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Extracción de antioxidantes de los residuos de la destilación a vapor e hidrodestilación de la *Lavandula angustifolia*

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TÍTULO: **EXTRACTION OF ANTIOXIDANTS FROM STEAM-, AND HYDRODISTILLED RESIDUES OF *LAVANDULA ANGUSTIFOLIA.***

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RESUMEN

Durante muchos años las plantas aromáticas han sido utilizadas en remedios terapéuticos, como saborizantes o perfumes. Lavandula angustifolia es una planta originaria de la zona mediterránea.

El aceite esencial de la lavanda obtenido de la destilación presenta actividad antioxidante. Pero de lo que trata esta investigación es de extraer los antioxidantes de residuos de la destilación a vapor e hidrodestilación para aprovechar estos residuos, para ello se comparan distintos métodos de extracción (extracción Soxhlet y extracción con tanque agitado) con diferentes disolventes como n-pentano, acetona, propanol, etil-acetato y etanol.

Los mejores rendimientos fueron obtenidos para los residuos de hidrodestilacion, mientras que la mayor actividad antioxidante la presentan los residuos de la destilación a vapor. El mejor disolvente para el caso de la extracción Soxhlet es el etanol y en la extracción de tanque agitado el agua. Así, se pueden obtener antioxidantes naturales cuya demanda ha crecido en los últimos años.

KEYWORDS

Antioxidantes, Extracción, *Lavandula angustifolia*, residuos, destilación.

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TABLE OF CONTENTS

1. INTRODUCTION

For thousand years aromatics plants have been mainly used as medical and therapeutical remedies, but they have been also employed as alimentary flavourings or as perfumes. Some of them are *Coriandrum sativum*, *Matricaria recutita*, *Rosemarinus officinalis*, *Origanum vulgare*. The one studied in this thesis is the *Lavandula angustifolia*, also called lavender [1].

Figure 1. Lavandula angustifolia

Lavandula angustifolia is a perennial flowering plant from Lavandula genus and Lamiaceae family, native to the Mediterranean area. It is commonly known as an ornamental plant because its colourful flowers and its fragrance [2].

Lavender essential oil is produced from the flowers, leaves and stems of lavender using different distillation techniques like steam distillation or hydrodistillation. Lavandula essential oil contains active constituents like linalool, linalyl acetate, 1,8-cineole, *cis*- and *trans*-ocimene, terpinen-4-ol and camphor, has been reported to have antimicrobial, anticholinesterase and antioxidant activities. Lavandula oil promotes healing symptoms for stress, exhaustion, migraines, anxiety, insomnia and depression and is also used in food manufacturing as a flavour, for cosmetics because of his preservatives properties [1-3].

The main topic of this work was, what can be obtained from the plant residue of the distillation to reduce waste and help the environment, choose the best solvent and method to obtain these compounds (antioxidants, polyphenols and tannins) and drawn conclusion at the end. Two residues were tested one obtained as dried hydrodistilled residue, while the other residue was a dried steam distilled residue. The plant residues from distillation can be submitted to different extraction techniques like supercritical fluid extraction, Soxhlet extraction, stirred tank extraction or maceration. Only two of extraction method were tested in this work: Soxhlet extraction and stirred tank extraction. Soxhlet extraction is a solvent extraction using a Soxhlet apparatus where the solvent is heated, solvent vapor is cooled which extracts non-volatile compounds from the plant material until no soluble compounds are left [4]. This was tested using solvents as ethanol, propanol, ethyl-acetate, acetone and *n*-pentane. While, stirred tank extraction is also another solvent extraction in which the solvent is in contact with the plant material submitted at a different temperature

and agitation, then filtered and evaporated [5]. As solvent ethanol-water solution in different ratio were used at 40 ºC for 3 hours.

The residue holds antioxidants and polyphenols that can be exploited by food, medical and cosmetic industries. In fact, demand of these natural antioxidants has growth since it has proved that artificial antioxidants are not so good for health. This residue can still be of value as it contains traces of essential oil along with non-volatile compounds, such as phenolics and lactones which can be extracted with different solvents. Nowadays these distilled-residues are principally used for soil replenishment or as a fuel source [4].

In this work the antioxidant activity of the extracts was evaluated, along with measuring the total polyphenol and tannin contents of the different extracts. For the antioxidant activity measurement, the DPPH method was used in which a stable free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was applied and the inhibition or quenching activity of tested extracts could be characterized. The total polyphenol and tannin contents of the extracts were also evaluated using standard spectrophotometric methods.

2. OBJETIVES

The principal objectives of this Bachelor work are listed in the next points:

1. Obtain the plant extracts of the residues of leaves, stems and flowers of differently processed *Lavandula angustifolia* with different extraction processes.
2. Steam distilled and hydrodistilled lavender residues were compared.
   2.1.Soxhlet extraction with organic solvents like:
      - Ethanol
      - *n*-pentane
      - Acetone
      - Propanol
      - Ethyl-acetate

   2.2.Stirred tank extraction with different ethanol percentages mentioned below:
      - 96% EtOH
      - 70% EtOH (30% H_20)
      - 50% EtOH (50% H_20)
      - Water
3. Compare the different amounts of extract obtained by different extraction process with different solvent and from different samples of *Lavandula angustifolia*, steam-, and hydrodistilled residues.
4. Determinate the antioxidant activity of the extracts using:
   - DPPH method.
5. Determination of the polyphenol and tannin content in all the extracts and comparation with the antioxidants activity results obtained before.
6. Draw conclusion from all the results obtained.

3. THEORICAL BACKGROUND

3.1. *Lavandula angustifolia*

The Lavandula genus, a medicinal plant, from Lamiaceae family is cultivated all around the world to get benefits of its essential oils which have applications in the food, fragrances and pharmaceutical industries [4]. The genus Lavandula has a long history as an ornamental and medicinal plant, just its scent prevents deterioration in work performance, improve memory or the state of people with Alzheimer's disease [6]. The Lavandula genus counts with more than 20 different species that differs in the habit where they were cultivated, morphological characters and chemical composition. Most of the production of lavender oil takes place in countries like Bulgaria, United Kingdom, France, China, Ukraine, Spain and Morocco and is around 200 tons per year [7].

It is popular for its ability to survive with low water consumption. It does not grow in damp soils, it prefers gravel ones and in neutral or alkaline conditions. It does best in Mediterranean climates similar to its native habitat, characterised by wet winters and dry summers. It resists at low temperatures [2].

From all of the species, the two most important species to obtain essential oils are the true lavender (*L.angustifolia=L.officinalis=L.vera*) and the grande lavender (*L.latifolia=L.spica*). Also there are hybrids of this two species called lavandins (*L.hybrida =L.intermedia*) [8].

The meaning of the name of *Lavandula* is a Latin word that come from *lavare* because years ago it was mainly used to perfume the washing. The specie *angustifolia* is Latin for narrow leaf [2].

Lavandula angustifolia, the specie used, known as the fine lavender, it grows at an altitude of 600-1400 m. From *Lavandula angustifolia* yields 15 kg of oil per hectare can be distilled and the price is around 100€/ kg [5].

It is a strongly aromatic shrub growing as high as 1 to 2 metres tall with square stems, somewhat hairy and generally with rounded angles [2]. The leaves are evergreen, 2-6 cm long, and 4-6 mm broad, very narrow and are curled on the edges [8]. The flowers are pinkish-purple (lavender-coloured), produced on spikes 2-8 cm long at the top of slender, leafless stems 10-30 cm long [2], they are grouped in biparous cymes on short peduncles, the corolla is bilabiate with the upper lip bifid and the lower lip trilobate [8].

No pharmacological experiments with animals have been conducted with the dried flower. *In vitro*, lavender oil has moderate antibacterial activity and, in the mouse, has a depressant activity. The drug may be used in the composition of phytomedicines. It is used for sunburns, superficial burns of limited area, to relieve nasal congestion in the common cold or as a mouthwash for oral hygiene. Orally is used for neurotronic disorders like sleeplessness or in Germany bath to improve circulatory problems [8].

The flowers and leaves are used as an herbal medicine in the form of lavender oil or as herbal tea. Dried lavender flowers are also used as a prevention against clothing moths which do not like their scent. It is used to make perfumes boxes, smoothing hand lotion, insects bites, to relieve sprains, ecological disinfectant or make infusions for insomnia and fever [2].

3.2. Lavender essential oil

The essential oil is stored as droplets in glandular structures on the surface of flowers and leaves and it is during the steam distillation when the pressurized water vapour releases the oil from these glandular structures. There are many types of essential oil distillation methods those will be explain subsequently and it is remarkable that the chemical composition of the essential oils differs according the used method [4].

Lavender oil from *Lavandula angustifolia* is a pale-yellow liquid with a fresh, sweet, floral, herbaceous odour on a woody balsamic base. Its properties can be resumed in a density between 0.876-0.892 kg/m^3, solubility: 1 vol in 5 vol of 70% ethanol at 20ºC, acid number: maximum 1, ester content of 35-60%. Other varieties of lavender yield more oil per hectare and can be grown at lower altitudes, however they produce a poorer quality oil, for example lavandin variety produces 50-100 kg/ha [9].

The main uses of the lavender essential oils are scents for perfumes, cosmetics, personal care and home maintenance. Another minor part is used as natural food flavours, as remedies against diseases (insomnia, alopecia or anxiety) or in therapeutical medicine. However, lavender oils have toxic effects at certain doses and are due to the linalool and linalyl acetate activity of lymphocyte proliferation and the abortifacient properties of the camphor. But not these compounds are only toxic, they have also beneficial actions, for example, the linalool and linalyl acetate are sedative and anaesthetic properties and the camphor is a good insecticide [5]. There are also some researches of the relation between the lavender oil and the tyrosinase. This amino acid in plants responsible for the effect of blackening in vegetables and fruits so it can be useful for agriculture and food industry. On the other hand, the human tyrosine by the melanogenesis process is transformed in melanin, so the lavender oil can be beneficial to reduce the melanogenesis to decrease the melanin from melanic spots [10].

In the lavender essential oil more than 100 molecules have been identified, the composition depends on the herb type, variety, plant part, climate conditions and extraction method. The principal compounds are in the terpene and terpenoid families. The terpenes are organic hydrocarbons form by units of isoprene (C_5H_8) and terpenoids are modified forms of terpenes, hydroxylated, esterifies or oxygenated forms [7]. Thus far, several attempts have been made to propagate Lavandula plants *in vitro*, species like the *Lavandula angustifolia*, and in a study conducted by Andrys and Kulpa it was found that the composition and the amount of oils differs from those of the oils obtained from

field grown plants, and it has usually higher antioxidant and antimicrobial activity in comparison with plants growing in field conditions [6].

Lavender essential oil must contain 25-38% linalool, 25-45% linalyl acetate, 0.1-0.5% limonene, 0.3-1.5% cineole, 0.2-0.5% camphor and 0.3-1% α-terpineol, determined by GC. The French standard has these specifications β-ocimenes (*cis*, 4-10%; *trans*, 1.5-6%), terpin-1-en-4-ol (2-6%), and octan-3-one (less than 2%); minimal levels of specific compounds (lavandulol and its acetate) are also requires (0.3 and 2 %, respectively). In 1981, the French government specified the requirements that a lavender oil must have to receive the appellation origin de Haute-Provence [4].

Classes	Major constituents	Percentage[a]			
		Fine lavender oil	Spike lavender oil	Lavandin oil Grosso var.	Lavandin oil Super var.
Terpenes					
Monoterpenes	Camphene	Traces–0.3	0.3–2.0	0.3	0.1
	Limonene	0.1–4.1	0.5–3.0	0.4–0.9	0.3–1.1
	Myrcene	0.1–1.8	0.2–1.0	1.5	0.3–1.2
	cis- and/or *trans*-Ocimene	0.2–17.0	Traces–0.5	0.8–2.1	1.7–3.1
	α- and β-Pinene	Traces–1.0	1.3–4.7	1.0–2.1	0.2–0.4
Monoterpenols	Borneol	0.7–7.5	1.1–4.9	1.1–3.2	1.7–5.5
	Linalool	17.8–50.0	15.1–54.7	22.5–35.5	29.9–43.7
	Terpinen-4-ol	0.6–19.5	Traces–2.0	2.3–3.4	1.0
	α- and/or γ-Terpineol	Traces–3.8	Traces–2.0	0.9–3.4	2.2–3.5
	Lavandulol	0.2–2.3	–	0.3–0.8	–
Terpenic esters	Lavandulyl acetate	0.5–14.0	Traces	1.6–2.9	–
	Linalyl acetate	10.1–54.0	Traces–9.0	23.6–35.4	26.0–42.5
Sesquiterpenes	Caryophyllene and/or its oxide	Traces–8.0	0.2–2.3	1.6–2.7	0.9–2.8
	Farnesene	Traces–3.0	Traces–0.3	1.1	–
Terpenoids					
Terpenic oxides	1,8-Cineole (eucalyptol)	Traces–5.8	11.0–47.9	4.0–10.9	1.9–4.6
Ketones	Camphor	Traces–5.5	8.0–18.6	6.3–12.2	5.1–19.8
	3-Octanone	Traces–1.4	–	Traces	Traces–0.1

Table 1. Percentage of compounds in different Lavandula essential oil [4].

3.3. Lavender distilled residues

The basic chemical composition (nitrogen, carbon, chlorine, hydrogen, oxygen and sulphur), ash and moisture content of lavender distilled straws (LLDS) were determined by the French Inter-Regional Centre for Experimentation in Medicinal and Aromatic Plants of the French Provence-Alpes-Côte d'Azur region, see in *Table 1* [4].

Elemental analysis	Percentage of dry matter
Nitrogen	1.3
Carbon	48.1
Chlorine	0.2
Hydrogen	5.8
Oxygen	37.8
Sulphur	0.1
Total ash content	6.7
Ash composition	
Silica expressed as SiO_2	22.1[a]
Iron expressed as Fe_2O_3	16.6[a]
Aluminium expressed as Al_2O3	10.7[a]
Phosphorus expressed as P_2O_5	4.5[a]
Calcium expressed as CaO	24.1[a]
Magnesium expressed as MgO	4.8[a]
Potassium expressed as K_2O	8.7[a]
Sodium expressed as Na_2O	0.6[a]
Manganese expressed as Mn_3O_4	0.1[a]
Sulphur sulphate expressed as SO_3	<0.3[a]
Titanium expressed as TiO_2	0.6[a]
Moisture content	61

Table 2.Composition of lavender distillation straws (LLDS) [4].

LLDS are basically used for soil replacement or as a fuel source (only if the moisture content is low), but more than 40 % is considered a waste. Other applications are to avoid the formation of algae in aquatic environments, to purify water or as thermal insulators for their high content in silica.

After distillation of lavender, still many phenolic compounds and lactones (anti-inflammatory) remains in the straws which can be extracted with organic solvents, the compounds obtained are different depending on the solvent used [5]. The exploitation of distilled plants constitutes an environmental sustainable measurement to reduce the residues generated every year from this industry, moreover the employment of distilled instead of non-distilled material minimizes the transmissions of odours and flavours [11]. Especially, aromas (coumarine) and antioxidants (rosmarinic acid) are found in LLDS. It also contains caffeic acid with antimutagenic activity, chlorogenic acid with anti-inflammatorys properties or sesquiterpenes like τ-cadinol, αcadinol and α-bisabolol which exhibit pharmacological properties [4].

3.4 Antioxidants

The antioxidant activity is used to protect food products from oxidative rancidity, loss of labile compounds, the formation of off-flavours in the food industry and moreover, to contribute additional physiological benefits over normal nutritional requirements. Nowadays, the most part of antioxidants used in food are artificial like butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) and are considered harmful for health, producing liver damage and carcinogenesis, are strongly allergenic, irritating and for these reasons consumers are demanding natural antioxidants. These artificial preservatives mentioned have been approved by the FDA (Food and Drug Administration) of the USA, and by the Regulation of the European Parliament and the European Commission (EC) no. 1223/2009 in the European Union with the condition that they cannot exceed 1% of volume products for phenoxyethanol and benzyl alcohol, and 0.6% for dehydroacetic acid [6]. Residue antioxidant activity results for a Soxhlet extraction with 96 % EtOH are in the *Table 3* [4].

	Extraction yield[a]	Total polyphenols[b]	Fe^{3+} reducing power[c]	DPPH inhibition activity[d]
Red grape pomace	n.d.	15.6	14.7	15.8
C. ladanifer SXEE	10.6	7.2	68.1	97.3
C. ladanifer USAEE	1.4	4.4	94.4	n.a.
L. × intermedia SXEE	14.8	5.3	55.5	81.5
L. × intermedia USAEE	10.5	1.6	n.a.	n.a.
S. rosmarinifolia SXEE	5.8	10.0	29.3	41.5
S. rosmarinifolia USAEE	1.9	6.0	89.3	87.2
T. mastichina SXEE	11.7	22.2	11.1	13.9
T. mastichina USAEE	7.1	7.7	33.5	37.4

n.d.: not determined.
n.a.: no available: absorbance below 0.5 A.U./activities below 50% in the range of concentrations assayed.
[a] Expressed as g dry extract/100 g dry plant material.
[b] Expressed as g/100 g dry extract.
[c] Concentration (μg/mL) required to obtain 0.5 A.U.
[d] IC_{50} (μg/mL).

Table 3. Extraction yields, polyphenols and antioxidant activity of *Lavandula angustifolia [4].*

As is seen the extraction yield from lavender residues was 14.8 g/100 g dry plant material.

3.5. Polyphenols

Phenolic compound are natural antioxidants that provide protection for plants against UV radiation, beneficial effects to the human body because of their anti-microbial, cardio protective, anti-allergenic and anti-inflammatory activities and they also preserve food against oxidation. They contain phenols, phenolic acids, coumarins and flavonoids [6]. The total phenolic content of the residue of *Lavandula angustifolia* was 5.3g/100 g dry mass according to literature [4].

The main compounds, characterized and quantified by gas chromatography-mass spectrometry are summarized in *Table 4*.

	Classes	Compounds	Percent
Cultivar Grosso	Lactones	Coumarin	16.9[a]
		Herniarin	16.0[a]
	Sesquiterpenes	τ-Cadinol	5.0[a]
		α-Cadinol	1.0[a]
		α-Bisabolol	9.6[a]
		10-Hydroxycadin-4-ene-3-one	1.2[a]
	Alcanes	Pentacosane	1.1[a]
		Hexacosane	5.1[a]
		Heptacosane	2.7[a]
Cultivar Super	Phenolic acids and derivatives	Protocatechuic acid	nd
		Caffeic acid	nd
		Rosmarinic acid	0.124[b]
		Rosmarinic acid methyl ester	nd
		Chlorogenic acid	0.215[b]
		Glycosylated *p*-coumaric acid	40.7[a]
		Glycosylated ferulic acid	24.6[a]
		Glycosylated caffeic acid	nd
		Glycosylated 3,4,5-trihydroxycinnamic acid	nd
	Flavonoids	Hexosides	
		Isoquercetin	nd
		Lutein-*O*-hexoside	3.3[a]
		Chrysoeriol-*O*-hexoside	nd
		Apigenin-*O*-hexoside	nd
		Glucuronides	
		Chrysoeriol-*O*-glucuronide	nd
		Luteolin-*O*-glucuronide	nd
		Aglycone	
		Apigenin	0.4[a]
		Luteolin	1.2[a]

Table 4. Percentage of phenolic compounds in Lavandula angustifolia residue [4].

3.6. Extraction techniques

3.6.1. Distillation

The distillation process consists in the separation of a volatile substance from a mixture with a non- volatile substance by using heat to evaporate it and a cooling to produce then its condensation. One of the applications of the distillation is to obtained the essentials oils of aromatic plants [12].

For essential oil distillation from plant sources, the plant material is soaked in water or a steam current is passed through the plant material and by simply heating, a steam current is generated containing volatile compounds according to their vapour-liquid equilibrium. The steam is then passed through to the condenser and two liquid phases appear and because of the exceed of maximum solubility of the essential oil in water, a miscibility gap is produced [12]. The most important condition to make possible this technique is that the volatile compound and impurities must be insolubility in water which will allow the separation of the product from the water [13].

It is a simple technique and it has some advantages like it does not produce any toxic waste and does not require any additives. But it has also disadvantages like the yields are often insufficient, at high temperatures some volatile compounds can be degraded (so is usually carried out at reduced temperatures) or that dissolved substances can affected the vapour-liquid equilibrium [12].

Two of the distillation techniques are described below, the steam distillation and the hydrodistillation.

