e-ISSN: 2757-8836
ORIGINAL ARTICLE
*J. Raw Mater. Process. Foods vol.1(2)* (2020) 55-71

Recieved: 14/12/2020 Revised: 25/01/2021 Accepted article published: 26/01/2021 Published online: 26/01/2021

# Effect of infusion time on the phenolic profile and some physicochemical properties of *Lavandula* x intermedia *cv.* 'SUPER'

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## Abstract

***Lavandula* x *intermedia*** **(lavandin) is a more abundant and fertile species obtained from natural crosses between species of *L. latifolia* and *L. angustifolia*. Depending on the infusion period, the color, total acidity, total phenolic content, antioxidant activity, fatty acid and phenolic profile of *Lavandula* x *intermedia* cv. 'SUPER' (Lamiaceae) grown in Adana is to elucidate in this study. Phenolic compounds and fatty acid profile were analyzed with LC-DAD-ESI-MS/MS and GC, respectively. A total of 20 phenolic compounds with total concentrations ranging from 236.06 to 522.17 mg/L and 149.0 to 186.25 mg/L in lavender flower and lavender stems, respectively, were identified and characterized by LC-DAD-ESI/MS. The highest ratio of these compounds was observed in all infusions obtained at lavender flower for 12 min. Among the phenolic compounds, coumaric acid-O-glucoside was the most abundant and was followed by salvianolic acid derivative 3 and rosmarinic acid. The results revealed significant differences among extracts of lavender flower and stalk regarding phenolics, antioxidant, and other chemical properties. With regard to fatty acids, oleic acid has the highest concentration in all the samples, followed by linoleic acid.**

***Keywords:*** *Lavender, Lavandula x intermedia cv 'SUPER', phenolics, antioxidant capacity,*

## 1. INTRODUCTION

Medicinal and aromatic plants have been widely used from time immemorial, in the treatment of many diseases, cosmetics, and for preserving and enhancing the flavor of foods (Sánchez-Vioquea et al., 2013; Contreras et al., 2017; Gayoso et al., 2018). The World Health Organization seeks to capitalize on the use of traditional medicines including, herbal medicines, in its 2014–2023 strategy, with the aim of keeping populations healthy through providing access to effective and affordable alternatives to medicine, and to provide healthcare choices consistent with people's cultural practices (WHO, 2013). The Lavender is the genus *Lavandula* belonging to the Lamiaceae family and the whole genus *Lavandula* comprises more than 40 different species and hundreds of cultivars and hybrids (Adaszyńska-Skwirzyńska & Dzięcioł, 2017; Alizadeha & Aghaeea, 2016; Bajalan & Pirbalouti, 2015; Hawrył et al., 2019). Lavender is a perennial plant that can grow up to 1 m, is not soil-selective, and can be produced as a semi-shrub, vegetative and generative plant widely grown in the Mediterranean regions (Héral et al., 2020; Rady & Saker, 2000). It is highly resistant to drought, heat and cold climate (Aslancan & Sarıbaş, 2011). Located in the east of the Mediterranean region, Adana has dry summers and mild and rainy winters. Lavender is usually harvested during the summer months and a warm, sunny day is chosen for the harvest (Kara et al., 2014). Lavender reaches full bloom in July according to species and varieties, climate and soil conditions, altitude and region and is harvested during this period (Aslancan & Sarıbaş, 2011). Lavender is mostly used in the cosmetic and perfume industry, pharmaceutical industry and in aromatherapy with its pain relieving, calming and insomnia properties. It can be consumed in the form of tea due to the sedative effect of lavender flowers in addition to the effect of increasing urine and relieving the pain of rheumatism (Aslancan &

