed a double-exponential equation in the presence of edible oils and oil fractions which suggested the presence of a fast- and slow-acting group of antioxidants (Robards , 2001)

Verbascum plants could serve as attractive mines of powerful antioxidants for various purposes. Antioxidants play an important role as health protecting factor. Scientific evidence suggests that antioxidants reduce the risk for chronic diseases including cancer and heart disease.

An antioxidant may be defined as ‘any substance that when present at low concentrations, compared with those of the oxidizable substrate, significantly delays or inhibits oxidation of that substrate’. For convenience, antioxidants have been traditionally divided into two classes, primary or chain-breaking antioxidants and secondary or preventative antioxidants. Secondary or preventative antioxidants are compounds that retard the rate of oxidation. This may be achieved in a number of ways including removal of substrate or singlet oxygen quenching. Primary antioxidants, AH, when present in trace amounts, may either delay or inhibit the initiation step by reacting with a lipid radical or inhibit the propagation step by reacting with peroxyl or alkoxyl radicals:

$L\bullet + AH \rightarrow LH + A\bullet$

$LOO\bullet + AH \rightarrow LOOH + A\bullet$

$LO\bullet + AH \rightarrow LOH + A\bullet$

The antioxidant free radical may further interfere with chain-propagation reactions by forming peroxy antioxidant compounds:

$A\bullet + LOO\bullet \rightarrow LOOA$

$A\bullet + LO\bullet \rightarrow LOA$

The activation energy of the above reactions increases with increasing A–H and L–H bond dissociation energy. Therefore, the efficiency of the antioxidant increases with decreasing A–H bond strength.

Chain-breaking antioxidants may occur naturally or they may be produced synthetically as in the case of BHT, BHA, TBHQ and the gallates. The synthetic antioxidants are widely used in the food industry and are included in the human diet. The use of naturally occurring antioxidants has been promoted because of concerns regarding the safety of synthetic antioxidants, with natural alternatives (e.g., plant biophenols) possessing antioxidant activity similar to or even higher than that of synthetic antioxidants.

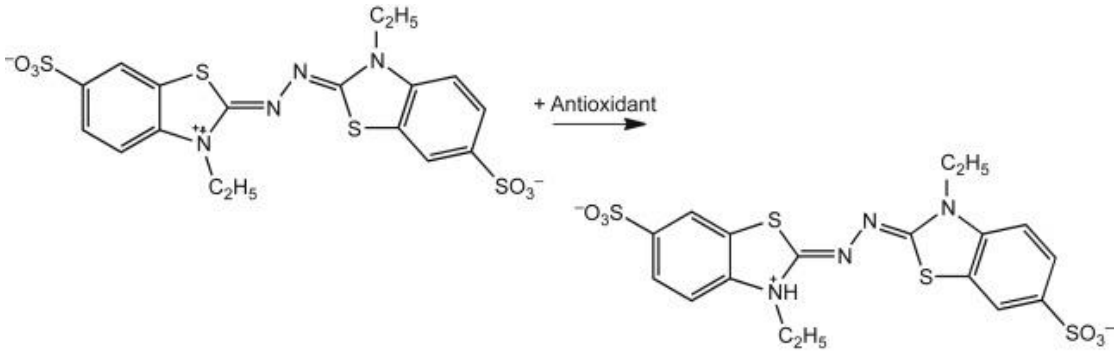
TBARS assay

Despite its limited analytical specificity and ruggedness, the thiobarbituric acid reactive substances (TBARS) assay has been widely used as a generic metric of lipid peroxidation in biological fluids. It is often considered a good indicator of the levels of oxidative stress within a biological sample, provided that the sample has been properly handled and stored. The assay involves the reaction of lipid peroxidation products, primarily malondialdehyde (MDA), with thiobarbituric acid (TBA), which leads to the formation of MDA-TBA_2 adducts called TBARS. TBARS yields a red-pink color that can be measured spectrophotometrically at 532 nm. The TBARS assay is performed under acidic conditions (pH = 4) and at 95 °C. Pure MDA is unstable, but these conditions allow the release of MDA from MDA bis(dimethyl acetal), which is used as the analytical standard in this method. The TBARS assay is a straightforward method that can be completed in about 2 h.