Hydrodistillation

In laboratory practise, the plant material is charged into a round bottom flask with water with a concentration around 0.5 kg of plant material per litre of water. This method involves distillation by keeping the plant material in direct contact with water during 3 hours [14]. After, it is subjected boiling. In this method, the action of water on the material is maximized, therefore hydrolysis and oxidation can occur. Useful for materials that tend to caking (small flowers). The heterogenic steams produced condensate and the essential oil is separated by the difference of density [15].

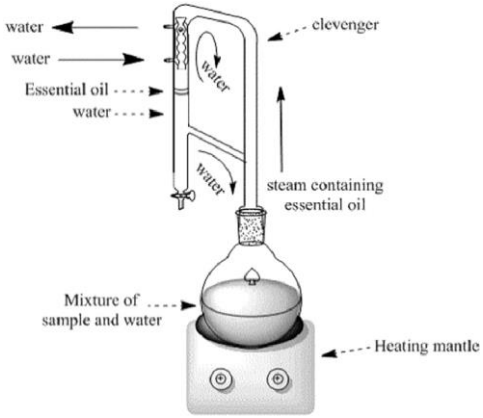

Figure 2. Hydro- distillation [15].

Steam distillation

The difference from the last one is that the water is not in contact with the plant material, the plant material is charged in a flask and a steam current is passed through carrying the volatile compounds and the following steps are the same as every distillation. The material must have the suitable size to stimulate the pass of the steam. Because of its low price, high yields and simplicity, this technique is the most appropriate for essential oils industry, it is recommended by several Pharmacopoeias [15].

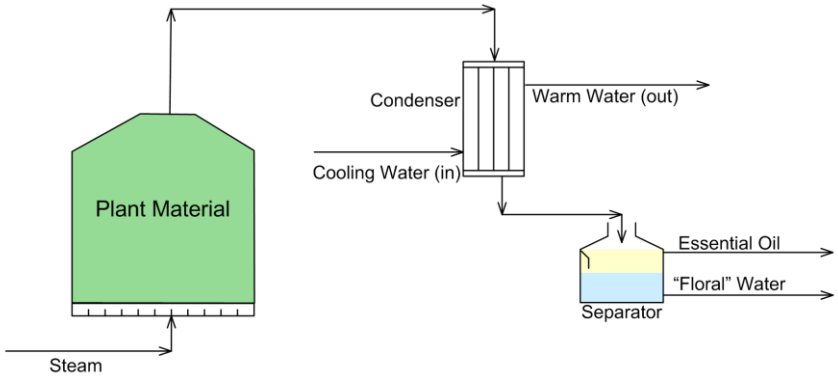

Figure 3. Steam distillation [15].

3.6.2. Solvent extraction

In solvent extraction the main objective is to separate certain substances contained in a solid. To separate these compounds the solid is contacted with a liquid phase. The two phases are in contact and the solute can diffuse from the solid to the liquid phase [16].

The choice of solvent for an extraction process depends on parameters such as solvent capacity, selectivity, and recovery costs. The toxicologic hazards associated with the use of a given solvent must be considered, even there is a growing awareness that certain solvents are carcinogenic. Because of this reason, aromatic solvents have been excluded from these methods and the residuals levels allowed in the product are fixed by the regulatory authorities [17]. Furthermore, there is a chance of thermal damage to the product during solvent recovery when solvents have a high boiling point. Aqueous alcohols or liquid carbon dioxide are solvents that avoid these problems [17].

This kind of extraction is used in industrial level in pharmaceutical industry for example: for penicillin production or in food industry for lipids extraction, decaffeination or flavours and aromas extraction [17].

Maceration

The plant material is soaked with a solvent (it can be water or an organic solvent) for a certain time at room temperature until it permeates and solves the soluble substances. Any covered vessel can be used. After 2- 14 days of periodic agitation, the liquid is filtered and the plant material is squeezed, the solvent is recovered in an evaporator and the extract is obtained. It is preferable to use an organic solvent because water can cause microbial degradation or rust formation [15].

Stirred tank

It is a similar method to maceration; the grounded plant material is put in contact with the solvent in a flask. The difference with the maceration is that temperature of extraction can be adjusted and is continuously stirred. The duration of the process is lower, around 3 hours, depending on extracting material. After all, the liquid is filtered and evaporated obtaining the extract [5].

Soxhlet extraction

It is developed using solvents with low boiling points, to avoid degradation of the sample. It is suitable for obtaining raw extracts of plants [13].

In laboratory scale, the ground material is placed in a porous bag, called thimble made of strong filter paper, which is placed in the chamber (E) of the Soxhlet apparatus (*Figure 4*). The extracting solvent in the flask (A) is heated and its steams condense in the condenser (D). The condensed solvent falls into the thimble containing the plant material and extracts it by contact. When the level of the liquid in the chamber rises to the top of the siphon tube (C), the liquid goes into the flask. This process is continuous and is carried

out until the solvent is totally clear. The advantage is that large amount of plant material can be extracted with much smaller quantity of solvent than in the others methods, having effects in terms of time, energy and financial inputs [18].

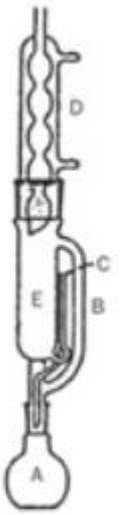

Figure 4.Soxhlet apparatus [18].

3.6.3. Supercritical fluid extraction (SFE)

When a gas is compressed to a high pressure, it becomes liquid but if a gas is heated beyond a specific temperature and no amount pf compression will cause it to become a liquid. This specific temperature is called critical temperature (T_C) and the corresponding vapour pressure is called critical pressure (P_C). A supercritical fluid is when the state of the substance exceed the critical temperature and pressure. In the critical region a substance exhibits a liquid- like density and much increased solvent capacity [19].

The most desirable SCF solvent for extraction of natural products for foods and medicines today is carbon dioxide (CO_2). It is an inert, inexpensive, easily available, odourless, tasteless, environment friendly and GRAS (generally regarded as safe) solvent [19].

Supercritical fluid extraction can be carried out in a high-pressure apparatus equipped with an extractor vessel and two separators connected in series, as in *Figure 5*.

Liquid CO_2 (1) is cooled (2), compressed to a desired pressure by a pump (3) and heated (4) to an extraction temperature and to bring it into the supercritical state it is passed into the extraction vessel with the plant material. The solution leaves the extractor and reduces its pressure. Then, it flows into the first separation vessel, where the supercritical fluid extract (SFE) is collected and the rest of the solution goes to the second separation vessel where CO_2 is evaporated, SFE (mostly volatile compounds) is recovered [19].

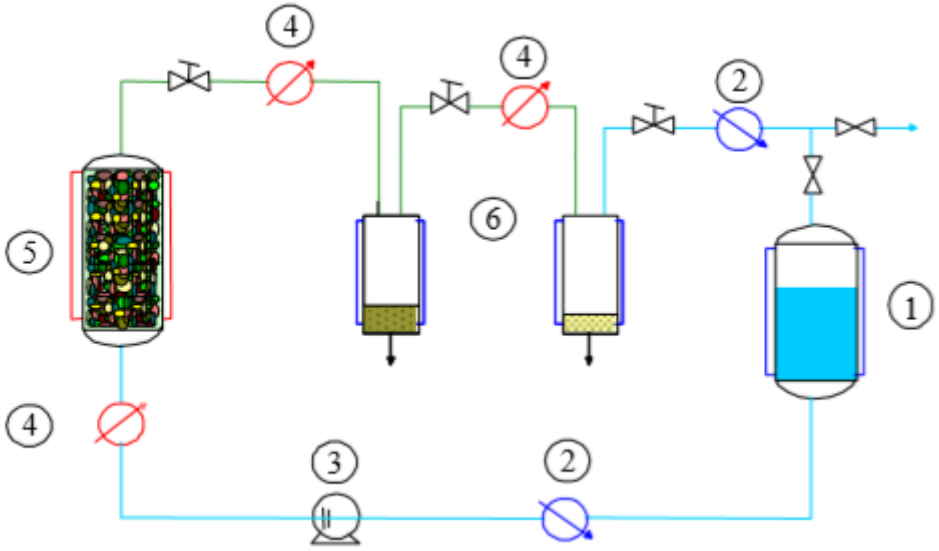

Figure 5. Supercritical fluids extraction diagram [19].

Some industrial applications are polymer recycling, decaffeination of coffee, extraction medical plants, hops, oils, neutralization and impregnation of paper and decontamination of soils [19].

4. MATERIALS AND METHODS

4.1. MATERIALS

4.1.1. Plant materials

Fresh lavender was steam distilled (L2/1) and hydrodistilled (L2/2) in pilot plant distillation apparatus in the lab in June 2017. Lavender steam distilled residue is light green with soft smell, while lavender hydrodistilled has a darker green colour and a soft smell. The distilled plant material was dried, packed and stored for further use. The dry distilled residues contain leaves, stems and flowers of lavender (*Figure 6*).

Before experiments, the residues were ground in Fritsch cutting mill using 1 mm sieve plate.

Figure 6. Dry lavender distilled residue sack before grinding.

4.1.2. Chemicals

Along the process, different chemicals were used to reach the objectives:

- Ethanol (C_2H_6) supplied by Molar Chemicals Kft. Purity: 96.08%.
- Ethyl-acetate ($C_4H_5O_2$) supplied by Molar Chemicals Kft. Purity: 99.98%.
- *n*-Pentane (C_5H_{12}) supplied by Molar Chemicals Kft. Purity: 98.03%.
- Acetone (C_3H_6O) supplied by Molar Chemicals Kft. Purity: 99.95%
- Propanol (C_3H_8O) supplied by Molar Chemicals Kft. Purity: 99.92%
- Distilled water: from laboratory.
- Hide Power (from bovine hide) supplied by Sigma-Aldrich Co.
- Sodium Carbonate (Na_2CO_3) supplied by Sigma-Aldrich Co.
- DPPH or 2,2-diphenyl-1-picrylhydrazyl, free radical ($C_{18}H_{12}N_5O_6$) supplied by Sigma-Aldrich Co (*Figure 7*).

- Folin-Ciocalteu's phenol reagent supplied by Merck Kft (*Figure 8*).
- Methanol (CH_4O) supplied by Molar Chemicals Kft. Purity: 99.99%.

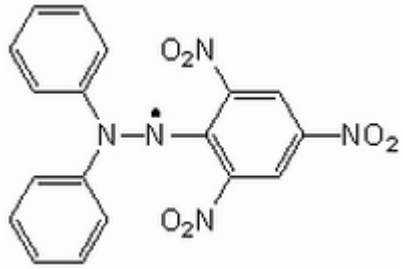

Figure 7. DPPH molecule.

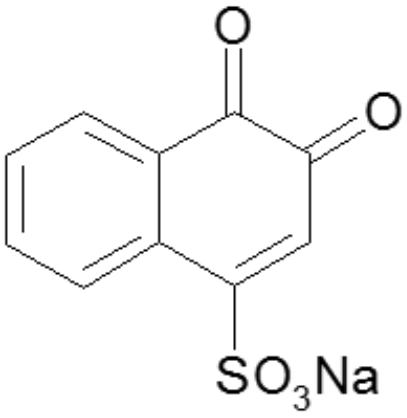

Figure 8. Folin Ciocalteu's molecule.

4.2. METHODS

4.2.1. Grinding

The hole sack full of plant material was grinded in Fritsh cutting-mill with different size of sieve inserts, from 1mm till 4 mm. Because of the shape and hardness of the stems, flowers and leaves of the distilled residue of *Lavandula angustifolia* the sieve insert of 1 mm was enough to grind everything.

The efficiency of extraction depends mostly on the particle size of raw material. Grinding and particle size distribution evaluation are crucial to characterise the plant material.

4.2.2. Particle size determination

With the objective of known the particle size distribution, the particles have been separated in different size fractions, therefore a Retsch vibratory sieve shaker was used (*Figure 9*).

1. A selection of the configuration of the sieves plates were chosen as follows: 1.4;1.25; 0.8; 0.63; 0.5; 0.4; 0.25; 0.1; <0.05 mm.
2. The sieves were weighted before the sieving.
3. The sieves were in the decreasing order and placed them onto the sieve stack.
4. Around 60 grams of ground *Lavandula angustifolia* residue were weighted on the top of sieves.

5. Top was clamped and machine was set for 20 minutes and at 40 Hz vibratory force.
6. After, each sieve was weighted back.
7. The percentages of particles on each sieve were calculated.

The experiment was repeated three times, calculating the average for both lavender samples.

Figure 9. Retsch vibratory sieve shaker.

4.2.3. Moisture content determination

The moisture was determined according to the gravimetric method.

1. Three parallels of similar quantity of ground plant material was weighted in a Petri glasses (M1).
2. The glasses were put in the oven for minimum of 24 hours at 105 ºC until mass-permanency.
3. The glasses were taken out of the oven and let them cool until room temperature.
4. Weigh the Petri glasses (M2).

The calculation of the moisture follows the next equation:

$$\%Moisture = \frac{M1 - M2}{M1} * 100$$

M1, is the weight of the plant before drying.

M2, the weight of dry (moisture-free) material.

The moisture content was calculated for both plant materials, the lavender steam-distilled residue and the hydrodistilled residue, respectively.

4.2.4. Extractions

4.2.4.1. Soxhlet extraction

It can be described as a method to obtain soluble compounds from a solid material, it is possible with a Soxhlet extractor unit. It uses a special Soxhlet apparatus in lab scale, which is suitable for extraction of solids with different solvents.

A Soxhlet apparatus is a laboratory device with it desired compounds from a solid material can be extracted accordingly the solubility power of applied solvent.

Unit setup (*Figure 10*):

- Heater. It is a vessel full of silicone oil with a resistance that contribute the necessary heat to boil the solvent.
- Glass rounded-bottom flask (A). It is the glass that contains the solvent and is submerged in the oil bath.
- Condenser (C). A jacketed vessel using water which induce the condensation of the solvent that arrives as a vapor and falls down in a liquid phase
- Paper Thimble. Filter made of paper that holds the plant material.
- Soxhlet apparatus (D). Glass where the thimble is placed and where the condensated solvent falls removing some compounds from the plant material.

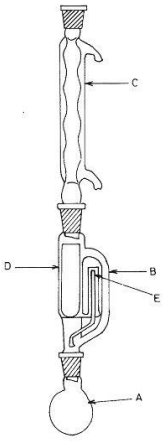

Figure 10. Soxhlet apparatus.

Process

- The paper thimble fill with the ground material and weighed between 15-20 g/each.
- A cotton wool was placed onto the top of ground material to avoid particles coming out from the paper filter.
- The thimble was placed into the glass Soxhlet apparatus.
- Depending of extraction, 250 ml of different solvents were poured in the rounded-bottom flask.
- Carefully, the Soxhlet extraction unit was assembled.
- Switch on water steam for cooling.

- Switch on heating element and control the temperature of oil bath above the boiling point of the used solvent.

When the solvent reaches its boiling point, it transforms to a vapour phase and rises up the tube of the Soxhlet apparatus arriving to the condenser, where the cooling water cools the solvent and changes into a liquid phase dropping onto the filled thimble vessel. The solvent crosses the filter taking the soluble particles with it. When the thimble vessel is almost fully, it is emptied by the siphon, the solvent returns to the flask, where it evaporates again. This cycle is repeated many times, over hours and days until the condensed solvent becomes clear.

Figure 11 shows the first stage of the extraction of lavender residue while *Figure 12* shows the end of the extraction.

Figure 11. First stage of the Soxhlet extraction.

Figure 12. Last stage of the Soxhlet extraction.

After extraction; the solvent was removed by rotary evaporator until the extract contained no solvent. The dry extract was then weighted back and the yield of extraction was calculated. Three parallel measurements were carried out.

4.2.4.2. Stirred tank extraction

During stirred tank extraction (*Figure 13*) the raw material and the solvent are in direct contact for the time of extraction at a chosen temperature with continuous stirring with a collapsible blade stirrer at a set stirring speed.

Different solvents and solvent mixtures can be applied with different extraction time and at different temperatures. The feed to solvent ratio can also be adjusted and optimized.

- Approximately 25 g of plant material was put on a glass round-bottom flask with 250 ml of solvent. In this work, EtOH (96 %), 70% EtOH:H_20; 50% EtOH:H_20 and water (100%) was used.
- This mixture was subjected to agitation at 250 rpm for 3 hours in a water bath at 40-45 ºC.

After the extraction finished; the mixture was filtrated using the vacuum filtration of Buchner to eliminate the residue of extraction, then the solvent was evaporated with a rotary-evaporator. The mass of extract was weighted and yields were calculated.

Figure 13. Stirred tank extraction.

4.2.4.3. Laboratory hydrodistillation

This separation process was used to recover any trace of essential oil left in the steam- and hydrodistilled residues.

- To proceed, around 150 g of lavender residue was weighted with 1000 ml of distilled water and put on a big glass rounded-bottom flask.
- It was connected to a Clevenger distillation unit (*Figure 14*) and a cooling apparatus. Distilled water was filled into the Clevenger unit to be able to recover the essential oil from the top of the water.

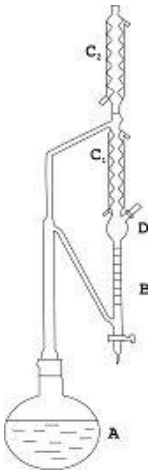

Figure 14. Clevenger distillation unit.

- Switch on the cooling water and the heating.
- Th duration of the distillation is 3 hours until essential oil appeared above the water, see in *Figure 15*.

Figure 15. Essential oil on the top of water in Clevenger distillation unit.

- For collecting the essential oil, first a valve at the bottom of Clevenger unit was opened, water withdrawn; then the volume of essential oil was measured in the volumetric burette unit and emptied.

In this process, the water boils and the steam drag the volatile compounds reaching the cooling and it is there where they transform into liquid phase both the water and the essential oil, because of their differences of density an interphase appears and it can be separated by decantation.

4.2.5. Chemical analysis

4.2.5.1. Antioxidant test

One of the most popular methods to measure the antioxidants is the DPPH method. The molecule of 1,1-diphenyl-2-picrylhydrazyl (DPPH) is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule. The delocalisation also gives rise to the deep violet colour, characterised by an absorption band in ethanol solution centred at about 520 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form with the loss of this violet colour. It should be evident that the method is a colorimetric titration, although the slowness of the overall reaction (with mixtures having to be left for 30 minutes before the absorbance reading is taken) complicates the experimental procedure [20].

Procedure:

- Set the spectrophotometer at 517 nm wavelength and use clear methanol (MeOH) solution as the blank.
- DPPH solution is prepared in methanol at a concentration of 0.4 mg/ml.
- Part of this solution is diluted with methanol in another flask to reach an absorbance between 0.7-0.9.
- Prepare the extract sample solution with a concentration of 0.5 mg/ml in MeOH of extract.
- In each cuvette 2.5 ml of the diluted DPPH solution were pipetted and an increasing volume of sample solution (0-500 µl) was added.
- Each cuvette must be homogenized in the vortex, covered with foil, kept in dark for 30 minutes at room temperature.
- Measure each cuvette in the spectrophotometer.
- Three parallel measurements for each concentration for each extract were carried out.

  To determine the antioxidant activity for each cuvette is necessary to use *Equation 1*.

$$Inhibition(\%) = \frac{A0 - A1}{A0} * 100$$

Equation 1. Antioxidant activity

A_0, is the absorbance of the diluted solution without extract sample (control DPPH solution).

A_1, is the absorbance of a cuvette with extract solution.

The data of interest is the "efficient concentration", called the IC_{50} value, and is defined as the concentration of substrate that causes 50% loss of the DPPH activity, it corresponds

to the endpoint of the titration. It is important that the lower is the value of IC50, the higher is the antioxidant activity [20].

The change of colour of DPPH solution indicated that the extract has antioxidant activity at the applied concentration which can also be measured by spectrophotometer (*Figure 16*).

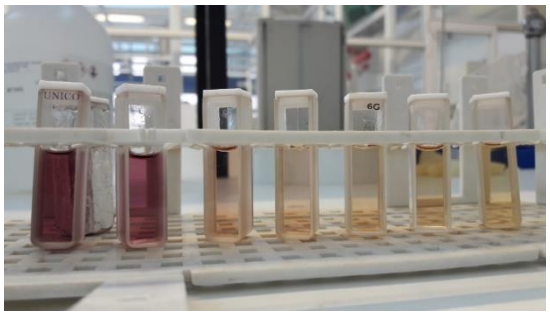

Figure 16. Cuvettes containing the samples at different concentrations.