Sarıbaş, 2011). Lavender is the most demanding plant in Turkey and the world and is actively cultivated in Aydın and Isparta in Turkey. According to TURKSTAT data, while there is a lavender production area in an area of 8.700 decares in our country, the country-wide lavender production areas have surpassed 10.000 decares as of 2019. It is possible to obtain approximately 1.500 tons of lavender flowers from these areas and obtain 20-30 tons of lavender oil from the flowers (TURKSTAT, 2019). Lavender has anti-microbial, anti-fungal, and carminative properties due to the essential oils (monoterpenes) (Gilani et al., 2000; Kirmizibekmez et al., 2009; Cavanagh et al., 2002; Sabara, & Kunicka-Styczyńska, 2009). Three varieties of *Lavandula*, geographically cultivated and of economic importance in the World is *Lavandula angustifolia* Miller, *Lavandula* latifolia and *Lavandula* x *intermedia* (Bajalan et al., 2016). Interest in the industrial cultivation, production and essential oil of the medicinal and aromatic plants *Lavandula angustifolia* and *Lavandula* x *intermedia* Emeric has been growing rapidly in recent years (Máthé, 2015; Quílez et al., 2020). In recent years, lavandin (*Lavandula* x *intermedia* var. Super (Isparta)) and lavender *(Lavandula angustifolia)* culture are done economically in Turkey (Aslancan, & Sarıbaş, 2011). *Lavandula* × *intermedia* (lavandin) is a species more abundantly andis more prolific obtained, which results from natural crosses between *L.latifolia* and *L. angustifolia* species (Raghavan, 2007; Yohalem & Passey, 2011). *Lavandula* × intermedia is used in soaps, washing agents and perfumes. Addition to this is also added as a flavor to food and beverages. Bioactive compounds are molecules containing a broad variety of groups, such as carotenoids, phenolic compounds and vitamins that have health benefits for living organisms, tissues or cells (Komes et al., 2010).

Phenolic compounds are secondary metabolites formed by phenylpropanoid metabolization in the shikimic acid of plants and pentose phosphate (Randhir et al., 2004; El-Haci et al., 2013). They contain benzene rings, with one or more hydroxyl substituents, and range from simple phenolic molecules to highly polymerized compounds (Velderrain-Rodríguez et al., 2014). Plant polyphenols and polyphenol-rich products modulate the metabolism of carbohydrates and lipids, attenuate hyperglycemia, dyslipidemia and insulin resistance, enhance the role of β-cells, promote insulin secretion, boost the metabolism of adipose tissue and relieve oxidative stress, pathways of stress-sensitive signaling and inflammatory processes (Lin et al., 2016).

Plants with various pharmacological activities include phenolic compounds, an important class of secondary metabolites (El-Haci et al., 2013). Phenolic compounds, such as phenolic acids, coumarins, flavonoids, stilbenes, hydrolysable and condensed tannins, lignans and lignins, are distinguished by being a very heterogeneous category with a wide variety of compounds. These molecules also have antioxidant, anti-tumor, anti-inflammatory and antiviral properties (Komes et al., 2010; Costa et al., 2013; Port et al., 2013). Most plants in the world have a strong antioxidant activity and a strong cleaning activity against free radicals (Gonçalves & Romano, 2013). Lavender is one of herbs with a high content of biologically active substances in the polyphenol group. In extracts and hydrolates obtained from this plant, polyphenols are present (Spiridon et al., 2011; Prusinowska et al., 2016), while other active compounds from the terpenoid group are found in essential lavender oil (Donadu et al., 2017). A wide variety of phenolic acids, including 4-hydroxybenzoic acid, vanillic acid, chlorogenic acid, syringic acid, *o*-coumaric acid, *p*-coumaric acid, ferulic acid, metoxy-cinnamic acid and sinapic acid, have been detected in methanolic extracts of *Lavandula angustifolia* (common lavender) (Blažeković et al. 2010; Spiridon et al. 2011; Sytar et al. 2016). According to study of Torras-Claveria et al. (2007), rosmarinic, chlorogenic, n-coumaric, 4-O- caffeoylquinic acids as hydroxycinnamic acids, and pinocembrin, apigenin glycosides, and luteolin as flavonoids were determined as phenolic compounds in the water-ethanol extract of

*Lavandula* x *intermedia* Emeric ex Loisel *(Lamiaceae)* cv. 'Bora' (Torras-Claveria et al., 2007). Gallic acid, *p*-hydroxybenzoic acid, catechin, vanillic acid, caffeic acid, ferulic acid, and naringenin were found in the *Lavandula vera* (Proestos et al., 2006). *O*-coumaric acid, coumarin, rosmarinic acid, herniarin, luteolin and apigenin have been detected with reverse phase HPLC/DAD in ethanolic extracts of *Lavandula officinalis* (Areias et al., 2000).