ABTS assay

In the ABTS$^{\bullet+}$ radical scavenging assay (an electron transfer-based assay), the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\bullet+}$), which has a dark blue color, is reduced by an antioxidant into colorless ABTS, which can be measured spectrophotometrically.

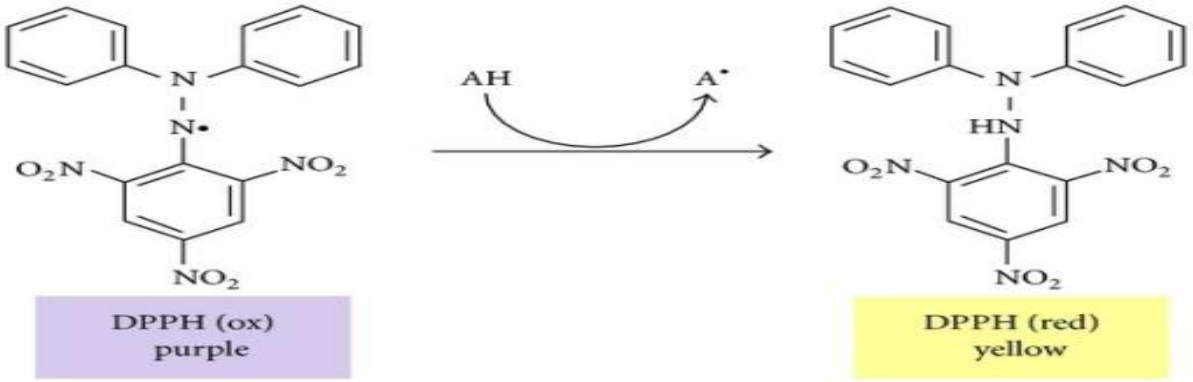
The ABTS assay measures the relative ability of antioxidants to scavenge the ABTS generated in aqueous phase, as compared with a Trolox (water soluble vitamin E analogue) standard. The ABTS is generated by reacting with a strong oxidizing agent (eg, potassium permanganate or potassium persulfate) with the ABTS salt.



DPPH radical

The DPPH radical absorbs at 517 nm and, in a second substrate-free system, antioxidant activity can be determined by monitoring the decrease in this absorbance. Results were reported as the EC50, that is, the amount of antioxidant necessary to decrease by 50% the initial DPPH concentration. The time taken to reach the steady state to EC50 concentration (TEC50) was also calculated. In recognition of the effect of both parameters on antiradical capacity, a new parameter, namely antiradical efficiency, which combined both factors, was defined.

DPPH is a stable radical, and solution of DPPH can be made in methanol. After mixing with a sample solution containing the antioxidant of interest, absorption is monitored for 30 min or until the absorption is stable. The concentration of antioxidant needed to reduce DPPH concentration by 50% is termed EC_{50} (moles of antioxidant per mole of DPPH). The reduction reaction of DPPH is seen in the reaction below. The hydrogen transfer leads to change of color from purple to yellow