4.2.5.2. Polyphenol measurements

The content of total polyphenols was measured by a spectrophotometric method at 760 nm, using pyrogallol as a reference standard, method described in Hungarian Pharmacopeia [21]. This method is based on the formation of blue-coloured products by redox reaction with Folin-reagent. Polyphenols reduces the yellow-coloured Folin-Ciocalteu reagent at base pH, therefore blue-coloured Mo- and W- oxides are produced, which has absorbance maximum at 760 nm.

The method is based on the quantitative measurement of produced blue-coloured complexes, which is equivalent of polyphenol content of extracts. As a reference material: pyrogallol is used and for calculation firstly a pyrogallol calibration curved is measured.

Pyrogallol calibration

First, it is necessary to make a pyrogallol calibration:

- Weight out 40 mg of pyrogallol in a volumetric flask of 20 ml, fill until the sign with distilled water. Repeat it three times.
- From the first solution, get 1 ml of this solution into 20 ml if volumetric flask and mix with 96% EtOH and another of 1,6 ml and add EtOH too.
- From the second solution, take 2.2. ml in one flask and 2.8 ml in another flask.
- From the third solution, take out 3.4 ml and fill the flask with EtOH.
- From each of the 5 flask, get 800 µl of this solution of pyrogallol and mix with 400µl Folin-Ciocalteu reagent, 4 ml distilled water and 14,8 ml Na_2CO_3(29 g/l) into a 20 ml of dark volumetric flask.

- Homogenize with the vortex.
- Leave at room temperature during 30 minutes.
- Take notes of the absorbance of the 5 solutions at 760 nm.
- Represents graphically the absorbance with the pyrogallol concentration, obtaining the slope (Appendix, *Figure 72*); which was used in the calculation.

Polyphenols content

- Extract solution is prepared with a concentration between 0.5-2.5 mg/ml of extract in 96% EtOH.
- This solution is put in the ultrasound bath to ensure all extract is solved in EtOH.
- In a dark volumetric flask of 20 ml put:
  - 800 µl of the sample solution
  - 4 ml of distilled water
  - 400 µl Folin-Ciocalteu reagent
  - Na_2CO_3 (29 g/l) solution until reach the line of the flask (set basic pH).
- Three parallel measurements were carried out for each extract solution.
- Homogenize with the vortex.
- Leave at room temperature during 30 minutes.
- Set the wavelength on the spectrophotometer at 760 nm and used distilled water as the blank.
- Pour some solution from each flask in a cuvette and measure the absorbance.
- The results were calculated on the slope of the pyrogallol curve and expressed as g pyrogallol equivalent / 100 g extract (%).

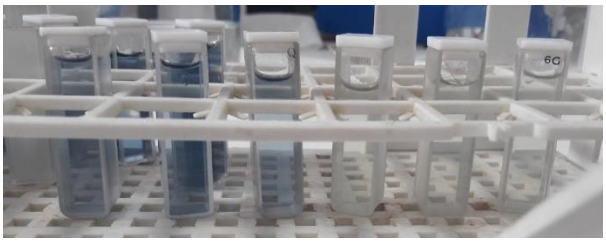

Figure 17. Cuvettes with samples to measure polyphenols content.

Colour also indicates the concentration of polyphenols in the extract. For example: EtOH extract is blue because contains high concentration of polyphenols while extract of pentane is clear (*Figure 17*).

4.2.5.3. Tannin measurements

The same procedure was used as in polyphenols measurements. Firstly, the tannins were adsorbed onto the surface of hide powder, therefore the concentration of the tannin-free polyphenols were evaluated.

- An extract solution with a concentration of 0.5-2.5 mg/ml of extract in EtOH 96%.
- Extract solution is put in the ultrasound bath.
- 10 ml of extract solution is mixed with 100 mg of hide power and placed again in the ultrasound bath for an hour.
- Filter the solution
- In a dark volumetric flask of 20 ml poured:
  - 800 µl of the sample solution
  - 4 ml of distilled water
  -400 µl Folin-Ciocalteu reagent.
  - Na_2CO_3 (29 g/l) solution until reach the line of the flask (to set basic pH)
- Three parallel measurements were carried out for each extract solution
- Homogenize with the vortex.
- Leave at room temperature during 30 minutes.
- Set the wavelength on the spectrophotometer at 760 nm and used distilled water has the blank.
- Pour some solution from each flask in a cuvette and measure the absorbance.

From measuring the tannin- free polyphenols (g pyrogallol equivalent/ 100 g extract), the concentration of tannin can be evaluated by:

$$\%\ of\ tannin = \Sigma\ polyphenols(\%) - \Sigma\ tannin\ free\ polyphenols(\%)$$

The tannin content is equivalent with the difference between the total polyphenol content and the polyphenol content remained after the tannins were absorbed by hide power.

5. RESULTS AND DISCUSSIONS

5.1. Results of Particle size determination

5.1.1. Lavender steam distilled residue (L2/1)

The results and estimations of each test are in *Appendix 8.1.1.*

The experiment was repeated three times to get an average and a standard deviation of the percentage of residue in each plate (*Table 5*).

Size (mm)	%average	Standard deviation
1,4	0,15	0,05
1,25	0,08	0,12
0,8	1,04	0,07
0,63	4,56	0,58
0,5	13,10	0,80
0,4	17,72	0,78
0,25	31,66	0,85
0,1	28,41	1,26
<0,05	2,98	0,51

Table 5. Results of the particles size.

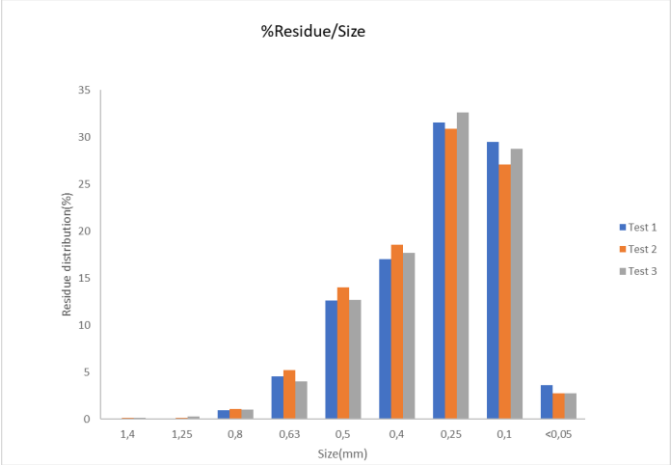

Figure 18. Abstract of the particle size.

It is seen that the size of more than 50% of ground particles of L2/1 were between 0.1-0.25 mm. The most common size of particle is 0.25 mm with a percentage of particles around 32 %.

5.1.2. Lavender hydrodistilled residue (L2/2)

The results of measurements are in *Appendix 8.1.2.*

Taking into account the three experimental cases, the abstract is represented in the following table., *Table 6.*

Size (mm)	%Average	Standard desviation
1,4	0,25	0,08
1,25	0,23	0,08
0,8	1,71	0,21
0,63	6,52	0,63
0,5	16,00	1,10
0,4	17,64	1,23
0,25	30,35	0,74
0,1	23,84	3,67
<0,05	2,89	0,11

Table 6. Results of the particle size

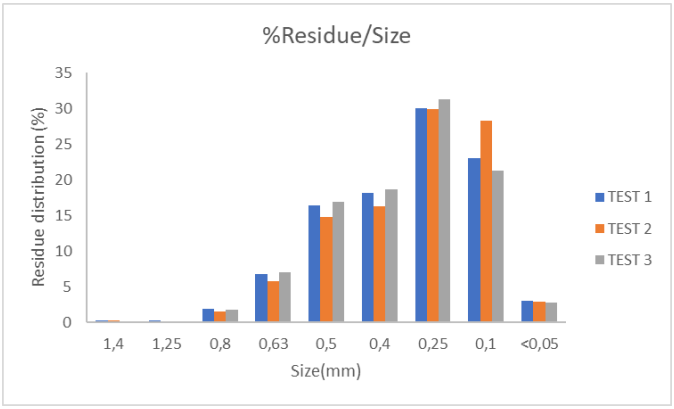

Figure 19. Abstract of the particle size.

In the second case, the most common size of particle is 0.25 mm but with a percentage around 30 %. Also, more than 50 % of particles has a particle size equal and smaller than 0.25 mm.

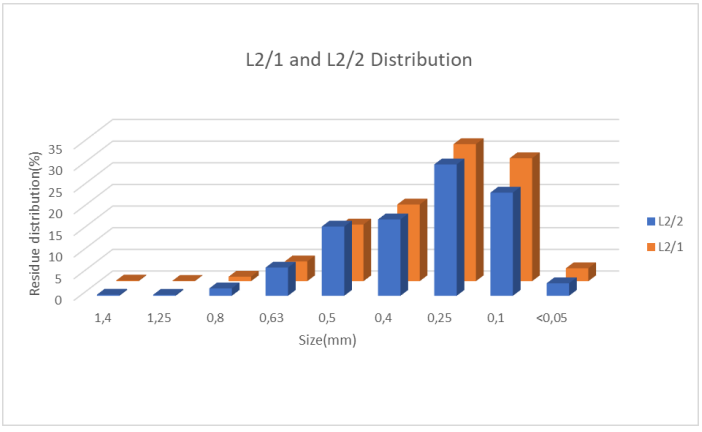

Figure 20. L2/1 and L2/2 Comparation

Comparing with the previous one, in Lavandula L2/1 the percentage in 0.25 mm particle size is 31 % and in the lavender L2/2 is lower with a percentage of 30%, but it is a small difference (*Figure 20*). The particle size distribution of both samples are the same, as same milling procedure and sieve plate was used during the grinding of both samples.

5.2. Results of Moisture determination

5.2.1. Lavender steam distilled residue (L2/1)

	Test 1	Test 2	Test 3
Glass mass (g)	116,73	115,13	105,15
Plant and glass mass (g)	126,33	126,18	115,61
Plant mass (g)	9,6	11,05	10,46
After drying mass (g)	125,54	125,29	114,78
Dry plant mass (g)	8,81	10,16	9,63
Dry mass (%)	91,77	91,95	92,07
Moisture (%)	8,23	8,05	7,93

Table 7. Moisture content of the lavender steam residue (3 parallel measurements)

To determinate the dry content, the average and standard deviation of the three measurements are estimated and showed in the following table, *Table 8*.

	Average	Standard desviation
Dry content (%)	91,93	0,15

Table 8. Average moisture content of lavender steam residue (L2/1)

In conclusion, the *Lavandula angustifolia* steam distilled residue has a 91.93 ± 0.15 % of dryness, the rest was moisture.

5.2.2. Lavandula hydrodistilled residue(L2/2)

	Test 1	Test 2	Test 3
Glass mass (g)	112,46	100,88	105,15
Plant and glass mass (g)	127,34	131,96	124,52
Plant mass (g)	14,88	31,08	19,37
After drying mass (g)	126,22	129,58	123,11
Dry plant mass (g)	13,76	28,7	17,96
Dry mass (%)	92,47	92,34	92,72
Moisture (%)	7,53	7,66	7,28

Table 9. Moisture content of lavender hydrodistilled residue (3 parallel measurements).

In this case, the average and deviation are the ones presented in *Table 10*.

	Average	Standard desviation
Dry content (%)	92,51	0,19

Table 10.Average moisture content lavender hydrodistilled residue (L2/2).

For the *Lavandula angustifolia* hydrodistilled residue the dry content is 92.51 ± 0.19 %.

So, comparing both types of lavender residues, the lavender steam residue contained more moisture than the lavender hydrodistilled, but the different is less than 1%.

5.3. Results of extractions

5.3.1. Soxhlet extraction

From the quantity of each extract obtained with different solvent extraction yield can be calculated representing as g of extract/ 100 g dry plant material.

5.3.1.1. Lavandula steam distilled residue (L2/1).

From three parallel measurements average yields with standard deviation were calculated (*Appendix 8.2.1*)

Solvent	Y(%)	Standard deviation
N-pentane	2,70	0,07
Etil-acetate	7,47	0,32
Acetone	8,56	0,07
Propanol	10,59	0,59
EtOH	22,91	0,58

Table 11.Yields of Soxhlet extraction using different solvent (L2/1)

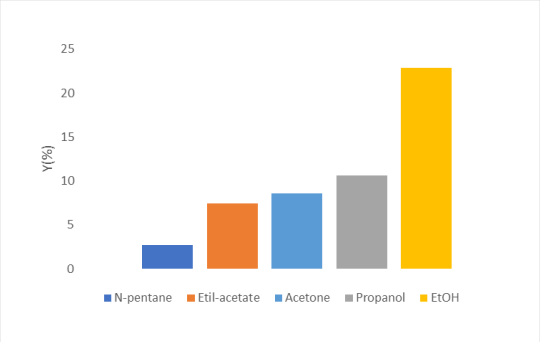

Figure 21. Extraction yield of L2/1 with different solvents.

In the previous graph is shown the yields of extract obtained with each solvent (*Figure 21*). It can be seen that the yields increased with the applied solvents in the following: *n*-pentane < ethyl-acetate< acetone< propanol< ethanol. The numeric data are represented in *Table 11*. The yields were between 2.7-22.9 g/100 g dry mass depending on the solvent used.

Figure 22. Extracts from Soxhlet extraction (L2/1)

As is showed in the picture (*Figure 22*), the extracts of the *n*-pentane, ethyl-acetate, acetone, propanol and EtOH solvents are shown respectively. The *n*-pentane extract has a dark colour with strong smell and high viscosity, the ethyl-acetate has an appearance like dust, is pea green and has a soft smell, the acetone is similar to ethyl-acetate it only differs in the colour that is darker, the propanol one is like dust but with bigger grains and darker colour, the smell is also soft. The EtOH-extract is gelatinous with high viscosity, dark green almost black and a strong smell of lavender. With EtOH almost 10 times more extract was obtained than with *n*-pentane.

5.3.1.2. Lavandula hydrodistilled residue (L2/2).

For lavender hydrodistilled residue yield of extract obtained in Soxhlet extraction for each solvent is reflected in *Table 12*. Detail results are in *Appendix 8.2.2.*

Solvent	Y(%)	Standard deviation
N-pentane	3,19	0,13
Etil-acetate	8,59	0,18
Acetone	9,59	0,16
Propanol	11,40	0,19
EtOH	26,08	0,33

Table 12. Yield of Soxhlet extraction using different solvents from L2/2 residue.

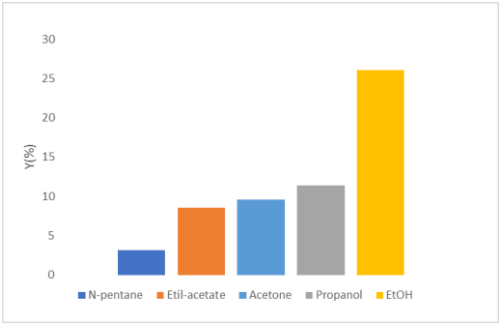

Figure 23. Extraction yields of L2/2 with different solvents.

For the lavender hydrodistilled residue, the solvents follow the same trend as the lavender steam distilled residue (Figure 23). The yields were between 3.2- 26.1 g/100 g dry mass respectively.

Figure 24. Extracts from Soxhlet extraction(L2/2)

The pentane extract has a sticky texture, dark green colour and strong smell, while ethyl-acetate extract is large particles of green extract with soft scent. The acetone one is similar to the ethyl-acetate, the only difference is that the particles are smaller. The propanol extract is the same as the previous one with a smaller size of particles. The EtOH extract is like dark green dust with strong scent, see each extract in *Figure 24*.

The appearances of extracts from L2/1 and L2/2 are very similar, although the extraction yields show differences.

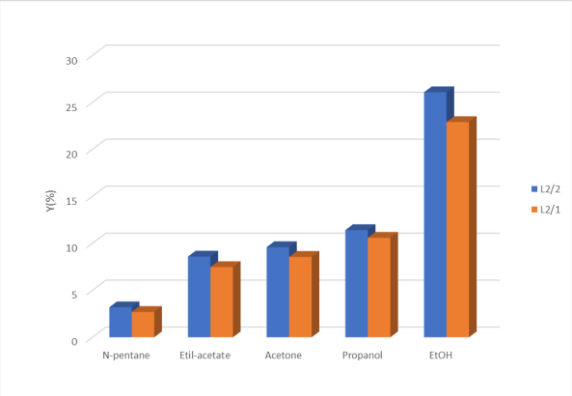

Figure 25. Extracts of L2/1 and L2/2

Comparing the extraction yields of two samples (*Figure 25*), it can be seen that higher yields were obtained from the lavender hydrodistilled residue (L2/2) by around 1% for all solvents were applied. In literature [4] 14.8 g/100 g dry material yield was obtained with 96% EtOH from steam distilled lavender, which correlates well with our results. We obtained almost twice amount of extracts from steam distilled and hydrodistilled residues comparing the results to the literature data. The difference might have caused by the differences in raw materials; growing conditions of plants; pre-treatment and distillation process used.

5.3.2. Stirred tank (ST) extraction

Below, yields obtained with different solvents are represented from lavender steam-, and hydrodistilled residue.

5.3.2.1. Lavender steam distilled residue (L2/1)

The following table, *Table 14* shows the extract yields of stirred tank extraction working with lavender steam distilled residue with different percentages of EtOH in water, *Table 13*. Detail measurements can be found in *Appendix 8.3.1*.

Solvent	Y(%)
EtOH 96%	6,88
EtOH 70%	18,89
EtOH 50%	23,86
Water	27,96

Table 13. Yields of lavender steam distilled residue (L2/1) at stirred tank experiments.

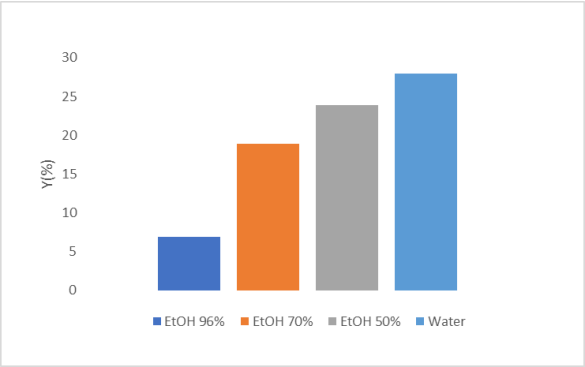

Figure 26. Extraction yield of L2/1 from ST experiments.

The yields increase with the percentage of water added to ethanol; it was the highest carrying out the extraction with 100 % water (27.96 %). It is needed to note that the evaporation of water from extract was extremely difficult and had taken couple of days.

Figure 27. Extract from ST extraction(L2/1)

EtOH 96 % extract has a browner colour, floury texture and soft smell. EtOH 70% has sticky and liquid texture and brownish colour, EtOH 50% has brown crystal texture and last one, the water extract is sticky, brown with strong scent, as is seen in *Figure 27*.

5.3.2.2. Lavender hydrodistilled residue (L2/2).

The yields of lavender hydrodistilled residue (L2/2) using ethanol: water solutions with different percentage of water are summarised below, *Table 14.* All data are shown in *Appendix 8.3.2*

Solvent	Y(%)
EtOH 96%	7,85
EtOH 70%	21,87
EtOH 50%	26,42
Water	17,94

Table 14. Yields of lavender steam distilled residue (L2/2) at stirred tank experiment.

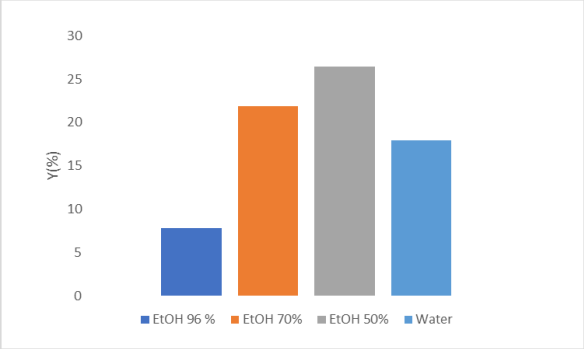

Figure 28. Extraction yields of L2/2.

It can be seen that the extraction yields increase with the percentage of water in ethanol: water solutions. The yield obtained in water extraction shows lower yield, because some part of extract was lost during the evaporation of water. Also, it is seen that the lavender hydrodistilled has more mass of extract with the exception of the water. The results are similar to those obtained by Soxhlet extraction.