Fatty acids such as $\alpha$-linolenic, linoleic, palmitic, oleic, stearic, arachidonic and vaccenic are found in lavender (Urwin & Mailer, 2008; Radu et al., 2020; Quilez et al., 2020). Lavender contains $\alpha$-linolenic acid and linoleic acid, two essential fatty acids for the health of humans. $\alpha$-Linolenic acid is involved in EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) synthesis as well (Gogus et al., 2010). These fatty acids are also reported to play a key role in cancer, stroke, inflammatory disorders and cardiovascular disease prevention (Wassell et al., 2010). In addition to the ever-popular green and black varieties, a number of plant species may produce tea with water infusions from the roots, leaves and flowers. These 'herbal teas' have a wealth of compounds and may play a major role in supplying nutrients and chemicals to compensate for diets of low quality (Poswal et al., 2019).

The literature on lavender infusion includes a limited number of studies. For the reason, the aim of the present study is to elucidate color, total acidity, total phenolic content, the fatty acid antioxidant activity and phenolic profile depending on the period of infusion of *Lavandula* x *intermedia* cv. 'SUPER'. Fatty acid profile and phenolic compounds of the samples were determined with GC and LC-ESI-MS / MS method, respectively.

## 2. MATERIALS AND METHODS

### *2.1. Materials*

The lavender plant (*Lavandula* x *intermedia* cv. 'SUPER') was collected in the Adana region (Kuyumcular village, Adana), Turkey. The lavender harvest was completed in early July. The stalk was cut to 30 cm long, then the stalk and flower part were separated. The samples were spread out without thick layers and allowed to dry at room temperature for 14 days in the dark. Samples were checked regularly and inverted. In a home-style coffee grinder, dry lavender flower and stalk were ground before analysis. The parts of the flower and stalk were prepared at various infusion times using a water bath (ISOLAB, Isolab Laborgeräte GmbH, Germany).

### *2.2. Methods*

#### *2.2.1. Preparation of Lavender Infusions*

Three grams of dry lavender flower and stalk were weighed and decanted into a 250 ml beaker, added 100 ml of water and then brewed for 3, 6 and 12 min at approximately 100°C. The samples were then centrifuged for 15 minutes at 4°C at 5.500 rpm and filtered through a 0.45 µm cellulose acetate filter (Millipore) prior to high-performance liquid chromatography analysis (HPLC). Extraction has been carried out in duplicate.

#### *2.2.2. Color analysis of Lavender Infusions*

The color of the lavender flower and stalk infusions were measured in the Konica Minolta CM-55 (Konica Minolta Optics Inc.). The results were reported using the Commission Internationale de l'Eclairage for the L*, a*, and b* colour system profiles (CIE) (Liang et al., 2004).

#### *2.2.3. pH and Total Acidity Analysis*

pH values of lavender infusions were measured using pH-meter (Isolab Laborgeräte GmbH, Germany). All measurements were carried out at room temperature after immersion of the electrode (that was firstly cleaned with distilled water) in the herb aqueous solution, until constant values were reached (Sadler & Murphy, 2010).

10 ml of infusions prepared from the lavender flower and stalk were transferred to the beaker for total acidity analysis and then titrated with a standard solution of sodium hydroxide (0.1 N) to pH 8.1 (Sadler & Murphy, 2010).

*2.2.4. Fatty Acid Analyses*

Using the Soxhlet extraction device, the lavender oil was extracted with an appropriate solvent. The fatty acid composition of the lavender oil was determined by gas chromatography coupled with split injection (1:50) and a flame ionization detector (FID). Fatty acid methyl esters were prepared by cold transmethylation (IOOC, 2001—COI/T. 20/Doc. no. 24). Separation was accomplished with a 60 m capillary column (DB23; Agilent Inc.) that had a 0.25 mm I.D. and 0.25 μm film thickness (Hashempour et al., 2010).

*2.2.5. Antioxidant activity*

*2.2.5.1. DPPH Assay*

The DPPH assay was determined by the DPPH method previously described by Brand-Williams et al., (1995) with modifications (Sanchez-Moreno et al.,1998). Briefly stated, 100 μl of each extract was mixed with a 3.9 ml of DPPH solution and incubated in the dark at room temperature for 1 h. The absorbance was then measured at 515 nm by a UV–visible spectrophotometer (Carry 60, Agilent, Malaysia). The antioxidant activities were calculated from a calibration curve and the result was expressed as a Trolox equivalent per liter.