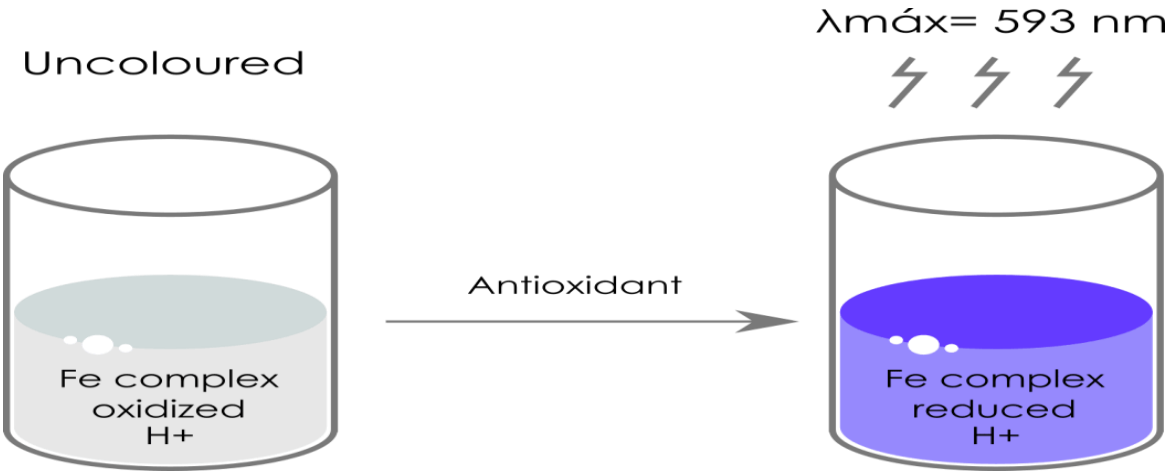
TRAP assay

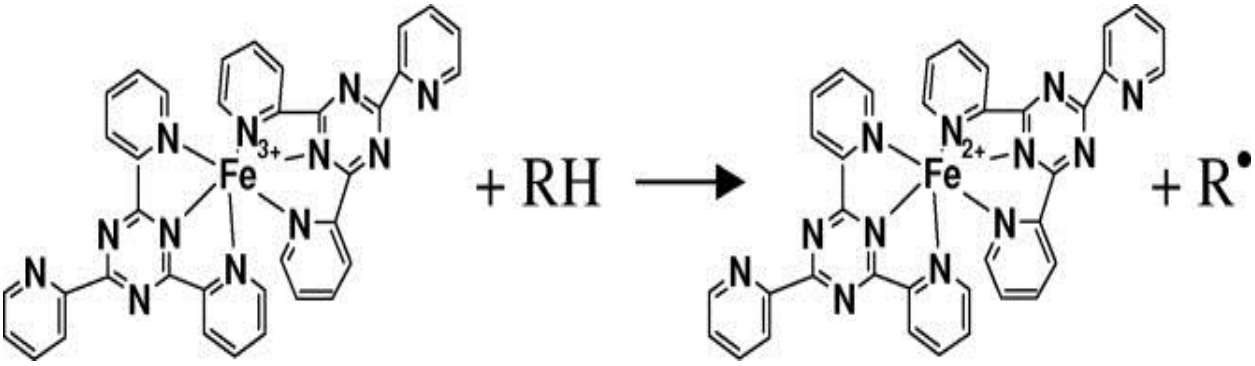
Total radical-trapping antioxidant parameter. The total radical-trapping antioxidant parameter (TRAP) assay of Wayner et al.248 has been widely used to determine TAA based on measuring oxygen consumption during a controlled lipid oxidation reaction induced by thermal decomposition of AAPH. The TRAP expresses results196 as the number of μmoles of peroxyl radicals trapped by 1 l of plasma. The measurement of serum TRAP196 was based on the determination of the length of time that a subject's serum was able to resist artificially induced oxidation.

Wayner and co-workers followed oxidation by monitoring oxygen consumption in a thermostated oxygen electrode cell during oxidation of linoleate by free radicals. A major problem with this method lies in the oxygen electrode end-point. An oxygen electrode will not maintain its stability over the period of time required (up to 2 h per sample) and the TRAP assay was modified to use luminol-enhanced chemiluminescence (CL) as the end-point. This led to enhanced precision and a greater ability for automation. In this system, peroxyl radicals enhance the CL reaction. When an antioxidant was added, the CL was extinguished, the duration of which was directly proportional to the radical trapping ability of the antioxidant sample.