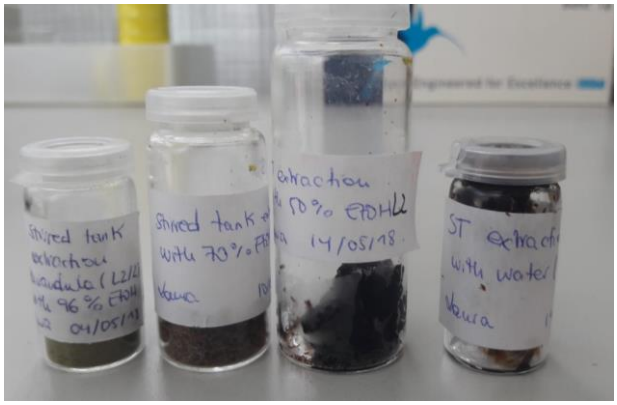

Figure 29. Extracts from ST extraction(L2/2)

EtOH 96 % extract has a dusty texture, green and with a soft smell, while the EtOH 70 % extract is like little brown crystals. The EtOH 50 %has a sticky texture but solid, while the water extract has a sticky and liquid texture.

In the stirred tank extraction experiments more extracts were obtained from the hydrodistilled lavender residue; by 1-3%; similarly, the yields obtained with Soxhlet extraction.

5.3.3. Laboratory hydrodistillation

Hydrodistillation of lavender residues L2/1 and L2/2 were carried out in laboratory apparatus.

From steam distilled lavender residue (L2/1) only 0.01 ml of yellow coloured oil was recovered. In the case of the lavender hydrodistilled residue (L2/2), after 3 hours of distillation 0.005 ml were obtained, the colour was clear and a little yellow.

Lavender residue	Y(%)
L2/1	0,007
L2/2	0,004

Table 15. Essential oil obtained in lab hydrodistillation

In conclusion, the L2/2 lavender was almost fully distilled in pilot plant distillation unit while the L2/1 contained little essential oil so that means that the steam distillation was not as effective as the hydrodistillation.

The calculations are presented in *Appendix 8.4.*

5.4. Results of chemical analysis

5.4.1. Results of antioxidant activity measurements.

5.4.1.1. Lavender steam distilled residue (L2/1) from Soxhlet extraction.

The results of antioxidant activity measurements of extracts obtained by Soxhlet extraction with different solvents from lavender steam distilled residue (L2/1) are summarised in *Table 16*. The rest of data can be found in Appendix 8.5.1.1. The scavenging activity on DPPH radical is expressed as IC_{50}, which the concentration (µg extract/ ml test solution) of extract that causes 50 % loss of the DPPH activity.

Solvent	IC50 (µg/ml)
N-pentane	176,62
Ethyl-acetate	94,45
Acetone	82,85
Propanol	76,20
EtOH	39,10

Table 16. IC_{50} (µg/ml) of L2/1 extracts.

The results show that we need the least concentration from Ethanol extract; which shows 50 % inhibition only in 39 µg/ml concentration. From the other extracts higher concentration is needed to achieve 50 % of loss of DPPH activity.

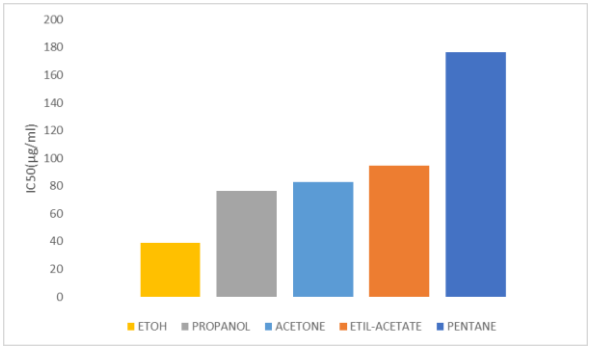

Figure 30. Antioxidant activity of L2/1 extracts of different solvents.

Ethanol is the best solvent to extract antioxidant- rich extract, which acts strongly in a DPPH test even in small concentration (39 µg/ml).

5.4.1.2. Lavender hydrodistilled residue (L2/2) from Soxhlet extraction.

The results of antioxidant activity measurements of extracts obtained by Soxhlet extraction with different solvents from lavender hydrodistilled (L2/2) are summarised in *Table 17* and results took in the lab can be found in *Appendix 8.5.1.2.*

Solvent	IC50 (µg/ml)
N-pentane	258,67
Ethyl-acetate	124,75
Acetone	94,41
Propanol	86,49
EtOH	47,30

Table 17. IC_{50}(µg/ml) of L2/2 extracts.

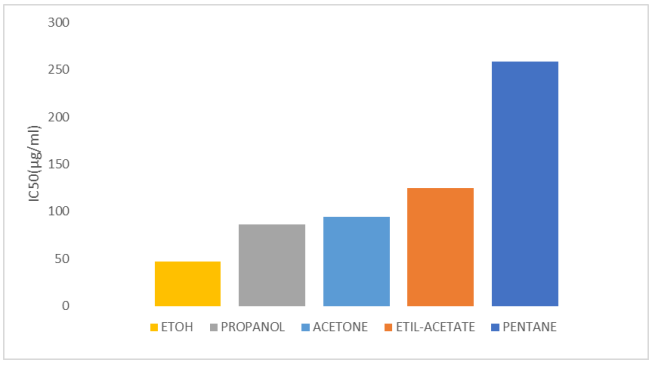

Figure 31. Antioxidant activity of L2/2 extracts with different solvents.

Seeing *Figure 31*, the order of the solvents is the same that of the previous lavender steam distilled residue, so it behaves in the same way. The lowest IC_{50} concentration is found in the ethanolic extract too (47.30 µg/ml).

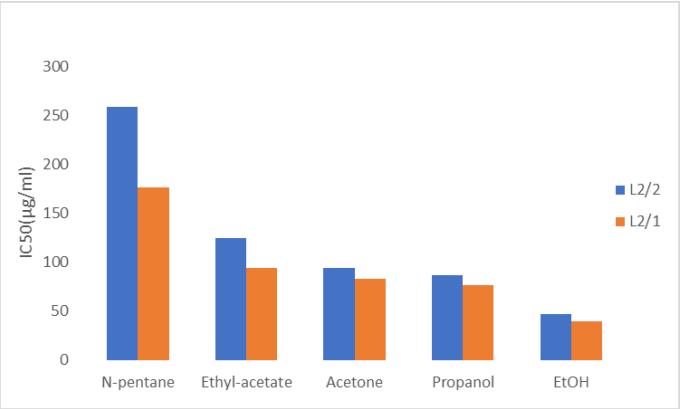

Figure 32. IC_{50} of L2/1 and L2/2.

As it is seen, graphs from both residues are similar that means that the solvents work the same independently of the type of residue, so the best solvent to obtain the antioxidants from lavender is also the EtOH.

Comparing the results from the same solvent it is seen that the lavender steam distilled residue has a smaller IC_{50} what means that the quantity of antioxidants obtained are more in the extracts of lavender steam distilled residue. It shows that the more gentle steam distillation is better to protect the compounds in the residue, which possess antioxidant activity.

5.4.1.3. Lavandula steam distilled (L2/1) from stirred tank extraction.

The antioxidant activity measured for the lavender steam distilled residue from stirred tank experiments with different percentages of ethanol: water is showed in *Table 18*, expressed as IC_{50} (µg/ml). See the detail results in *Appendix 8.5.2.1*

Solvent	IC50 (µg/ml)
Water	11,01
EtOH 50%	20,57
EtOH 70%	22,90
EtOH 96%	45,47

Table 18. IC_{50} of extracts from ST experiments (L2/1).

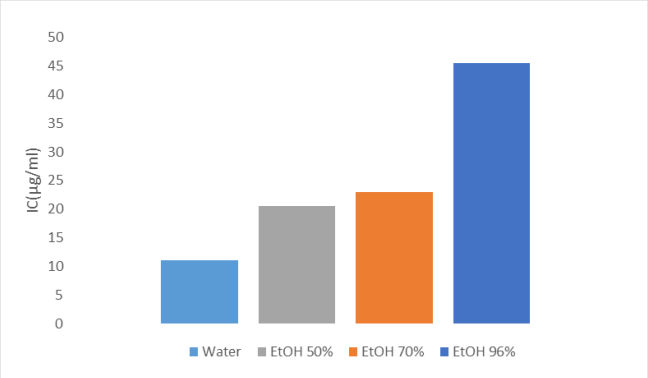

Figure 33. Antioxidant activity of extracts from ST experiments of (L2/1 residues).

It can be seen that the antioxidant activity is better in the presence of extracts obtained with higher water content H_20: EtOH solutions. The least amount of extract was needed for 50 % loss of DPPH activity from H_20 extract; which caused a 50 % loss of DPPH activity just at 11 µg/ ml concentration. The molecules, which show antioxidant activity in this test method are not heat- sensible.

5.4.1.4. Lavandula hydrodistilled residue(L2/2) from stirred tank extraction.

The detail results are in *Appendix 8.5.2.2* but below in *Table 19* a summary of the results can be found, in which the antioxidant activity for hydrodistilled residue from Stirred tank extraction is reflected with different percentages of H_20: EtOH, expressed as IC_{50} (µg/ml).

Solvent	IC50 (µg/ml)
Water	19,00
EtOH 50%	20,65
EtOH 70%	27,93
EtOH 96%	45,49

Table 19.IC_{50} (%) of L2/2 obtained at stirred tank experiment.

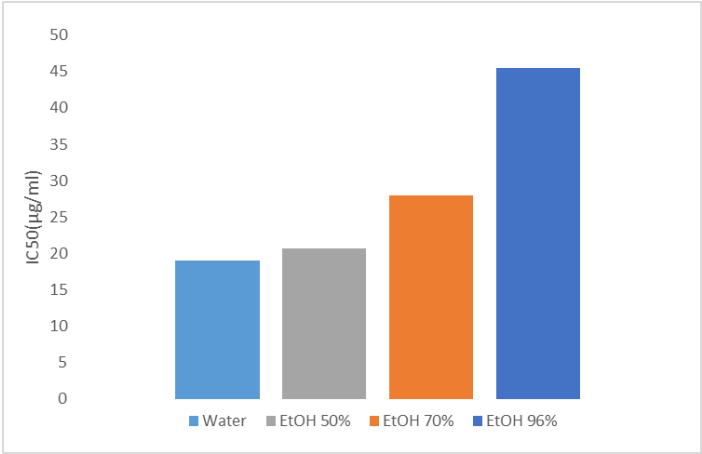

Figure 34. Antioxidant activity of L2/2 extracts by ST experiment.

In this case happens the same, higher percentage of water higher antioxidant content, so the better extraction must be done with 100 % of water to obtained the highest level of antioxidant activity. The concentration which shows 50 % inhibition for 100 % water is 19 µg/ml. Also, we see that the extracts obtained with 96 % EtOH either by Soxhlet extraction or in stirred tank apparatus showed similar antioxidant activity in extracts from steam distilled residue 39.1 µg/ml (Soxhlet extraction); 45.47 µg/ml (ST).

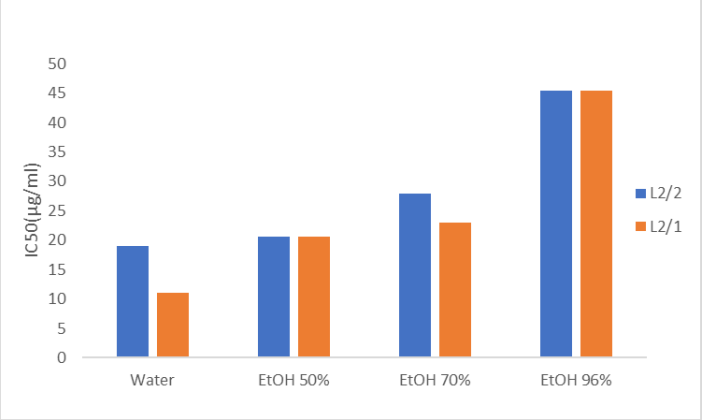

Figure 35. IC_{50} of L2/1 and L2/2.

From the compared results, it can be concluded that almost no difference was measured among the IC_{50}(%) values of steam-, and hydrodistilled residues. Also, is shown that there is a slight increase in the IC_{50} of the lavender hydrodistilled (L2/1) what means that this residue might contain less antioxidants. Even the extracts obtained with the water extraction showed the strongest antioxidant activity, but the evaporation of water was very difficult and time consuming. It is advisable to make the extractions with little of EtOH to ease the measurement procedure.

5.4.2. Polyphenols in the extracts

5.4.2.1. Lavender steam distilled residue (L2/1) from Soxhlet extraction

Table 20 shows the polyphenol content obtained from the extract of lavender steam distilled residue by Soxhlet extraction, expressed in percentage (g pyrogallol equivalent polyphenols/ 100 g of extract). Detail results can be found in *Appendix 8.6.1.1*.

L2/1		
Solvent	**Polyphenols(%)**	**Stan. Deviation**
N-pentane	1,56	0,03
Ethyl-acetate	3,61	0,09
Acetone	3,97	0,14
Propanol	4,98	0,17
EtOH	6,96	0,07

Table 20. Polyphenols (%) in extracts of Soxhlet extraction from L2/1.

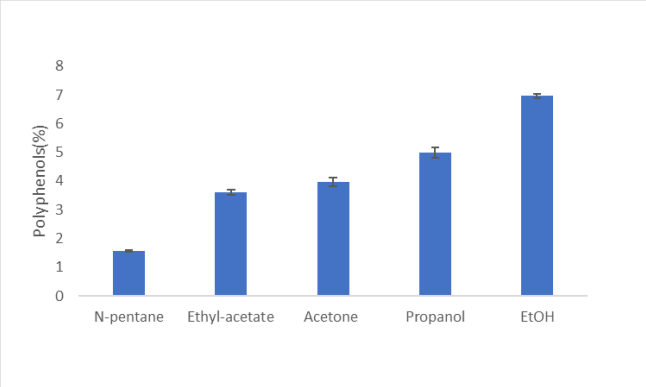

Figure 36. Polyphenols (%) in extracts of L2/1 by Soxhlet extraction (L2/1)

It can be seen that the concentration of polyphenols increases with the polarity of solvent; the lowest level of polyphenols was obtained with the most apolaric solvent: *n*-pentane (1.6 %), while the highest amount 7% of polyphenols were measured in the extract of EtOH.

5.4.2.2. Lavender hydrodistillation residue (L2/2) from Soxhlet extraction

Polyphenol content (g pyrogallol equivalent polyphenols/100 g of extract) is summarised in *Table 21* for extract obtained from hydrodistillation residue by Soxhlet extraction. Data took in lab are in *Appendix 8.6.1.2.*

	L2/2	
Solvent	**Polyphenols(%)**	**Stan. Deviation**
N-pentane	1,46	0,10
Ethyl-acetate	3,53	0,11
Acetone	3,92	0,07
Propanol	4,08	0,05
EtOH	6,46	0,13

Table 21. Polyphenols (%) in Soxhlet extraction (L2/2)

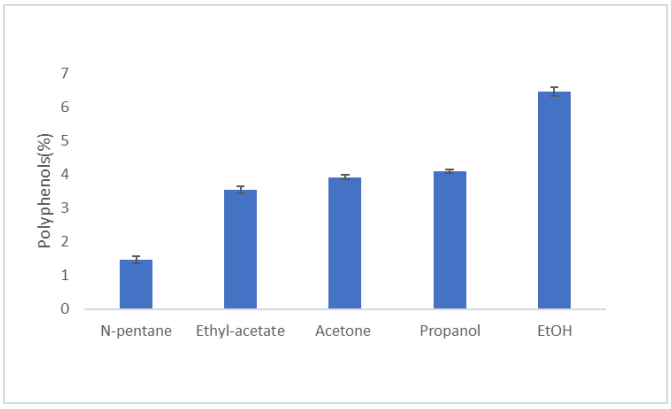

Figure 37. Polyphenols (%) in extracts obtained by Soxhlet extraction (L2/2)

As in the case of L2/2, in ascending order of polyphenols quantity, the solvents used are in the same position. So, the top solvent to take out the polyphenols is the EtOH with 6.5 % of polyphenols, and the worst is the n-pentane with a level of 1.5 % of polyphenols.

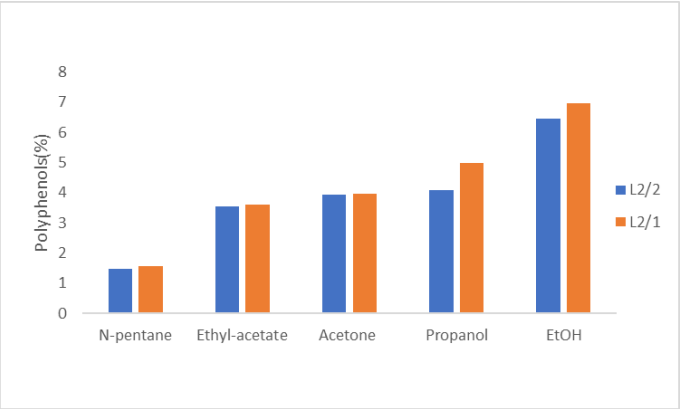

Figure 38. Polyphenols (%) of L2/1 and L2/2

If both residues are compared, the solvents work the same in both samples, the only difference is that the lavender steam distilled residue (L2/1) contains a higher level of polyphenols, but only with a little difference (< 1%).

In the literature 5.3 g/ 100 g extract total polyphenols were measured in the ethanolic extract, it well correlates with my data: 6.5-7 % from both samples.

5.4.2.3. Lavandula steam distilled (L2/1) from stirred tank extraction.

Table 22 reflects data took in lab of polyphenols content (%) from the extract of the lavender steam distilled by stirred tank extraction. Detail data is showed in *Appendix 8.6.2.1*.

L2/1		
Solvent	**Polyphenols(%)**	**Stan. Deviation**
96% EtOH	4,71	0,12
70% EtOH	6,83	0,08
50 %EtOH	10,15	0,09
Water	13,34	0,30

Table 22. Polyphenols (%) in extracts of stirred tank extraction(L2/1)

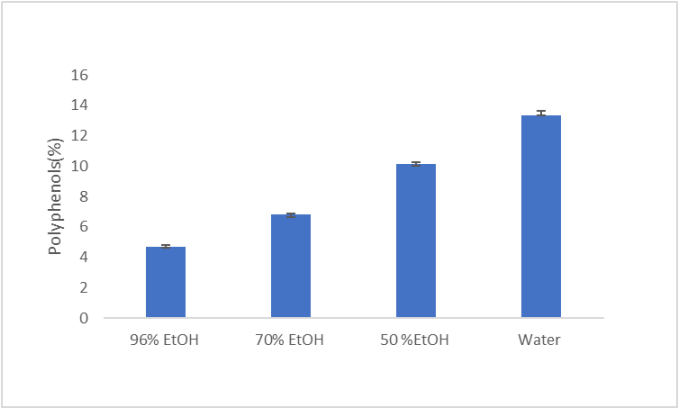

Figure 39. Polyphenols (%) in extracts of stirred tank extraction (L2/1)

It is seen that with an increase of water content in H_20: EtOH extraction solution, higher % of polyphenols can be obtained. The highest percentage of polyphenols obtained is 13.34% using 100 % of water as the solvent.

5.4.2.4. Lavender hydrodistilled residue (L2/2) from stirred tank extraction.

Results took in lab are in *Appendix 8.6.2.2* and a little summary of polyphenols content is showed below, *Table 23.*

L2/2		
Solvent	**Polyphenols(%)**	**Stan. Deviation**
96% EtOH	4,43	0,09
70% EtOH	6,27	0,08
50 %EtOH	9,15	0,06
Water	12,64	1,02

Table 23. Polyphenols (%) in stirred tank extraction (L2/2)

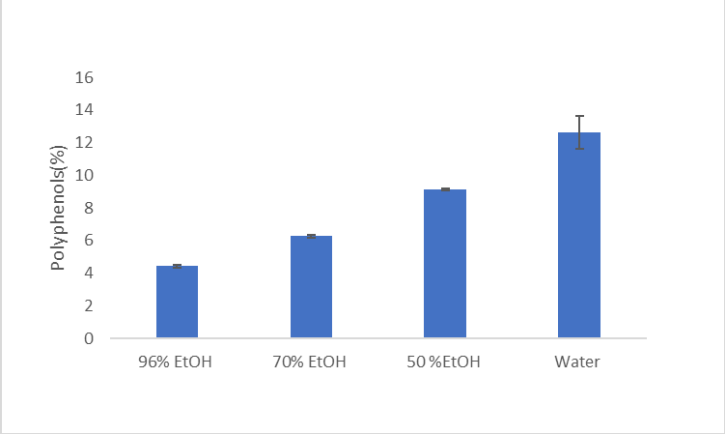

Figure 40. Polyphenols (%) in stirred tank extraction (L2/2)

For the extracts from lavender hydrodistilled residue similar trend can be observed; the concentration of polyphenols increases with the content of water in the EtOH:H_20 extraction solution. The polyphenol contents were between 4.4-12.6 %.

As it can be seen on *Figure 41* from steam distilled residue 7.0 % PE in Soxhlet extraction and 4.7 % of stirred tank were measured. From hydrodistilled residue 6.5 % from Soxhlet extraction and 4.4 % from stirred tank extraction. It can be seen from the results that in the lavender steam distilled residue (L2/1) contains slightly more polyphenols than that of in hydrodistilled residue (L2/2). It can be also concluded that the polyphenol concentration in extracts obtained with Soxhlet extraction and stirred tank extraction using 96% EtOH solvent, are slightly different. More polyphenols can be extracted using Soxhlet extraction at higher temperature.

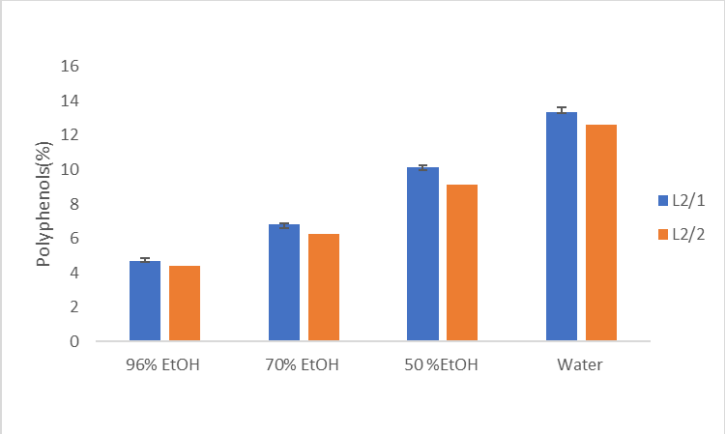

Figure 41.Polyphenols(%) in extracts of L2/1 and L2/2.

There is a parallelism between the antioxidants and the polyphenols because the solvent that gets with huge quantity of antioxidants gets also high mass of polyphenols. So, the order of the solvents from the best to the worst for taking out these compounds is the same.

5.4.3. Tannins contents in the extracts

5.4.3.1. Lavender steam distilled residue (L2/1) from Soxhlet extraction.

Table 24 represent the tannins content (%) of extract from lavender steam distilled residue by Soxhlet extraction, is expressed in g pyrogallol equivalent tannins/ 100 g of extract. In Appendix 8.7.1.1. can be found the data took in the lab.

L2/1	
Solvent	**Tannins(%)**
N-pentane	0,51
Acetone	1,03
Ethyl-acetate	1,1
Propanol	1,85
EtOH	2,2

Table 24. Tannins (%) in extracts obtained by Soxhlet extraction (L2/1)

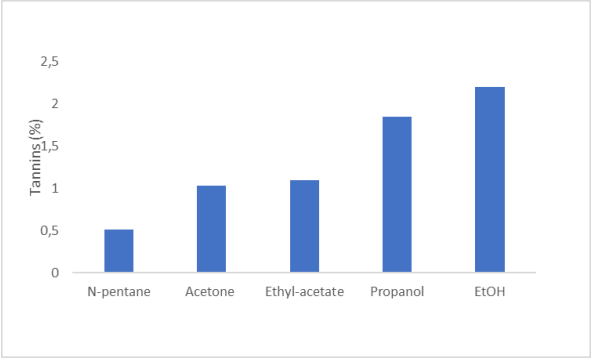

Figure 42. Tannins (%) in extracts obtained by different solvents (L2/1)

The content of tannins increase depending on the solvent used in the following order:

n-pentane< acetone<ethyl-acetate<propanol< ethanol.

Tannin contents are also increased by the increase of solvent power of applied solvents. The highest tannin content was measured in the ethanolic extract 2.2 % of the extract from steam distilled lavender residue (*Figure 42*).

5.4.3.2. Lavender hydrodistilled residue (L2/2) from Soxhlet extraction.

Table 25 reflects the tannins content in percentage of extract from lavender hydrodistilled residue from Soxhlet extraction. See detail results in Appendix 8.7.1.2.

L2/2	
Solvent	**Tannins(%)**
EtOH	0,07
N-pentane	0,14
Propanol	0,6
Acetone	0,91
Ethyl-acetate	0,95

Table 25. Tannins in extracts by Soxhlet extraction(L2/2)

As is shown in *Figure 43* the growing order of tannins content for each solvent is:

Ethanol<n-pentane<propanol<acetone< ethyl-acetate

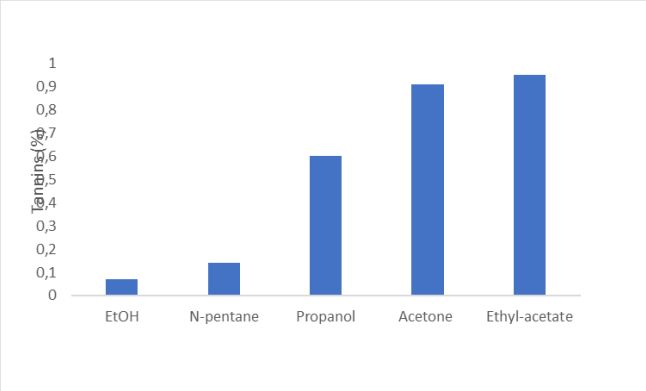

Figure 43. Tannins in extracts by Soxhlet extraction(L2/2)

From hydrodistilled residue, the results of tannin content are totally different from those obtained with steam distilled residue. As the tannin content is expressed by the difference between total polyphenol content and tannin-free polyphenol content, in the case of L2/2 residue the measured polyphenol contents were smaller, so measuring error can cause bigger deviation among the data.

Comparing both plant materials generally, the percentage of tannins is bigger in the extracts of steam distilled residue.

5.4.3.3. Lavender steam distilled residue (L2/1) from stirred tank extraction.

Tannins content (%) can be found in *Table 26*, expressed as percentage of tannins from extract of the lavender steam residue stirred tank made with different percentage of ethanol in water. See data took in lab in Appendix 8.7.2.1.

L2/1	
Solvent	**Tannins(%)**
96% EtOH	1,24
70% EtOH	2,53
50 %EtOH	4,81
Water	6,39

Table 26. Tannins in extracts of stirred tank extraction (L2/1)

An increase in the water percentage involves an increase in tannins content (%), so the best result was obtained using 100 % water as solvent.

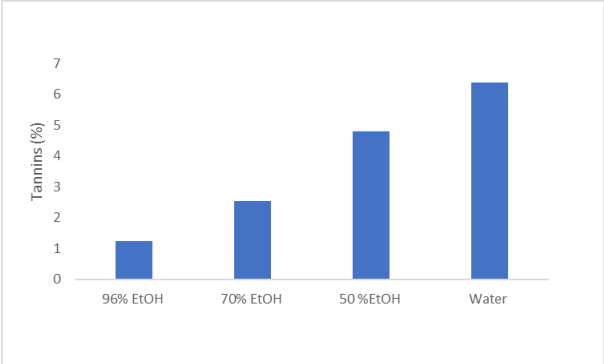

Figure 44. Tannins in extracts of stirred tank extraction (L2/1)

5.4.3.4. Lavender hydrodistilled residue (L2/2) from stirred tank extraction.

Tannins percentage of the extract obtained by stirred tank extraction of lavender hydrodistilled residue are in *Table 27* and detail results in Appendix 8.7.2.2.

L2/2	
Solvent	**Tannins(%)**
96% EtOH	0,86
70% EtOH	0,3
50 %EtOH	1,32
Water	4,47

Table 27. Tannins in extracts of stirred tank extraction (L2/2)

In extracts obtained from hydrodistilled residue of lavender (L2/2) the tannin content increased also by the decrease of ethanol in EtOH:H_20 solution.

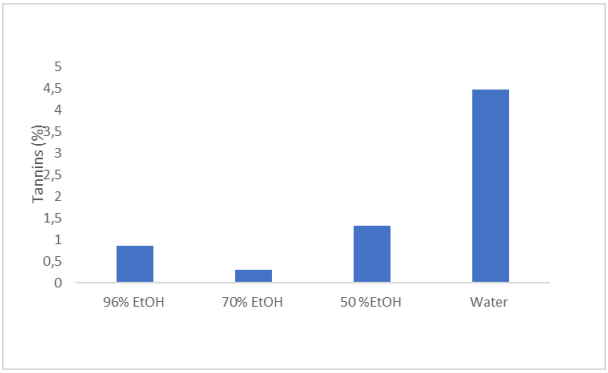

Figure 45. Tannins in extracts of stirred tank extraction (L2/2)

The tannin contents (%) were higher in extracts for steam distilled residue than from hydrodistilled residue. The tannin content obtained with water were 6.39 and 4.47 % for steam distilled residue and hydrodistilled residue, respectively. This probably was caused by the more gentle distillation technique was used, so less tannin compounds degraded.

6. CONCLUSION

In this thesis work the extraction of antioxidants from lavender steam-, and hydrodistilled residues were studied. Different extraction methods and different solvents were compared.

First of all, the particle size distribution of both residues was evaluated. The particle sizes of both ground residues were almost the same. More than 50 % of ground particles were between 0.1-0.25 mm.

Meanwhile, the dry content of hydrodistilled residue (92.51 ± 0.19%) was slightly higher than that from hydrodistilled residue (91.93 ± 0.15%).

Moreover, the essential oil quantity obtained with laboratory hydrodistillation was higher from steam distilled residue (0.007 ml/100 g dry mass). The residue from the more gentle steam distillation still contained a very little amount of essential oil, while the other residue contained no essential oil. The results showed that with hydrodistillation all essential oil was distilled in a pilot plant apparatus previously.

Generally, the yields obtained using hydrodistilled residue were higher than that of steam distilled residue for both extraction methods. Five solvents: ethanol, propanol, acetone, ethyl-acetate and *n*-pentane were applied in Soxhlet extraction. The highest yields were achieved with 96 % EtOH solvent 23.91 and 26.08 % from steam distilled and hydrodistilled residues, respectively. In stirred tank extraction experiments using ethanol solution with different contents of water showed that the yields increased with the increase of water content from 6.88-27.96 % from both residues. Comparing the two different extraction method, using the same solvent (96% EtOH) the results showed that 3.3x more extracts were obtained with Soxhlet extraction from both steam-, and hydrodistilled residues.

The extracts obtained from two residues showed similar antioxidant activities of the extracts obtained with both methods, it was a little stronger for the extracts from the steam distilled residue. In Soxhlet extraction the strongest antioxidant activity indicated by the lowest IC_{50} was obtained by 96% EtOH (39.1 μg/ml) from the steam distilled residue. From the results of stirred tank, as the water was increased higher antioxidant activity was measured from 11-45 μg/ml. It is remarkable that compounds resulting IC_{50} are not heat- sensitive because the results were similar of the extracts obtained by Soxhlet extraction and stirred tank extraction using 96% EtOH at different temperature.

Polyphenols contents were higher in the extracts of the steam distilled residue too. From Soxhlet extraction the highest percentage of polyphenols was in the ethanol extract from steam distilled residue (7.0 %). And from the stirred tank experiments, higher water percentage resulted in more polyphenols contents, water extract from steam distilled contains 13.3 % of polyphenols. From results of IC_{50} and polyphenols can be concluded, that antioxidant activity influenced not only by the presence of polyphenols, there must be other different compounds which possess antioxidant activity.

Tannins were also higher quantities in the extracts of steam distilled residue. In stirred tank extraction, an increase in water percentage in EtOH:H_20 solution meant an increase in tannin content from 1.24-6.39 % of tannins in the extract from steam distilled residue.

Generally, extracts with strong antioxidant activities and with high content of polyphenols were obtained from both steam-, and hydrodistilled residues of lavender. Therefore, these residues can be successfully applied for extraction of natural antioxidants.

Obtaining these natural extracts from an industrial waste using environmentally friendly solvents we can add value to an otherwise disposable material. These natural extracts can be used in food, pharmaceutical or cosmetics industries and they are more favourable due to their non-toxicity compare them with synthetic antioxidants.

7. LITERATURE

[1] Oil Extraction Technologies. *https://oilextech.com*

[2] Wikipedia. https://en.wikipedia.org/wiki/Lavandula_angustifolia

[3] Blumenthal, M. The Complete German Commission E Monographs – Therapeutic Guide to Herbal Medicines. American Botanical Council: Texas, USA; 1998. 159-160.

[4] L.Lesage-Meesen; M.Bou; J.C.Sigoillot; C.B.Faulds;A.Lomascolo. *Essential oils and distilled straws of lavender and lavandin: a review of current use and potential application in white biotechnology*. *Appl Microbiol Biotechnol* 2015,3375-3385.

[5] V.G. Pangarkar; A.A.Yawalkar; M.M.Sharma; A.A.C.M.Beenackers. *Particle-Liquid Mass Transfer Coefficient in Two-/Three-Phase Stirred Tank Reactors*.2002.4141-4167.

[6] H. Yalcin1; H. Kavuncuoğlu1; E. Tulukcu; Z. Eroğlu. *The effect of harvest time on the bioactive properties and volatile components of lavender (Lavandula officinalis). Wanenigein Academic publishers.*2017,273-283.

[7] R.Sanchez-Vioque;M.Polissiou;K.Astraka;M.de los Mozos-Pascual ;P.Tarantilis ;D.Herraiz-Peñalver ;O.Santana-Méridas. *Polyphenol composition and antioxidant and metal chelating activities of the solid residues from the essential oil industry*. European Journal of Lipid Science and Tecnhology.2013,150-159.

[8] J.Bruneton. *Pharmacognosy Phytochemistry Medicinal Plants.* Lavoisier Publishing; France.1999,528-529.

[9] K.Bauer; D.Gart; H.Surburg. *Common Fragance and Flavor Materials.* VCH Publishers.Germany.1990,160-161.

[10] F.Andrei; A.Ersilia; C.Tulcan; A.Dragomirescu. *Chemical Composition and the Potential of Lavandula angustifolia L.Oil as a Skin Depigmentant ACG publications records of natural products.*2018,340-349.

[11] R.Sanchez-Vioque. *Chemical composition and antioxidant capacities of four Mediterranean industrial essential oils and their resultant distilled solid by-products.* European Journal of Lipid Science and Tecnhology.2017

[12] I.Lukin; J.Merz; G. Schembecker. *Techniques for the recovery of volatile aroma Lavandula angustifolia compounds from biochemical broth*. Flavour fragance journal.2018,1-14.

[13]H.A.Peredo-Luna; E.Palou-García; A.Lopez-Malo. *Aceites esenciales: métodos de extracción.Temas selectos de Ingeniería de alimentos 3.*2009,24-32.

[14] G.D.K. Babu; A.Sharma; B.Singh. *Volatile composition of Lavandula angustifolia produced by different extraction techniques. Journal of essential oil research.*2016.1-13.

[15] A.A.Gonzalez. *Obtención de aceites esenciales y extractos etanólicos de plantas del Amazonas.*2004,5-14.

[16] C.J.Geankoplis. *Transport processes and separation process. Pearson. Unites States of America*.2014.803-809.

[17] T.C.Lo; M.H.I.Baird; C.Hanson. *Hanbook of Solvent Extraction. Wiley-Interscience Publication*.1983.593-603.

[18] S. S. Handa; S. P. S. Khanuja; G. Longo; D. D. Rakesh. *Extraction Technologies for Medicinal and Aromatic Plants*. International centre for science and high technology. Trieste.2008.23-24.

[19] E.Vagi.*Supercritical fluid extraction of plants and the functional properties of the extracts*. PhD dissertation, Budapest.2005.5-51.

[20] P.Molyneux. *The use of the stable free radical diphenylpicryl-hydrazyl(DPPH) for estimating antioxidant activity*. Songklanakarin J. Sci. Technol.2004.211-219.

[21] Hungarian Pharmacopeia 2.8.14.2008.232.

8. APPENDIX

8.1. Particle size determination

8.1.1. Lavender steam distilled residue(L2/1)

Below, three experimental essays are shown as the procedure to calculate the of plant material of each particle size.

Test 1

TEST 1				
Sample	Lavandula residue L2/1			
Weight(g)	1524,34-1466,46=57,88			
Amplitude	35	Time(min)	20	
Size (mm)	Tare (g)	Tare+Residue	Residue (g)	%
1,4	380,31	380,37	0,06	0,10
1,25	323,46	323,48	0,02	0,03
0,8	398,86	399,42	0,56	0,97
0,63	381,4	384,04	2,64	4,59
0,5	306,22	313,49	7,27	12,65
0,4	358,87	368,64	9,77	17,00
0,25	285,91	304,03	18,12	31,52
0,1	241,77	258,73	16,96	29,51
<0,05	362,48	364,56	2,08	3,62
		TOTAL	57,48	

Table 28.Results of size determination.

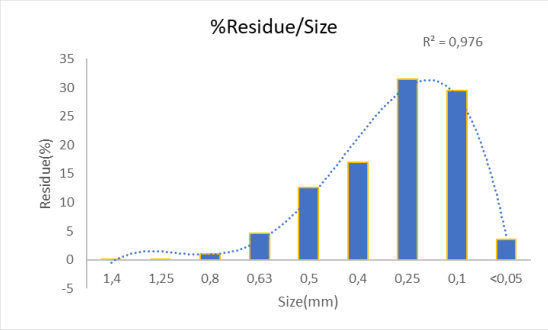

Figure 46. Results of size determination.

Test 2

TEST 2				
Sample	Lavandula residue L2/1			
Weight(g)	1466,24-1398,64=67,6			
Amplitude	40	Time(min)	20	
Size (mm)	Tare (g)	Tare+Residue	Residue (g)	%
1,4	380,33	380,46	0,13	0,19
1,25	323,46	323,59	0,13	0,19
0,8	398,89	399,64	0,75	1,11
0,63	381,5	385,01	3,51	5,20
0,5	306,32	315,8	9,48	14,05
0,4	359,03	371,54	12,51	18,54
0,25	286,09	306,94	20,85	30,91
0,1	241,99	260,24	18,25	27,05
<0,05	362,52	364,37	1,85	2,74
		TOTAL	67,46	

Table 29. Results of size determination.

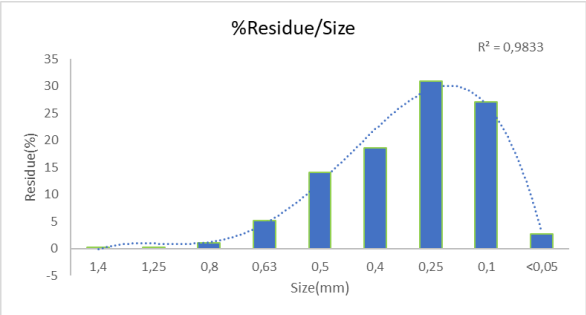

Figure 47. Results of size determination.

Test 3

TEST 3				
Sample	Lavandula residue L2/1			
Weight(g)	1344,69-1303,5=41,19			
Amplitude	40	**Time(min)**	20	
Size (mm)	**Tare (g)**	**Tare+Residue**	**Residue (g)**	**%**
1,4	380,38	380,46	0,08	0,20
1,25	323,51	323,62	0,11	0,27
0,8	398,96	399,38	0,42	1,03
0,63	381,6	383,25	1,65	4,04
0,5	306,39	311,57	5,18	12,68
0,4	359,04	366,26	7,22	17,68
0,25	286,14	299,45	13,31	32,59
0,1	241,89	253,64	11,75	28,77
<0,05	362,59	363,71	1,12	2,74
		TOTAL	40,84	

Table 30. Results of size determination.

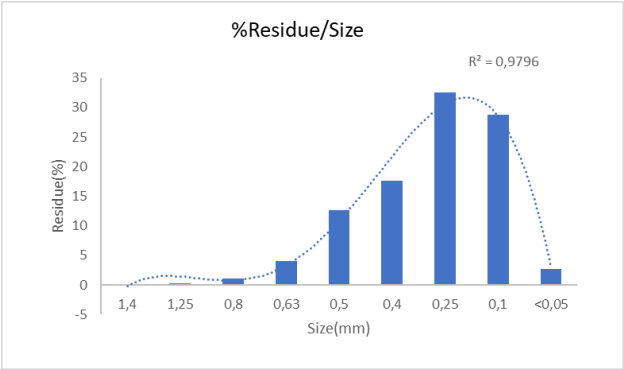

Figure 48. Results of size determination.

8.1.2. Lavender hydrodistilled residue(L2/2)

Test 1

TEST 1				
Sample	Lavandula residue L2/2			
Weight(g)	1627,57-1587,56=40,01			
Amplitude	40	Time(min)	20	
Size (mm)	Tare (g)	Tare+Residue	Residue (g)	%
1,4	380,38	380,51	0,13	0,354
1,25	323,51	323,63	0,12	0,327
0,8	398,92	399,61	0,69	1,880
0,63	381,51	384,02	2,51	6,837
0,5	306,36	312,4	6,04	16,453
0,4	358,98	365,66	6,68	18,197
0,25	286,05	297,05	11	29,965
0,1	241,86	250,29	8,43	22,964
<0,05	362,52	363,63	1,11	3,024
		TOTAL	36,71	

Table 31. Results of size determination.