FRAP method

The ferric ion reducing antioxidant power assay (FRAP) is based on reducing power of an antioxidant to reduce a ferric salt (Fe^{3+}) to ferrous salt (Fe^{2+}) by electron transfer reaction. The ferric salt usually used is ferric (TPTZ; 2,4,6-tripydidyl-*s*-triazine) chloride. When this ferric salt is reduced to ferrous salt, a blue color is formed, the intensity of which can be measured spectrophotometrically. The redox potential of ferric salt is comparable to that of $ABTS^{\bullet+}$ radical cation. Thus, FPAP is similar to TEAC assay, except TEAC assay is conducted at neutral pH, whereas FPAP assay is usually performed in acidic pH (3.6). TEAC is an end point assay, and, due to its simplicity, it is widely used in various laboratories for measuring total antioxidant capacity of various compounds and food constituents.





2.6: Toxicity

In two model systems, in vitro in BEAS 2B epithelial bronchial cells and in vivo in zebrafish embryos, a research was conducted to investigate the possible toxic and antioxidant effects of V. thapsus water extracts. In both model systems, the findings obtained showed that V. thapsus flower water extract showed high acute toxicity and no antioxidative effects (Dragićević ,2019)

There are no data on genotoxicity, carcinogenicity, reproductive, and developmental mullein flower (European Medical Agency 2009).

2.7: Extraction

Extraction is the first crucial step in preparation of plant formulations. Modern methods of extraction are effective in advancing the development of traditional herbal remedies. The development of modern sample-preparation techniques with significant advantages over conventional methods for the extraction and analysis of medicinal plants is likely to play an important role in the overall effort of ensuring availability of high-quality herbal products to consumers worldwide.

Sample preparation is of utmost importance to the development of analytical methods for the analysis of constituents present in the botanicals and herbal preparations.

A typical extraction process may contain following steps (Handa et al., 2008):

1. Collection and authentication of plant material & drying.

2. Size reduction.

3. Extraction.

4. Filtration.

5. Concentration.

6. Drying & reconstitution Quality of an extract is influenced by several factors such as, plant parts used as starting material, solvent used for extraction, extraction procedure, and plant material : solvent ratio etc. From laboratory scale to pilot scale all the parameters are optimized and controlled during extraction. Extraction techniques separate the soluble plant metabolites through selective use of solvents.

The properties of the extraction solvent, the particle size of the raw materials, the solvent-to-solid ration, the extraction temperature and the extraction duration will affect the extraction efficiency.

The selection of the solvent is crucial for solvent extraction. Selectivity, solubility, cost and safety should be considered in selection of solvents. Based on the law of similarity and intermiscibility (like dissolves like), solvents with a polarity value near to the polarity of the solute are likely to perform better and vice versa. Alcohols (EtOH and MeOH) are universal solvents in solvent extraction for phytochemical investigation.

Chapter three: Experimental part

This part is consisted of:

- Collecting of Mullein flowers.
- Drying of Mullein flower.
- Storage at freezing temperature.
- Grinding and extracting.
- Extraction: Three solvents are used separately: Acetonitrile, ethanol, Methanol.

Acetonitrile Extraction

One and half gr of grinded Mullein is mixed with 15 ml of CH_3CN solvent. It is mixed by magnetic stirrer for four hours at 40°C. This step is followed by filtration step. The obtained transparent yellowish extract is stored at freezer for two days for chemical composition test on MS / GC apparatus.

Ethanol Extraction

10 gram of grinded Mullein flowers are weighed and mixed with 120 ml ethanol .The solvent extraction experiment is conducted at 40° C under mixing on hot plate equipped with magnetic stirrer. This last for four hours.

The Mullein ethanol solution is filtered. The solvent is removed by vacuum rotary evaporator. About three gram of viscous thick brown sticky material is obtained..

Methanol Extraction:

The procedure of methanol extraction is carried out entirely similar to above ethanol extraction experiment. The yield here is about 3.2 gr.

GC/MS analysis of Mullein flower and leave extract

Two separate samples of acetonitrile are prepared one from mullein flowers and the other from Mullein leaves The tested Mullein flower samples doesn't demonstrate any result when tested on GC/MS analyzer, While the extracted leaves produced measurable concentrations of different compounds.

Chapter Four: Results and Analysis

As we mentioned earlier, the tests on the flowers of the mullein plant did not reveal the presence of any of chemical compounds. But when we repeated the test under the same conditions on plant leaves, we confirmed the presence of many compounds. You will find the results of the test in Table 1

Table 1: GC/MS Test Result.