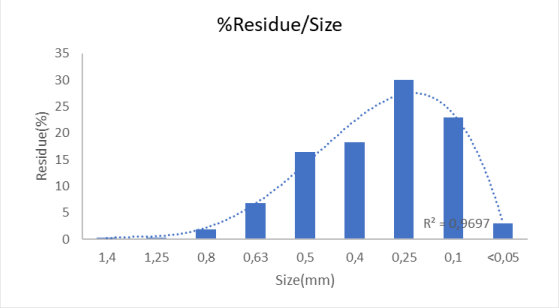

Figure 49. Results of size determination.

Test 2

TEST 2				
Sample	Lavandula residue L2/2			
Weight(g)	1587,50-1539,88=47,62			
Amplitude	40	Time(min)	20	
Size (mm)	Tare (g)	Tare+Residue	Residue (g)	%
1,4	380,38	380,5	0,12	0,249
1,25	323,51	323,62	0,11	0,228
0,8	398,91	399,63	0,72	1,491
0,63	381,5	384,32	2,82	5,841
0,5	306,35	313,5	7,15	14,809
0,4	358,94	366,81	7,87	16,301
0,25	286,07	300,51	14,44	29,909
0,1	241,87	255,54	13,67	28,314
<0,05	362,54	363,92	1,38	2,858
		TOTAL	48,28	

Table 32. Results of size determination.

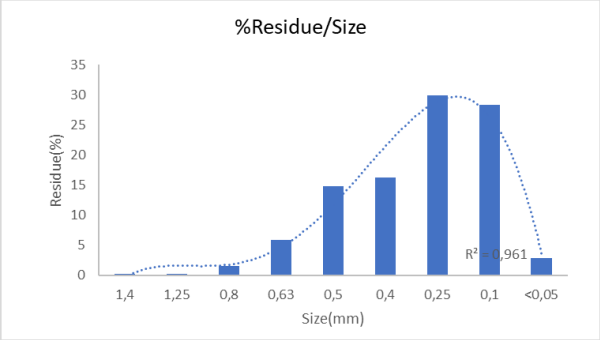

Figure 50. Results of size determination.

Test 3

TEST 3				
Sample	Lavandula residue L2/2			
Weight(g)	1539,99-1483,77=56,22			
Amplitude	40	**Time(min)**	20	
Size (mm)	**Tare (g)**	**Tare+Residue**	**Residue (g)**	**%**
1,4	380,38	380,49	0,11	0,197
1,25	323,53	323,63	0,1	0,179
0,8	398,93	399,95	1,02	1,825
0,63	381,51	385,42	3,91	6,997
0,5	306,34	315,78	9,44	16,893
0,4	358,93	369,33	10,4	18,611
0,25	286,15	303,59	17,44	31,210
0,1	241,86	253,75	11,89	21,278
<0,05	362,52	364,09	1,57	2,810
		TOTAL	55,88	

Table 33. Results of size determination.

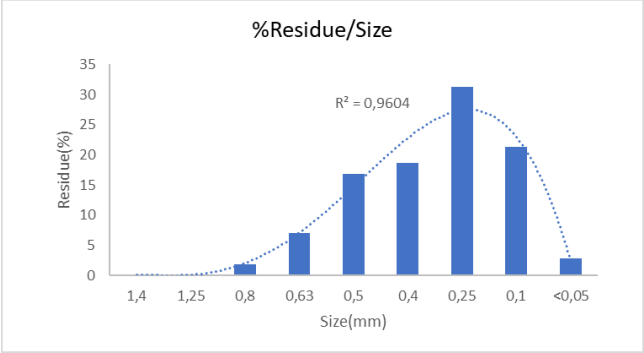

Figure 51. Results of size determination.

8.2. Extracts from Soxhlet extraction

8.2.1. Lavender steam distilled residue(L2/1)

N-PENTANE			
	SAMPLE 1	SAMPLE 2	SAMPLE 3
mglass	120,96	114,84	118,02
mplant	15,27	15,01	17,18
mdryplant	14,04	13,80	15,79
mglass+extract	121,35	115,22	118,44
mextract	0,39	0,38	0,42
Y(%)	2,75	2,74	2,63
Average	2,70		
Standard desviation	0,07		

Table 34. N-pentane yield of extract measurements.

ETIL-ACETATE			
	SAMPLE 1	SAMPLE 2	SAMPLE 3
mglass	113,97	114,84	118,02
mplant	17,70	18,12	17,25
mdryplant	16,27	16,65	15,86
m glass+extract	115,15	116,15	119,19
mextract	1,18	1,31	1,17
Y(%)	7,23	7,85	7,36
Average	7,47		
Standard desviation	0,32		

Table 35. Ethyl-acetate yield of extract measurements.

ACETONE			
	SAMPLE 1	SAMPLE 2	SAMPLE 3
mglass	113,54	114,85	139,55
mplant	17,32	15,29	15,16
mdryplant	15,92	14,05	13,93
m glass+extract	114,91	116,06	140,73
mextract	1,37	1,21	1,18
Y(%)	8,58	8,61	8,48
Average	8,56		
Standard desviation	0,07		

Table 36.Acetone yield of extract measurements.

PROPANOL			
	SAMPLE 1	SAMPLE 2	SAMPLE 3
mglass	121,01	115,75	94,52
mplant	15,51	16,71	17,50
mdryplant	14,26	15,37	16,09
m glass+extract	122,43	117,43	96,28
mextract	1,42	1,68	1,76
Y(%)	9,93	10,97	10,94
Average	10,59		
Standard desviation	0,59		

Table 37.Propanol yield of extract measurements.

ETOH			
	SAMPLE 1	SAMPLE 2	SAMPLE 3
mglass	139,54	122,34	130,89
mplant	16,46	15,14	17,56
mdryplant	15,13	13,92	16,14
m glass+extract	142,91	125,59	134,63
mextract	3,37	3,25	3,74
Y(%)	22,25	23,33	23,16
Average	22,91		
Standard desviation	0,58		

Table 38.EtOH yield of extract measurements.

8.2.2. Lavender hydrodistilled residue(L2/2)

N-PENTANE			
	SAMPLE 1	SAMPLE 2	SAMPLE 3
mglass	118,03	113,97	122,34
mplant	13,33	15,47	14,48
mdryplant	12,33	14,32	13,40
mglass+extract	118,44	114,41	122,76
mextract	0,41	0,44	0,42
Y(%)	3,34	3,08	3,16
Average	3,19		
Standard desviation	0,13		

Table 39. N-pentane yield of extract measurements.

ETIL-ACETATE			
	SAMPLE 1	SAMPLE 2	SAMPLE 3
mglass	113,49	107,87	117,55
mplant	16,92	11,40	14,98
mdryplant	15,65	10,54	13,86
m glass+extract	114,87	108,76	118,73
mextract	1,38	0,89	1,18
Y(%)	8,81	8,49	8,49
Average	8,59		
Standard desviation	0,18		

Table 40. Ethyl-acetate yield of extract measurements.

ACETONE			
	SAMPLE 1	SAMPLE 2	SAMPLE 3
mglass	113,56	107,87	117,58
mplant	14,43	15,25	13,48
mdryplant	13,35	14,11	12,47
m glass+extract	114,86	109,20	118,79
mextract	1,30	1,33	1,20
Y(%)	9,72	9,41	9,66
Average	9,59		
Standard desviation	0,16		

Table 41. Acetone yield of extract measurements.

PROPANOL			
	SAMPLE 1	SAMPLE 2	SAMPLE 3
mglass	98,21	112,67	94,52
mplant	14,92	14,03	13,72
mdryplant	13,80	12,98	12,70
m glass+extract	99,78	114,13	95,99
mextract	1,57	1,46	1,48
Y(%)	11,35	11,25	11,62
Average	11,40		
Standard desviation	0,19		

Table 42. Propanol yield of extract measurements.

ETOH			
	SAMPLE 1	SAMPLE 2	SAMPLE 3
mglass	113,49	107,86	139,52
mplant	13,06	14,46	15,99
mdryplant	12,08	13,37	14,80
m glass+extract	116,60	111,39	143,39
mextract	3,11	3,53	3,86
Y(%)	25,73	26,40	26,11
Average	26,08		
Standard desviation	0,33		

Table 43. EtOH yield of extract measurements.

8.3. Extracts from stirred tank extraction

8.3.1. Lavender steam distilled residue (L2/1)

EtOH 96%			
DRY PLANT		EXTRACT	
mglass (g)	111,11	mglass (g)	111,24
mglass+ dry plant (g)	132,13	mglass+extract (g)	112,69
mdry plant (g)	21,02	mextract (g)	1,45
Y(%)		6,88	

Table 44. EtOH 96% yield of extract measurements.

EtOH 70%			
DRY PLANT		EXTRACT	
mglass (g)	111,33	mglass (g)	114,85
mglass+ dry plant (g)	130,01	mglass+extract (g)	118,38
mdry plant (g)	18,68	mextract (g)	3,5278
Y(%)		18,89	

Table 45.EtOH 70% yield of extract measurements.

EtOH 50%			
DRY PLANT		EXTRACT	
mglass (g)	111,24	mglass (g)	100,96
mglass+ dryplant (g)	133,11	mglass+extract (g)	106,18
mdry plant (g)	21,87	mextract (g)	5,22
Y(%)		23,86	

Table 46. EtOH 50 % yield of extract measurements.

Water			
DRY PLANT		EXTRACT	
mglass (g)	100,89	mglass (g)	98,06
mglass+ dryplant (g)	118,56	mglass+extract (g)	104
mdry plant (g)	17,67	mextract (g)	5,94
Y(%)		33,62	

Table 47.Water yield of extract measurements.

8.3.2. Lavender hydrodistilled residue (L2/2)

EtOH 96%			
DRY PLANT		EXTRACT	
mglass (g)	115,73	mglass (g)	93,63
mglass+ dryplant (g)	140,45	mglass+extract (g)	95,57
mdry plant (g)	24,72	mextract (g)	1,94
Y(%)		7,85	

Table 48. EtOH 96% yield of extract measurements.

EtOH 70%			
DRY PLANT		EXTRACT	
mglass (g)	103,46	mglass (g)	94,47
mglass+ dryplant (g)	121,57	mglass+extract (g)	98,43
mdry plant (g)	18,11	mextract (g)	3,96
Y(%)		21,87	

Table 49. EtOH 70% yield of extract measurements.

EtOH 50%			
DRY PLANT		EXTRACT	
mglass (g)	103,51	mglass (g)	120,66
mglass+ dryplant (g)	122,21	mglass+extract (g)	125,60
mdry plant (g)	18,70	mextract (g)	4,94
Y(%)		26,42	

Table 50. EtOH 50 % yield of extract measurements.

Water			
DRY PLANT		EXTRACT	
mglass (g)	116,72	mglass (g)	107,89
mglass+ dryplant (g)	134,50	mglass+extract (g)	111,08
mdry plant (g)	17,78	mextract (g)	3,19
Y(%)		17,94	

Table 51. Water yield of extract measurements.

8.4. Laboratory hydrodistillation

Lavender residue	Plant material (g)	Plant material dry (%)	Essential oil volume (ml)	Y(%)
L2/1	149,52	137,45	0,01	0,007
L2/2	150,03	138,80	0,005	0,004

Table 52.Essential oi obtained by lab hydrodistillation.

8.5. Antioxidants measurements

8.5.1. Soxhlet extraction

8.5.1.1 Lavender steam distilled residue (L2/1)

1.N-Pentane							
Concentration of 1 (µl)	Absorbance 2	Absorbance 3	Activity 2	Activity 3	Average	Stan. desviation	Extract Concentration (µg/ml)
0	0,912	0,912			0		0
200	0,743	0,815	18,531	10,636	13,515	5,582	37,037
350	0,639	0,748	29,934	17,982	22,468	8,451	61,404
500	0,607	0,708	33,443	22,368	26,807	7,831	83,333
750	0,576	0,648	36,842	28,947	32,421	5,582	115,385
1000	0,566	0,603	37,939	33,882	35,796	2,869	142,857
					IC50 (µl)	1465,627	
					IC50(µg/ml)	176,616	

Table 53. Absorbances of N-pentane extract.

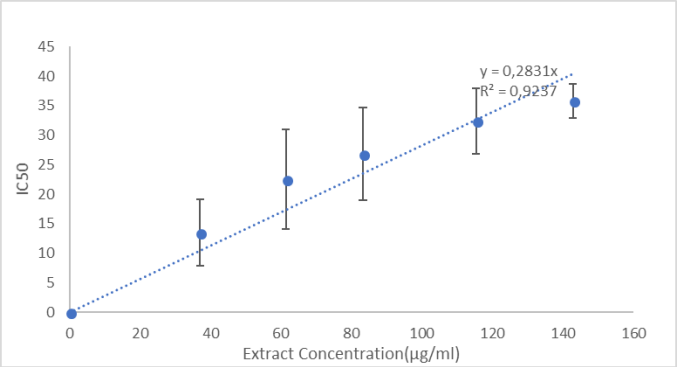

Figure 52. Inhibition curve of n-pentane.

2.Ethyl-acetate sample									
Concentration of 2 (µl)	Absorbance 1	Absorbance 2	Absorbance 3	Activity 1	Activity 2	Activity 3	Average	Stan. deviation	Extract Concentration (µg/ml)
0	0,729	0,727	0,732						
50	0,688	0,718	0,718	5,624	1,238	1,913	1,989	2,362	9,804
100	0,656	0,65	0,639	10,014	10,591	12,705	10,989	1,417	19,231
200	0,591	0,557	0,582	18,930	23,384	20,492	20,777	2,260	37,037
300	0,539	0,514	0,517	26,063	29,298	29,372	28,157	1,889	53,571
400	0,452	0,465	0,467	37,997	36,039	36,202	36,725	1,087	68,966
500	0,421	0,409	0,4	42,250	43,741	45,355	43,745	1,553	83,333
							IC50(µl)	550,983	
							IC50(µg/ml)	94,447	

Table 54.Absorbances of Ethyl-acetate extract.

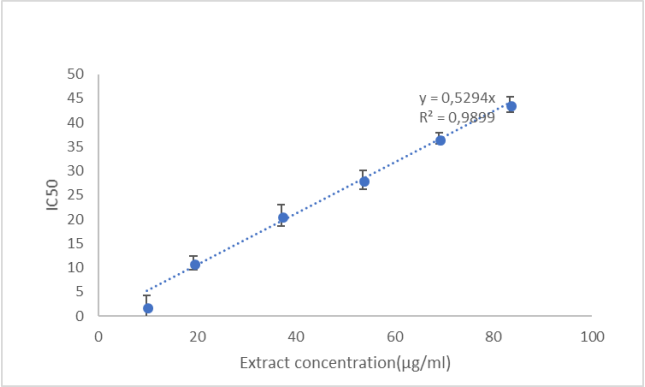

Figure 53. Inhibition curve of Ethyl-acetate.

5.Acetone sample									
Concentration of 5 (µl)	Absorbance 1	Absorbance 2	Absorbance 3	Activity 1	Activity 2	Activity 3	Average	Stan. deviation	Extract Conc. (µg/ml)
0	0,615	0,618	0,617						
50	0,588	0,585	0,577	4,390	5,340	6,483	5,270	1,048	9,804
100	0,548	0,533	0,515	10,894	13,754	16,532	13,334	2,819	19,231
200	0,463	0,477	0,468	24,715	22,816	24,149	23,866	0,975	37,037
300	0,418	0,409	0,416	32,033	33,819	32,577	32,793	0,916	53,571
400	0,36	0,361	0,361	41,463	41,586	41,491	41,513	0,064	68,966
500	0,312	0,318	0,312	49,268	48,544	49,433	49,079	0,473	83,333
							IC50(µl)	488,143	
							IC50(µg/ml)	82,850	

Table 55.Absorbances of Acetone extracts.

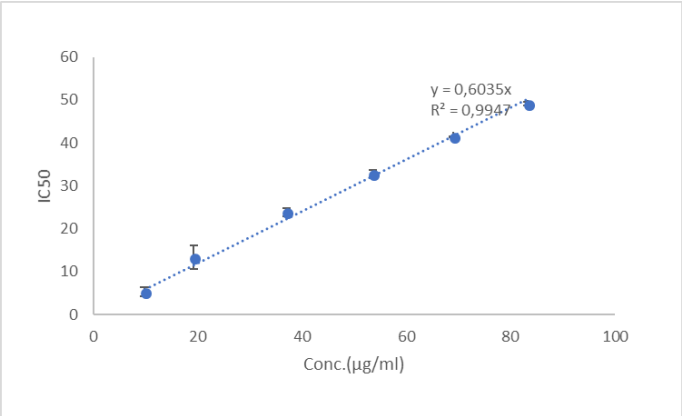

Figure 54. Inhibition curve of acetone.

3.Propanol sample									
Concentration of 3 (µl)	**Absorbance 1**	**Absorbance 2**	**Absorbance 3**	**Activity 1**	**Activity 2**	**Actvity 3**	**Average**	**Stan. deviation**	**Extract concentration (µg/ml)**
0	0,741	0,688	0,688						
200	0,532	0,521	0,525	28,205	24,273	23,692	25,240	2,455	37,037
250	0,5	0,495	0,486	32,524	28,052	29,360	29,864	2,299	45,455
300	0,475	0,463	0,461	35,897	32,703	32,994	33,805	1,766	53,571
350	0,431	0,428	0,411	41,835	37,791	40,262	39,892	2,039	61,404
450	0,347	0,326	0,347	53,171	52,616	49,564	51,734	1,942	76,271
500	0,328	0,322	0,325	55,735	53,198	52,762	53,867	1,606	83,333
							IC50(µg/ml)	76,196	
							IC50(µl)	450,567	

Table 56.Absorbances of propanol extracts.

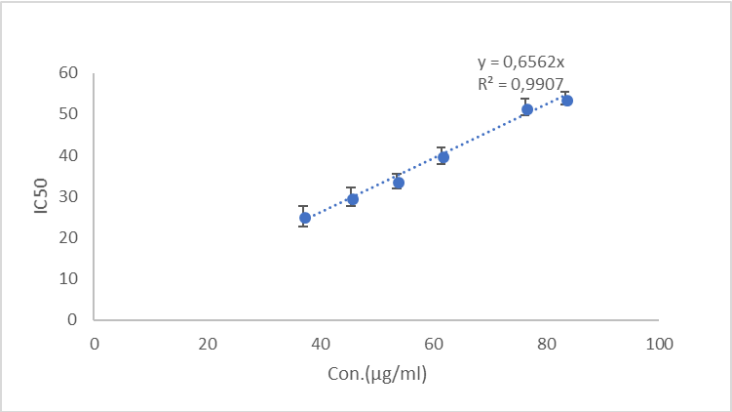

Figure 55. Inhibition curve of propanol

4.EtOH sample									
Concentration of 4 (µl)	**Absorbance 1**	**Absorbance 2**	**Absorbance 3**	**Activity 1**	**Activity 2**	**Actvity 3**	**Average**	**Stand. Deviation**	**Extract Concentration (µg/ml)**
0	0,619	0,615	0,613						
50	0,518	0,521	0,529	16,317	15,285	13,703	15,023	1,316	9,804
75	0,478	0,485	0,48	22,779	21,138	21,697	21,850	0,834	14,563
100	0,473	0,448	0,478	23,586	27,154	22,023	24,071	2,630	19,231
150	0,377	0,404	0,399	39,095	34,309	34,910	35,984	2,607	28,302
175	0,347	0,357	0,367	43,942	41,951	40,131	41,950	1,906	32,710
200	0,331	0,325	0,345	46,527	47,154	43,719	45,750	1,829	37,037
							IC50(µl)	212,841	
							IC50(µg/ml)	39,102	

Table 57.Absorbances of EtOH extracts.

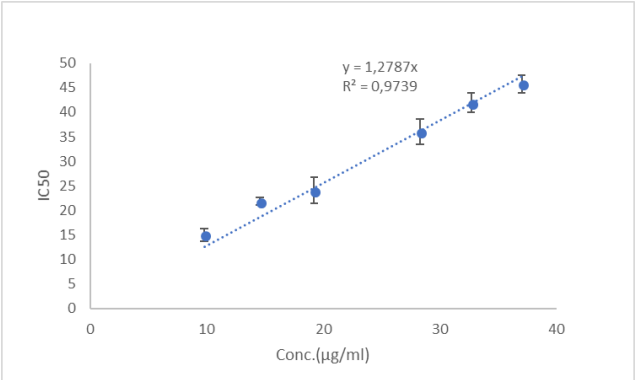

Figure 56. Inhibition curve of EtOH.

8.5.1.2. Lavender hydrodistilled residue (L2/2).

1.Pentane							
Concentration of 1 (µl)	**Absorbance 2**	**Absorbance 3**	**Activity 2**	**Activity 3**	**Average**	**Stan. deviation**	**Extract deviation (µg/ml)**
0	0,896	0,887					
200	0,838	0,812	6,473	8,455	7,333	1,402	37,037
350	0,783	0,793	12,612	10,598	11,517	1,424	61,404
500	0,734	0,736	18,080	17,024	17,536	0,747	83,333
750	0,695	0,68	22,433	23,337	22,876	0,639	115,385
1000	0,651	0,66	27,344	25,592	26,439	1,239	142,857
					IC50 (µl)	1804,970	
					IC50(µg/ml)	258,665	

Table 58. Absorbances of N-pentane extract.

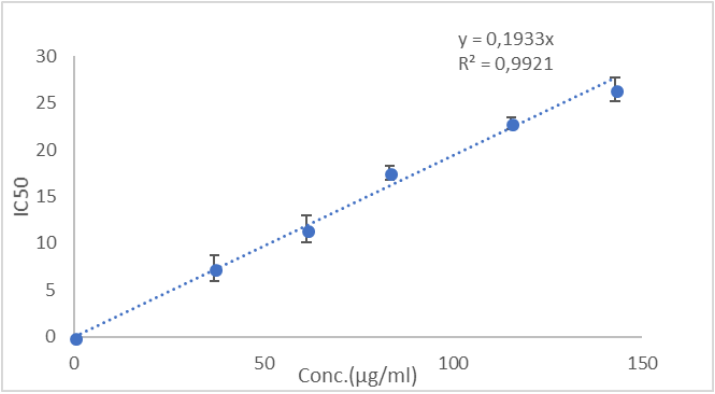

Figure 57.Inhibition curve of n-pentane.

2.Ethyl-acetate sample									
Concentration of 2 (µl)	**Absorbance 1**	**Absorbance 2**	**Absorbance 3**	**Activity 1**	**Activity 2**	**Activity 3**	**Average**	**Stan. deviation**	**Extract concentration (µg/ml)**
0	0,874	0,874	0,828						
100	0,76	0,758	0,722	13,043	13,272	12,802	13,036	0,235	19,231
200	0,725	0,712	0,691	17,048	18,535	16,546	17,336	1,035	37,037
400	0,608	0,617	0,608	30,435	29,405	26,570	28,708	2,001	68,966
500	0,591	0,573	0,562	32,380	34,439	32,126	32,950	1,269	83,333
750	0,499	0,483	0,454	42,906	44,737	45,169	44,249	1,201	115,385
							IC50(µl)	815,924	
							IC50(µg/ml)	124,750	

Table 59.Absorbances of Ethyl-acetate extracts

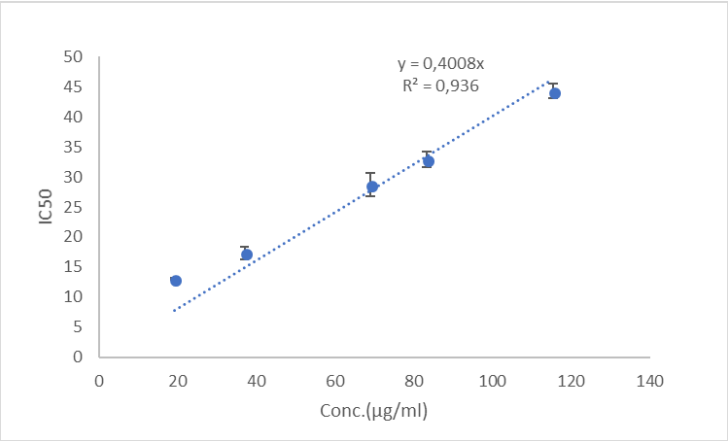

Figure 58. Inhibition curve of Ethyl-acetate.

5.Acetone sample									
Concentration of 5 (µl)	Absorbance 1	Absorbance 2	Absorbance 3	Activity 1	Activity 2	Activity 3	Average	Stan. deviation	Extract concentration (µg/ml)
0	0,809	0,808	0,808						
200	0,651	0,63	0,655	19,530	22,030	18,936	20,079	1,642	37,037
300	0,567	0,57	0,567	29,913	29,455	29,827	29,731	0,243	53,571
400	0,508	0,515	0,505	37,206	36,262	37,500	36,982	0,647	68,966
450	0,481	0,493	0,492	40,544	38,985	39,109	39,533	0,866	76,271
550	0,433	0,423	0,426	46,477	47,649	47,277	47,129	0,599	90,164
							IC50(µl)	563,651	
							IC50(µg/ml)	94,411	

Table 60.Absorbances of Acetone extracts.

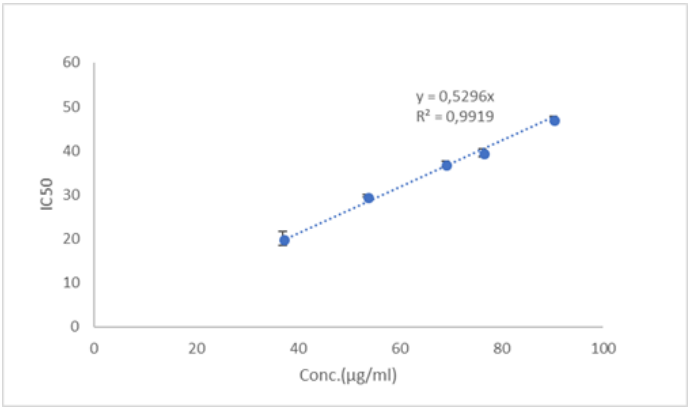

Figure 59.Inhibition curve of Acetone.

3.Propanol sample									
Concentration of 3 (µl)	Absorbance 1	Absorbance 2	Absorbance 3	Activity 1	Activity 2	Actvity 3	Average	Stan. desviation	Concentration extract (µg/ml)
0	0,817	0,812	0,81						
100	0,712	0,693	0,683	12,852	14,655	15,679	14,298	1,431	19,231
200	0,629	0,624	0,621	23,011	23,153	23,333	23,165	0,162	37,037
300	0,554	0,556	0,549	32,191	31,527	32,222	31,977	0,393	53,571
400	0,486	0,499	0,487	40,514	38,547	39,877	39,629	1,004	68,966
500	0,424	0,446	0,441	48,103	45,074	45,556	46,207	1,628	83,333
							IC50(µg/ml)	86,490	
							IC50(µl)	535,859	

Table 61.Absorbances of propanol extracts.

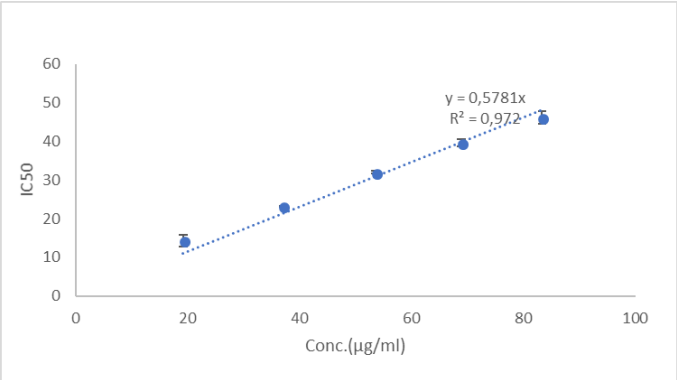

Figure 60.Inhibition curve of propanol sample.

4.EtOH sample new									
Concentration of 4 (µl)	Absorbance 1	Absorbance 2	Absorbance 3	Activity 1	Activity 2	Actvity 3	Average	Stand. Deviation	Extract concentration (µg/ml)
0	0,795	0,795	0,789						
50	0,706	0,71	0,72	11,195	10,692	8,745	10,094	1,294	9,804
100	0,579	0,643	0,606	27,170	19,119	23,194	22,689	4,025	19,231
150	0,496	0,545	0,543	37,610	31,447	31,179	33,163	3,638	28,302
200	0,491	0,486	0,485	38,239	38,868	38,530	38,544	0,315	37,037
300	0,352	0,377	0,352	55,723	52,579	55,387	54,526	1,727	53,571
							IC50(µl)	262,531	
							IC50(µg/ml)	47,304	

Table 62. Absorbances of EtOH extracts.

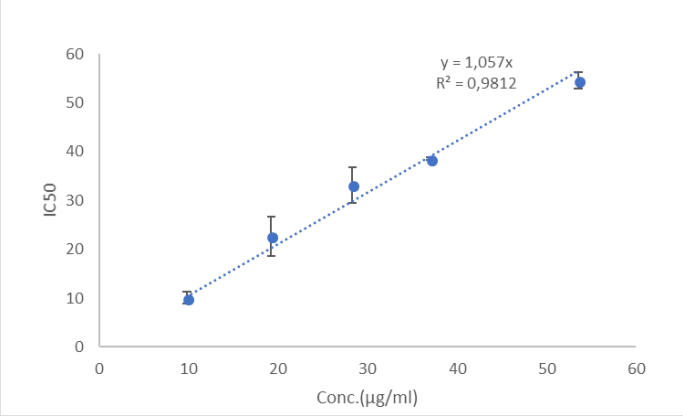

Figure 61. Inhibition curve of EtOH sample.

8.5.2. Stirred tank extraction

8.5.2.1. Lavender steam distilled residue(L2/1).

1.EtOH 96%									
Concentration of 1 (µl)	Absorbance 1	Absorbance 2	Absorbance 3	Activity 1	Activity 2	Actvity 3	Average	Stand. Deviation	Extraxt concentration (µg/ml)
0	0,768	0,721	0,719						
50	0,681	0,651	0,617	11,328	9,709	14,186	11,461	2,267	9,804
100	0,566	0,527	0,521	26,302	26,907	27,538	26,906	0,618	19,231
200	0,421	0,376	0,385	45,182	47,850	46,453	46,470	1,334	37,037
300	0,305	0,263	0,271	60,286	63,523	62,309	62,010	1,635	53,571
400	0,19	0,141	0,192	75,260	80,444	73,296	76,217	3,693	68,966
500	0,112	0,09	0,12	85,417	87,517	83,310	85,380	2,104	83,333
							IC50(µl)	255,905	
							IC50(µg/ml)	45,467	

Table 63.Absorbances of EtOH 96% extracts.

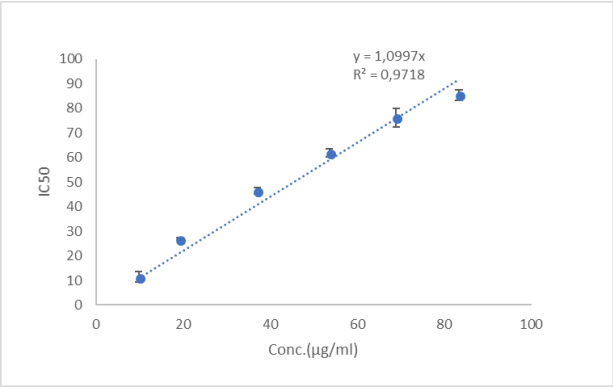

Figure 62.Inhibition curve of EtOH 96% samples.

2.EtOH 70 %									
Concentration of 2 (µl)	Absorbance 1	Absorbance 2	Absorbance 3	Activity 1	Activity 2	Actvity 3	Average	Stand. Deviation	Extract concentration (µg/ml)
0	0,765	0,765	0,765						
50	0,63	0,65	0,63	17,647	15,033	17,647	16,680	1,509	9,804
100	0,455	0,431	0,43	40,523	43,660	43,791	42,603	1,850	19,231
125	0,318	0,348	0,344	58,431	54,510	55,033	55,938	2,129	23,810
150	0,279	0,283	0,286	63,529	63,007	62,614	63,048	0,459	28,302
175	0,199	0,219	0,23	73,987	71,373	69,935	71,726	2,054	32,710
200	0,154	0,174	0,176	79,869	77,255	76,993	78,018	1,590	37,037
							IC50(µl)	121,959	
							IC50(µg/ml)	22,901	

Table 64.Absorbances of EtOH 70% extracts.

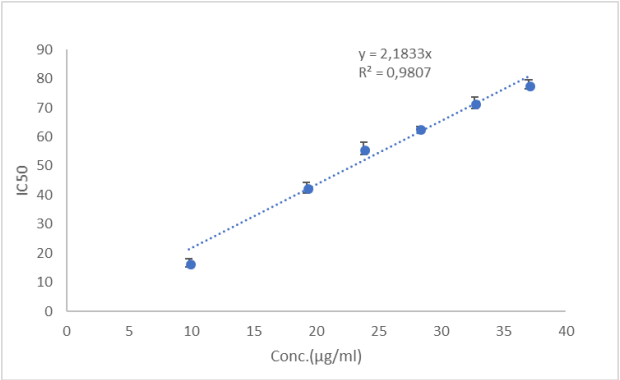

Figure 63.Inhibition curve of EtOH 70% samples.

3.EtOH 50%									
Concentration of 3 (µl)	Absorbance 1	Absorbance 2	Absorbance 3	Activity 1	Activity 2	Actvity 3	Average	Stand. Deviation	Extract concentration (µg/ml)
0	0,759	0,745	0,74						
50	0,591	0,595	0,609	22,134	20,134	17,703	19,824	2,219	9,804
100	0,37	0,341	0,351	51,252	54,228	52,568	52,654	1,492	19,231
150	0,213	0,22	0,221	71,937	70,470	70,135	70,839	0,958	28,302
175	0,153	0,149	0,172	79,842	80,000	76,757	78,838	1,829	32,710
200	0,083	0,089	0,109	89,065	88,054	85,270	87,433	1,965	37,037
							IC50(µl)	108,955	
							IC50(µg/ml)	20,538	

Table 65.Absorbances of EtOH 50% extracts

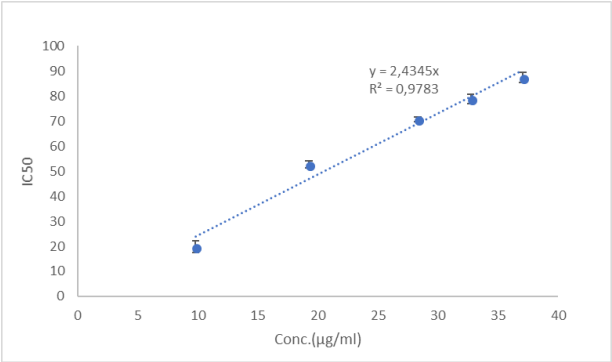

Figure 64.Inhibition curve of EtOH 50% samples.

4.Water									
Concentration of 4 (µl)	Absorbance 1	Absorbance 2	Absorbance 3	Activity 1	Activity 2	Actvity 3	Average	Stand. Deviation	Extract concentration (µg/ml)
0	0,862	0,866	0,866						
10	0,712	0,747	0,734	17,401	13,741	15,242	15,318	1,840	1,992
25	0,644	0,677	0,657	25,290	21,824	24,134	23,660	1,764	4,950
40	0,536	0,527	0,546	37,819	39,145	36,952	37,951	1,105	7,874
50	0,451	0,449	0,467	47,680	48,152	46,074	47,285	1,090	9,804
75	0,353	0,331	0,311	59,049	61,778	64,088	61,569	2,522	14,563
							IC50(µl)	56,929	
							IC50(µg/ml)	11,009	

Table 66.Absorbances of wáter extracts.

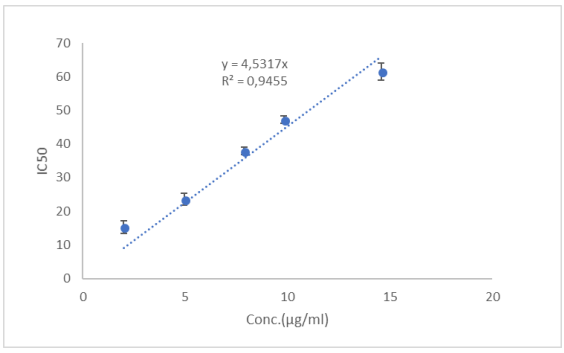

Figure 65.Inhibition curve of water samples.

8.5.2.2. Lavender hydrodistilled residue(L2/2).

1.EtOH 96%									
Concentration of 1 (µl)	Absorbance 1	Absorbance 2	Absorbance 3	Activity 1	Activity 2	Actvity 3	Average	Stand. Deviation	Extract concentration (µg/ml)
0	0,847	0,942	0,942						
50	0,794	0,873	0,869	6,257	7,325	7,749	7,053	0,769	9,804
100	0,741	0,838	0,839	12,515	11,040	10,934	11,453	0,883	19,231
200	0,645	0,744	0,741	23,849	21,019	21,338	21,999	1,550	37,037
400	0,521	0,526	0,52	38,489	44,161	44,798	42,284	3,474	68,966
							IC50(µl)	471,914	
							IC50(µg/ml)	82,008	

Table 67. Absorbances of EtOH 96% extracts.

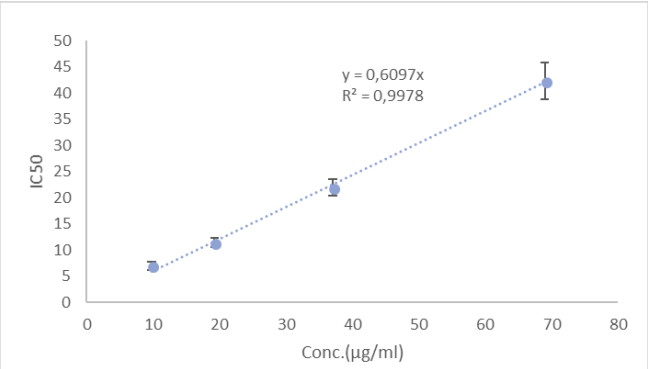

Figure 66. Inhibition curve of EtOH 96% samples.

2.EtOH 70 %									
Concentration of 2 (µl)	Absorbance 1	Absorbance 2	Absorbance 3	Activity 1	Activity 2	Actvity 3	Average	Stand. Deviation	Extract concentration (µg/ml)
0	0,843	0,823	0,843						
50	0,808	0,761	0,769	4,152	7,533	8,778	6,154	2,394	9,804
100	0,646	0,564	0,606	23,369	31,470	28,114	27,239	4,070	19,231
125	0,482	0,472	0,489	42,823	42,649	41,993	42,485	0,438	23,810
150	0,391	0,389	0,399	53,618	52,734	52,669	53,003	0,530	28,302
200	0,239	0,25	0,229	71,649	69,623	72,835	71,344	1,624	37,037
							IC50(µl)	147,885	
							IC50(µg/ml)	27,931	

Table 68. Absorbances of EtOH 70% extracts.

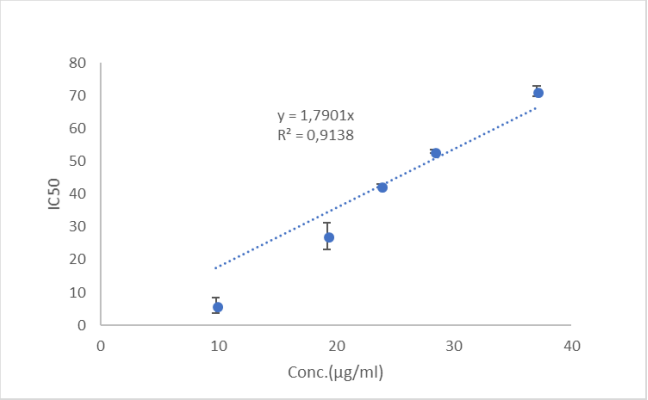

Figure 67. Inhibition curve of EtOH 70% samples.