% Of Area	Area	R.I	R.T	Name
23.11	**5112548**	**950**	**7.11**	**(8)-Annulene**
0.98	**216842**	**900**	**35.15**	**(2-Phenylcyclobutyl)benzene(Cis)**
4.12	**911650**	**826**	**35.9**	**5-Phenyl-2-Pentenal**
32.17	**7118705**	**947**	**36.69**	**(2-Phenylcyclobutyl)benzene(trance)**
39.62	**8767326**	**778**	**52.6**	**Azetidine,1-Benzyl-3,3-Dimethyl-2Phenyl-**
100.00	**22127071**			

The chemical composition of Mullein leave extract is shown on the table above. The obtained spectrum contains different parameters such as retention index, peak height and the area under each peak.

Retention time (RT) is the time taken for a solute to go through a chromatography column. It is calculated as the time from injection to detection. The RT for a compound is not constant as many factors can affect it even if the same GC and column are used.

Retention time (RT) in chromatogram is the time between sample introduction (beginning of the chromatogram) and the maximum signal of the given compound at the detector.

Height of each peak is proportion to the amount of the specific component present in the sample mixture injected into the chromatograph and area of a peak is proportional to amount of the compound present.

After studying the compounds found in the leaves of mullein, it became clear that there is some similarity between them and what was mentioned by the scientific references. But we still need to carry out the antioxidant test at the next semester to emphasize the anticancer capacity of the plant. The common thing is the presence of polyphenols and deferent sugar derivative that present in table 2.

Table 2: Comparison between GC/MS Test Result and Literature Review.

GC/MS Test Result	%	Literature Review	%
(8)-Annulene	23.1	D-galactose	47
(2-Phenylcyclobutyl)benzene(Cis)	0.9799	Arabinose	25
5-Phenyl-2-Pentenal	4.12	D-glucose	14
(2-Phenylcyclobutyl)benzene(trance)	32.1719	D-xylose	6
Azetidine,1-Benzyl-3,3-Dimethyl 2Phenyl-	39.622	L-rhamnose	4
		D-mannose	2
		L-fucose	1
		Uronic acids	1.25
		Flavonoids	1.54
		Invert sugar	11

Chapter Five: Conclusion

The use of plants for treating the diseases dates back to the mankind birth on earth, Mullein plant have very high antioxidant, antibacterial, antifungal, and antimicrobial properties, the active ingredients of which have been used for their expectorant and anti-cough attributes as well as for the treatment of some pulmonary discomforts like bronchitis and pertussis.

Since this plant is characterized by protection and healing the respiratory system and lungs from influenza, bacterial diseases, and infections. It can be both a protective and an impermeable dam against the Coronavirus, as it is expected to work against it

The aim of our study was to investigate the active ingredients occurred in Mullein flowers and leaves using the GC/MS technique. The next semester will be proper to emphasize whether these substances have anticancer capacity or not by using antioxidant test and/or direct test on cancer cells.

Based on the test results and based on what we know from the therapeutic results of folk medicine we will decide of medicinal formulae. This is because the healing effects of the active constituents of Mullein on diseases is known. Then preparing the proper formula, and designing a suitable dosage form to apply it in a suitable manner is a new challenge.

This project was done by Collecting of Mullein flowers, Drying of Mullein flower, Storage at freezing temperature, Grinding and extracting. Extraction: Three solvents are used separately: Acetonitrile, ethanol, Methanol.

At the end, several compounds were obtained in the Mullin flower: (8)-Annulene, (2-Phenylcyclobutyl) benzene (Cis), 5-Phenyl-2-Pentenal, (2-Phenylcyclobutyl) benzene (trance), Azetidine,1-Benzyl-3,3-Dimethyl-2Phenyl-.

Chapter six: Future Work

The implementation of the practical part of this project depends on our ability to work in the laboratories. The pandemic continues to pose a difficult challenge for us and limit our ability to operate. However, we aspire to make the following measurements**:**

Ant- oxidant test

Any possible medicinal formula to help healing asthma or bronchiolitis problems in a very safe dosage.