3.EtOH 50%									
Concentration of 3 (µl)	Absorbance 1	Absorbance 2	Absorbance 3	Activity 1	Activity 2	Actvity 3	Average	Stand. Deviation	Extract concentration (µg/ml)
0	0,734	0,733	0,733						
50	0,698	0,689	0,695	4,905	6,003	5,184	5,325	0,571	9,804
100	0,496	0,477	0,451	32,425	34,925	38,472	35,102	3,039	19,231
150	0,205	0,201	0,211	72,071	72,578	71,214	71,950	0,690	28,302
175	0,123	0,115	0,131	83,243	84,311	82,128	83,218	1,091	32,710
200	0,058	0,038	0,029	92,098	94,816	96,044	94,290	2,019	37,037
							IC50(µl)	123,615	
							IC50(µg/ml)	20,654	

Table 69. Absorbances of EtOH 50% extracts

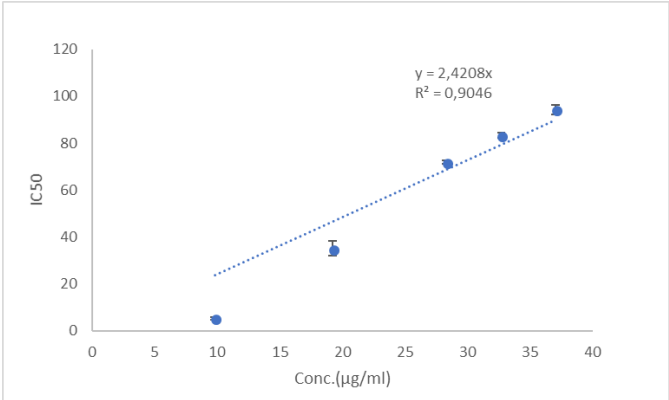

Figure 68. Inhibition curve of EtOH 50% samples.

4.Water									
Concentration of 4 (µl)	Absorbance 1	Absorbance 2	Absorbance 3	Activity 1	Activity 2	Actvity 3	Average	Stand. Desviation	Concentration extract (µg/ml)
0	0,762	0,766	0,766						
25	0,662	0,654	0,64	13,123	14,621	16,449	14,607	1,666	4,950
50	0,604	0,617	0,627	20,735	19,452	18,146	19,387	1,294	9,804
75	0,479	0,468	0,472	37,139	38,903	38,381	38,127	0,906	14,563
100	0,321	0,339	0,315	57,874	55,744	58,877	57,469	1,600	19,231
200	0,043	0,031	0,029	94,357	95,953	96,214	95,501	1,005	37,037
							IC50(µl)	93,488	
							IC50(µg/ml)	18,996	

Table 70. Absorbances of wáter extracts.

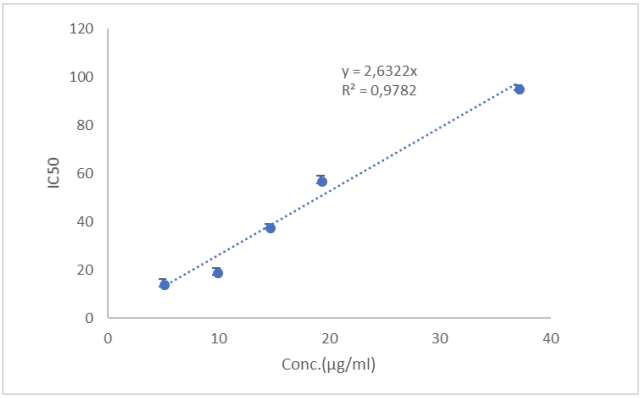

Figure 69. Inhibition curve of water samples.

8.6. Polyphenols measurements- pyrogallol calibration curve.

Volume solution (ml)	Pirogallol mass(mg)	Pirogallol conc. in flask (mg/ml)	Pirogallol conc. in cuvette (mg/ml)	Absorbance			Absorbance Average	Standard deviation
1	40,72	0,102	0,004	0,607	0,589	0,580	0,592	0,014
1,6	40,72	0,163	0,007	0,918	0,860	0,869	0,882	0,031
2,2	39,48	0,217	0,009	1,095	1,097	1,066	1,086	0,017
2,8	39,48	0,276	0,011	1,343	1,332	1,334	1,336	0,006
3,4	39,56	0,336	0,013	1,621	1,702	1,735	1,686	0,059

Table 71.Calculations for calibration curve of polyphenols.

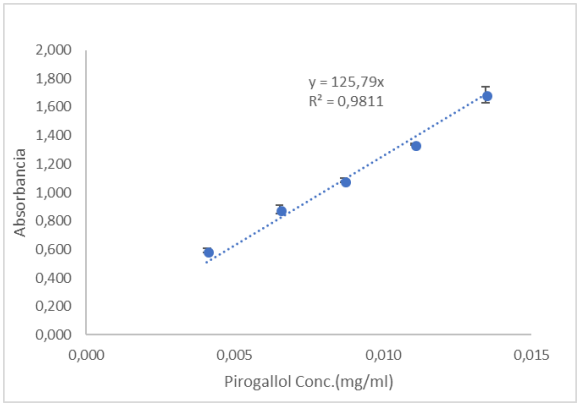

Figure 70.Calibration curve for polyphenols.

8.6.1. Soxhlet extraction

8.6.1.1 Lavender steam distilled residue (L2/1)

Slope of the calibration line:	125,79														
All polyphenols	N-pentane L2/1			Ethyl-acetate L2/1			Acetone L2/1			Propanol L2/1			EtOH L2/1		
Mass of dry matter [mg]	24,7			23,4			24,7			26,4			24,8		
Sample volume [ml]	15,0			15,0			15,0			15,0			15,0		
Pattern absorbance [-]	0,131	0,126	0,131	0,290	0,274	0,287	0,315	0,329	0,344	0,434	0,426	0,462	0,571	0,582	0,585
Concentration in the cuvette [mg / ml]	0,00104	0,00100	0,00104	0,0023	0,0022	0,0023	0,00250	0,00262	0,00273	0,0035	0,0034	0,0037	0,0045	0,0046	0,0047
In a 20 ml solution, equivalent to pyrogallol (mg)	0,02083	0,02003	0,02083	0,0461	0,0436	0,0456	0,05008	0,05231	0,05469	0,0690	0,0677	0,0735	0,0908	0,0925	0,0930
From a 800 microliter extract, an equivalent weight of pyrogallol (mg)	0,02083	0,02003	0,02083	0,0461	0,0436	0,0456	0,05008	0,05231	0,05469	0,0690	0,0677	0,0735	0,0908	0,0925	0,0930
In a sample solution, the weight equivalent to pyrogallol [mg]	0,39	0,38	0,39	0,86	0,82	0,86	0,94	0,98	1,03	1,29	1,27	1,38	1,70	1,74	1,74
The pyrogallol equivalent weight [mg]	0,39	0,38	0,39	0,86	0,82	0,86	0,94	0,98	1,03	1,29	1,27	1,38	1,70	1,74	1,74
100 g of pyrogallol equivalent equivalent (g)	1,58	1,52	1,58	3,69	3,49	3,66	3,80	3,97	4,15	4,90	4,81	5,22	6,86	7,00	7,03
Average [%]	1,56			3,61			3,97			4,98			6,96		
Stan. Deviation	0,03			0,09			0,14			0,17			0,07		

Table 72.Polyphenols measurements.

8.6.1.2. Lavender hydrodistilled residue(L2/2)

Slope of the calibration line:	125,79														
All polyphenols	Pentane L2/2			Ethyl-acetate L2/2			Acetone L2/2			Propanol l2/2			EtOH L2/2		
Mass of dry matter [mg]	23,6			25,5			22,9			23,2			23,7		
Sample volume [ml]	15,0			15,0			15,0			15,0			15,0		
Pattern absorbance [-]	0,105	0,118	0,124	0,303	0,312	0,290	0,306	0,293	0,304	0,312	0,322	0,318	0,526	0,513	0,501
Concentration in the cuvette [mg / ml]	0,00083	0,00094	0,00099	0,00241	0,00248	0,00231	0,00243	0,00233	0,00242	0,0025	0,0026	0,0025	0,00418	0,00408	0,00398
In a 20 ml solution, equivalent to pyrogallol (mg)	0,01669	0,01876	0,01972	0,04818	0,04961	0,04611	0,04865	0,04659	0,04833	0,0496	0,0512	0,0506	0,08363	0,08156	0,07966
From a 800 microliter extract, an equivalent weight of pyrogallol (mg)	0,01669	0,01876	0,01972	0,04818	0,04961	0,04611	0,04865	0,04659	0,04833	0,0496	0,0512	0,0506	0,08363	0,08156	0,07966
In a sample solution, the weight equivalent to pyrogallol [mg]	0,31	0,35	0,37	0,90	0,93	0,86	0,91	0,87	0,91	0,93	0,96	0,95	1,57	1,53	1,49
The pyrogallol equivalent weight [mg]	0,31	0,35	0,37	0,90	0,93	0,86	0,91	0,87	0,91	0,93	0,96	0,95	1,57	1,53	1,49
100 g of pyrogallol equivalent equivalent (g)	1,33	1,49	1,57	3,54	3,65	3,39	3,98	3,81	3,96	4,01	4,14	4,09	6,62	6,45	6,30
Average [%]	1,46			3,53			3,92			4,08			6,46		
Stan. Deviation	0,10			0,11			0,07			0,05			0,13		

Table 73. Polyphenols measurements.

8.6.2. Stirred tank extraction

8.6.2.1 Lavender steam distilled residue (L2/1)

Slope of the calibration line:	125,79														
All polyphenols	96%L2/1			70%L2/1			50%L2/1			30%EtOHL2/1			Water L2/1		
Mass of dry matter [mg]	24,8			24,7			24,8			24,8			25,0		
Sample volume [ml]	15,0			15,0			15,0			15,0			15,0		
Pattern absorbance [-]	0,378	0,397	0,401	0,561	0,575	0,562	0,838	0,839	0,855	0,462	0,447	0,472	1,083	1,135	1,138
Concentration in the cuvette [mg / ml]	0,0030	0,0032	0,0032	0,00446	0,00457	0,00447	0,0067	0,0067	0,0068	0,0037	0,0036	0,0038	0,00861	0,00903	0,00905
In a 20 ml solution, equivalent to pyrogallol (mg)	0,0601	0,0631	0,0638	0,08920	0,09142	0,08986	0,1332	0,1334	0,1359	0,0733	0,0711	0,0756	0,17219	0,18062	0,18094
From a 800 microliter extract, an equivalent weight of pyrogallol (mg)	0,0601	0,0631	0,0638	0,08920	0,09142	0,08986	0,1332	0,1334	0,1359	0,0733	0,0711	0,0756	0,17219	0,18062	0,18094
In a sample solution, the weight equivalent to pyrogallol [mg]	1,13	1,18	1,20	1,67	1,71	1,68	2,50	2,50	2,55	1,38	1,33	1,41	3,23	3,39	3,39
The pyrogallol equivalent weight [mg]	1,13	1,18	1,20	1,67	1,71	1,68	2,50	2,50	2,55	1,38	1,33	1,41	3,23	3,39	3,39
100 g of pyrogallol equivalent equivalent (g)	4,54	4,77	4,82	6,77	6,94	6,78	10,07	10,09	10,28	5,55	5,37	5,67	12,91	13,55	13,57
Average [%]	4,71			6,83			10,15			5,53			13,34		
Stan. Deviation	0,12			0,08			0,09			0,12			0,30		

Table 74. Polyphenols measurements.

8.6.2.2. Lavender hydrodistilled residue(L2/2)

Slope of the calibration line:	125,79														
All polyphenols	96% EtOH L2/2			70% L2/2			50% L2/2			30% L2/2			Water L2/2		
Mass of dry matter [mg]	24,7			23,0			23,1			24,0			25,0		
Sample volume [ml]	15,0			15,0			15,0			15,0			15,0		
Pattern absorbance [-]	0,364	0,359	0,377	0,492	0,482	0,478	0,706	0,716	0,706	0,426	0,423	0,432	0,963	1,046	1,172
Concentration in the cuvette [mg / ml]	0,00289	0,00285	0,00300	0,0039	0,0038	0,0038	0,00561	0,00569	0,00561	0,0034	0,0034	0,0034	0,00766	0,00832	0,00932
In a 20 ml solution, equivalent to pyrogallol (mg)	0,05787	0,05708	0,05994	0,0782	0,0766	0,0760	0,11225	0,11384	0,11225	0,0677	0,0673	0,0687	0,15311	0,16631	0,18634
From a 800 microliter extract, an equivalent weight of pyrogallol (mg)	0,05787	0,05708	0,05994	0,0782	0,0766	0,0760	0,11225	0,11384	0,11225	0,0677	0,0673	0,0687	0,15311	0,16631	0,18634
In a sample solution, the weight equivalent to pyrogallol [mg]	1,09	1,07	1,12	1,47	1,44	1,42	2,10	2,13	2,10	1,27	1,26	1,29	2,87	3,12	3,49
The pyrogallol equivalent weight [mg]	1,09	1,07	1,12	1,47	1,44	1,42	2,10	2,13	2,10	1,27	1,26	1,29	2,87	3,12	3,49
100 g of pyrogallol equivalent equivalent (g)	4,39	4,33	4,55	6,38	6,25	6,20	9,11	9,24	9,11	5,29	5,25	5,37	11,48	12,47	13,98
Average [%]	4,43			6,27			9,15			5,30			12,64		
Stan. Deviation	0,09			0,08			0,06			0,05			1,02		

Table 75. Polyphenols measurements.

8.7. Tannins measurements.

8.7.1. Soxhlet extraction

8.7.1.1 Lavender steam distilled residue (L2/1)

Tannin free polyphenol	N-pentane L2/1			Ethyl-acetate L2/1			Acetone L2/1			Propanol L2/1			EtOH L2/1		
Sample absorbance [-]	0,093	0,087	0,080	0,210	0,173	0,208	0,226	0,258	0,246	0,276	0,281	0,275	0,396	0,383	0,408
Concentration in the cuvette [mg / ml]	0,00074	0,00069	0,00064	0,00167	0,00138	0,00165	0,00180	0,00205	0,00196	0,00219	0,00223	0,00219	0,00315	0,00304	0,00324
In a 20 ml solution, the weight equivalent to pirogallol (mg)	0,01479	0,01383	0,01272	0,03339	0,02751	0,03307	0,03593	0,04102	0,03911	0,04388	0,04468	0,04372	0,06296	0,06090	0,06487
In the 800 microliter extract the weight equivalent to pyrogallol (mg)	0,01479	0,01383	0,01272	0,03339	0,02751	0,03307	0,03593	0,04102	0,03911	0,04388	0,04468	0,04372	0,06296	0,06090	0,06487
In the sample solution, the equivalent weight of pirogallol (mg)	0,28	0,26	0,24	0,63	0,52	0,62	0,67	0,77	0,73	0,82	0,84	0,82	1,18	1,14	1,22
In the dry sample, the equivalent weight of pyrogallol [mg]	0,28	0,26	0,24	0,63	0,52	0,62	0,67	0,77	0,73	0,82	0,84	0,82	1,18	1,14	1,22
In the 100 g sample the equivalent weight of pyrogallol [g]	1,12	1,05	0,97	2,68	2,20	2,65	2,73	3,11	2,97	3,12	3,17	3,11	4,76	4,60	4,90
Average [%]	1,05			2,51			2,94			3,13			4,76		
Stan.deviation	0,06			0,22			0,16			0,03			0,12		
Tannin(%)	0,51			1,10			1,04			1,84			2,21		

Table 76. Tannins measurements

8.7.1.2. Lavender hydrodistilled residue(L2/2)

Tannin free polyphenol	Pentane L2/2			Ethyl-acetate L2/2			Acetone L2/2			Propanol L2/2			EtOH L2/2		
Sample absorbance [-]	0,096	0,101	0,114	0,212	0,222	0,228	0,246	0,220	0,228	0,258	0,279	0,275	0,502	0,518	0,505
Concentration in the cuvette [mg / ml]	0,00076	0,00080	0,00091	0,00169	0,00176	0,00181	0,00196	0,00175	0,00181	0,00205	0,00222	0,00219	0,00399	0,00412	0,00401
In a 20 ml solution, the weight equivalent to pirogallol (mg)	0,01526	0,01606	0,01813	0,03371	0,03530	0,03625	0,03911	0,03498	0,03625	0,04102	0,04436	0,04372	0,07982	0,08236	0,08029
In the 800 microliter extract the weight equivalent to pyrogallol (mg)	0,01526	0,01606	0,01813	0,03371	0,03530	0,03625	0,03911	0,03498	0,03625	0,04102	0,04436	0,04372	0,07982	0,08236	0,08029
In the sample solution, the equivalent weight of pirogallol (mg)	0,29	0,30	0,34	0,63	0,66	0,68	0,73	0,66	0,68	0,77	0,83	0,82	1,50	1,54	1,51
In the dry sample, the equivalent weight of pyrogallol [mg]	0,29	0,30	0,34	0,63	0,66	0,68	0,73	0,66	0,68	0,77	0,83	0,82	1,50	1,54	1,51
In the 100 g sample the equivalent weight of pyrogallol [g]	1,21	1,28	1,44	2,48	2,60	2,67	3,20	2,86	2,97	3,32	3,59	3,53	6,31	6,52	6,35
Average [%]	1,31			2,58			3,01			3,48			6,39		
Stan.deviation	0,10			0,08			0,14			0,12			0,09		
Tannin(%)	0,15			0,95			0,91			0,60			0,06		

Table 77. Tannins measurements

8.7.2. Stirred tank extraction

8.7.2.1 Lavender steam distilled residue (L2/1)

Tannin free polyphenol	96% L2/1			70% L2/1			96% L2/1			30% EtOH L2/1			Water L2/1		
Sample absorbance [-]	0,276	0,305	0,284	0,357	0,364	0,347	0,446	0,450	0,437	0,337	0,362	0,352	0,550	0,601	0,597
Concentration in the cuvette [mg / ml]	0,00219	0,00242	0,00226	0,00284	0,00289	0,00276	0,00355	0,00358	0,00347	0,00268	0,00288	0,00280	0,00437	0,00478	0,00475
In a 20 ml solution, the weight equivalent to pirogallol (mg)	0,04388	0,04849	0,04515	0,05676	0,05787	0,05517	0,07091	0,07155	0,06948	0,05358	0,05756	0,05597	0,08745	0,09556	0,09492
In the 800 microliter extract the weight equivalent to pyrogallol (mg)	0,04388	0,04849	0,04515	0,05676	0,05787	0,05517	0,07091	0,07155	0,06948	0,05358	0,05756	0,05597	0,08745	0,09556	0,09492
In the sample solution, the equivalent weight of pirogallol (mg)	0,82	0,91	0,85	1,06	1,09	1,03	1,33	1,34	1,30	1,00	1,08	1,05	1,64	1,79	1,78
In the dry sample, the equivalent weight of pyrogallol [mg]	0,82	0,91	0,85	1,06	1,09	1,03	1,33	1,34	1,30	1,00	1,08	1,05	1,64	1,79	1,78
In the 100 g sample the equivalent weight of pyrogallol [g]	3,32	3,67	3,41	4,31	4,39	4,19	5,36	5,41	5,25	4,05	4,35	4,23	6,56	7,17	7,12
Average [%]	3,47			4,30			5,34			4,21			6,95		
Stan.deviation	0,15			0,08			0,07			0,12			0,28		
Tannin(%)	1,25			2,53			4,80			1,32			6,40		

Table 78. Tannins measurements

8.7.2.2. Lavender hydrodistilled residue(L2/2)

Tannin free polyphenol	96% EtOH L2/2			70% L2/2			50% L2/2			30% L2/2			Water L2/2		
Sample absorbance [-]	0,289	0,300	0,299	0,454	0,457	0,471	0,594	0,616	0,611	0,411	0,412	0,418	0,671	0,673	0,712
Concentration in the cuvette [mg / ml]	0,00230	0,00238	0,00238	0,00361	0,00363	0,00374	0,00472	0,00490	0,00486	0,00327	0,00328	0,00332	0,00533	0,00535	0,00566
In a 20 ml solution, the weight equivalent to pirogallol (mg)	0,04595	0,04770	0,04754	0,07218	0,07266	0,07489	0,09444	0,09794	0,09715	0,06535	0,06551	0,06646	0,10669	0,10700	0,11320
In the 800 microliter extract the weight equivalent to pyrogallol (mg)	0,04595	0,04770	0,04754	0,07218	0,07266	0,07489	0,09444	0,09794	0,09715	0,06535	0,06551	0,06646	0,10669	0,10700	0,11320
In the sample solution, the equivalent weight of pirogallol (mg)	0,86	0,89	0,89	1,35	1,36	1,40	1,77	1,84	1,82	1,23	1,23	1,25	2,00	2,01	2,12
In the dry sample, the equivalent weight of pyrogallol [mg]	0,86	0,89	0,89	1,35	1,36	1,40	1,77	1,84	1,82	1,23	1,23	1,25	2,00	2,01	2,12
In the 100 g sample the equivalent weight of pyrogallol [g]	3,49	3,62	3,61	5,88	5,92	6,10	7,67	7,95	7,89	5,11	5,12	5,19	8,00	8,03	8,49
Average [%]	3,57			5,97			7,83			5,14			8,17		
Stan.deviation	0,06			0,10			0,12			0,04			0,23		
Tannin(%)	0,85			0,30			1,32			0,17			4,47		

Table 79.Tannins measurements.