The studying of any new papers are important in this issue.

Chapter seven: References

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Chapter eight: Appendices

Figure 1: Mullien plant

Figure 2: mullien mixing

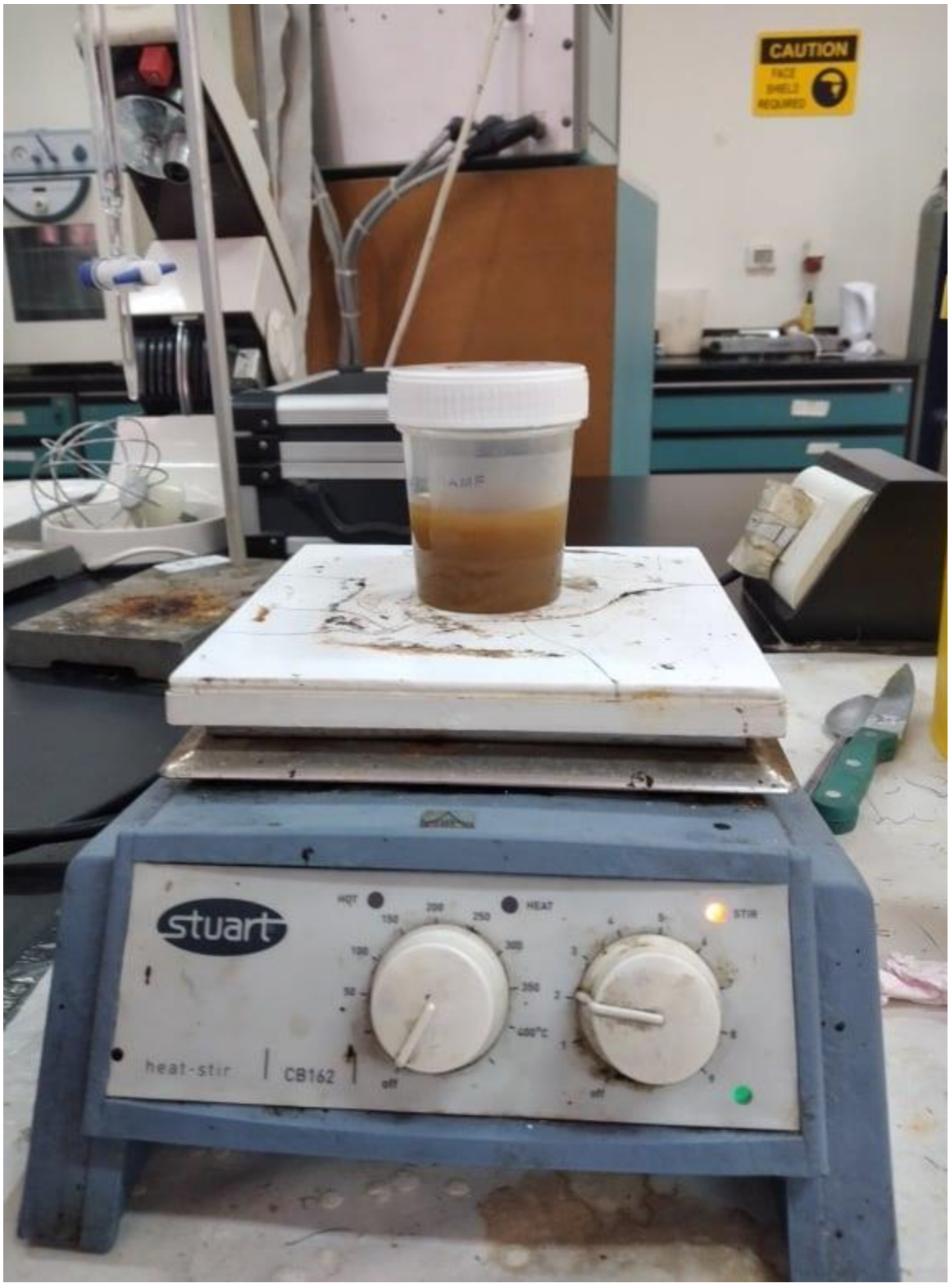

Figure 3: on hot plate equipped with magnetic stirrer

Figure 4: Mullien extract

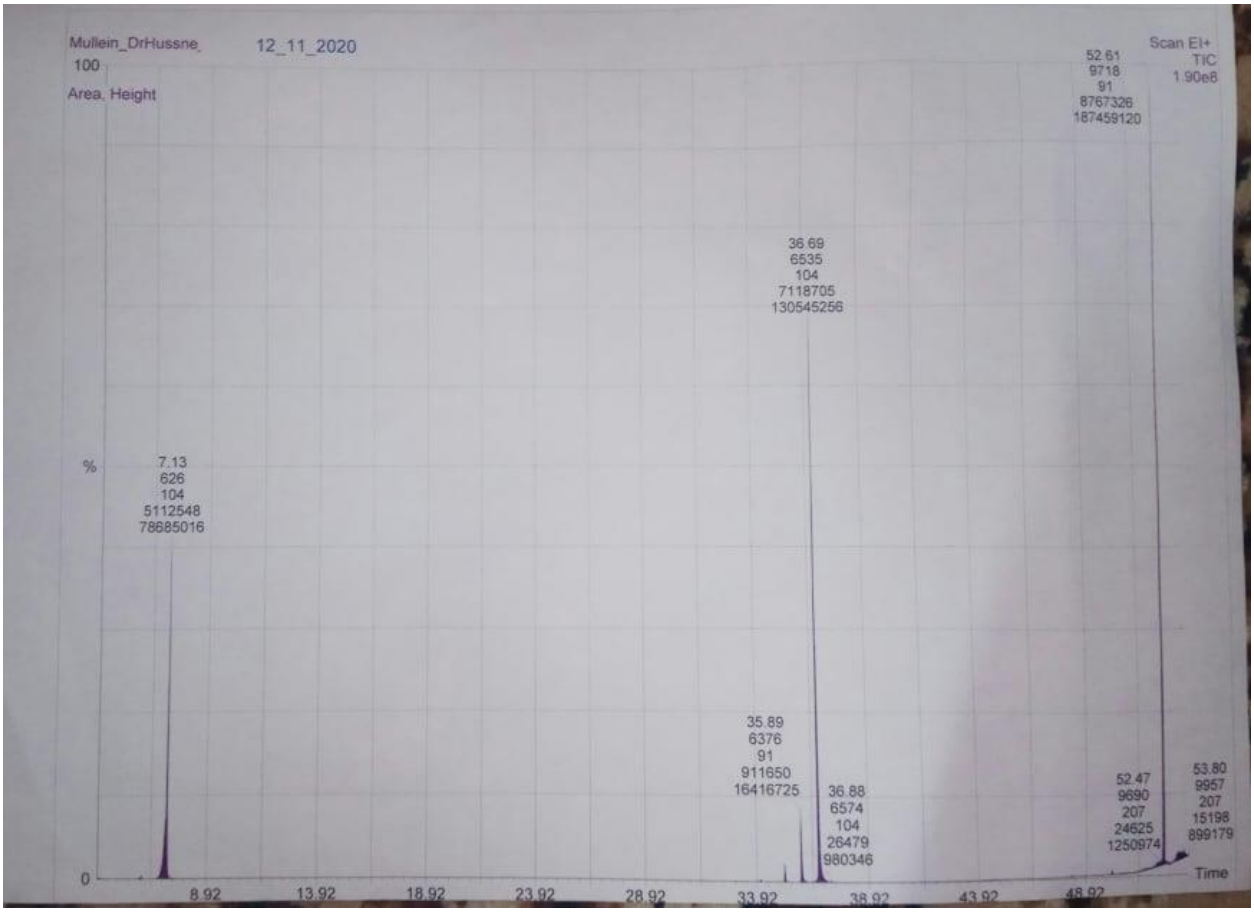

Figure 5: GC/MS Test Result.