*2.2.5.2. ABTS Assay*

The antioxidant capacities of the samples were evaluated by a method based on the decolonization of radical cation of 2,2'-azino bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) (Kelebek et al., 2013). The ABTS radical cation was prepared by the reaction of 7 mM ABTS with 2.54 mM potassium persulfate, after incubation at room temperature for 12–16 h. Prior to the assay, the ABTS solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. A total of 3.9 mL of the diluted ABTS solution was added to 0.1 mL of each sample. The reaction mixture was allowed to stand at room temperature for 30 min and absorbance at 734 nm was immediately recorded.

*2.2.6. Total Phenolic Content Analyses*

TPC analyses were determined using a Folin–Ciocalteu reagent previously described by Cemeroğlu (2014) and Shahidi & Ambigaipalan (2015). In brief, 100 μl of extract or standard 7.5 ml of distilled water was added, mixed for 3 minutes with 0.5 ml of Folin-Ciocalteu reagent, then added 1 ml of % (w/v) sodium carbonate and 0.9 ml of distilled water. Until absorbance at 765 nm was measured, the mixture was allowed to stand for 1 hour at room temperature. TPC in mg / L extract was expressed as gallic acid equivalents (GAE).

*2.2.7. Analysis of Lavender Infusions Phenolic Compounds Using HPLC/ESI-MS/MS*

Before injection, lavender infusions were filtered through a 0.45 μm membrane filter. According to Kelebek (2016), an LC-MS/MS study was performed The Agilent 1100 HPLC framework (Agilent Technologies) was fitted with ChemStation software based on Windows NT. The HPLC equipment consists of an auto sampler (G1367 E, 1260 HIP ALS), a binary pump (G1312 B, 1260 Bin pump), a degasser (G1322 A, 1260 Degasser), and a diode array detector (G1351D 1260 DAD VL). A Phenomenex Luna reverse phase C-18 column (4.6 mm × 250 mm, 5 μm) was used as the column in the HPLC system. Analysis conditions were as follows: water/ formic acid (99:1; v/ v, Solvent A) and methanol/ formic acid A (60:40; v/ v, Solvent B) as a mobile phase, a flow rate 0.5 ml/ min and at a temperature of 25°C, respectively. The DAD was adjusted at 280, 320, and 360 nm to observe peak intensity as real time, and the full spectra (190–650 nm) were continuously recorded for component identification of the extracts. Each phenolic compound was identified by the comparison of a retention index and UV spectra to pure standards and the identification was also confirmed by an Agilent 6430 LC–MS/MS spectrometer equipped with an electrospray ionization source. Mass spectra (over the range of m/z 100–2000) were simultaneously acquired in the positive and negative ionization modes. Identification and quantification of mass spectrum data of phenolic

compounds were collected in negative ion and MRM mode. Each sample was analyzed in triplicate. Identification and quantification of phenolic compounds were performed according to the method described by Kelebek (2016). The regression coefficient ($R^2$) value of the standards was given as above 0.995 in all cases. In the absence of reference compounds, the calibration of structurally related substances was used, taking into account the molecular weight correction factor. The limits of detection (LOD) and quantification (LOQ) were calculated at signal-to-noise ratios (S/N) of 3 and 10, respectively (Sen & Sonmezdag, 2020).

*2.2.8. Statistical Analysis*

Statistical data analysis was conducted using SPSS 22.0 with One-way ANOVA (SPSS Inc). Duncan's test measured the variations in the content levels of the results. Means with p-values below 0.05 have been found to be statistically important.

## 3. RESULTS AND DISCUSSION

*3.1. Color Properties of Lavender Infusions*

One of the most important food characteristics to be evaluated is color. It is generally known that color is an important factor affecting the food selection process of customers and is one of the organoleptic properties of herbal teas that are important for consumer acceptance (Jin et al., 2016; Ya-Lin et al., 2021). The literature examining the color change in infusions prepared with dried lavender flower and stalk, however, is quite limited. In this research, color variations were attempted to be explained based on various infusion times (3 min, 6 min, 12 min). Based on infusion times, color parameters (L*, a*, b*, c) differed significantly ($p < .05$). The L* is related to the light transmittance of the material (L* =0 black, L* = 100 white) and as can be seen from Table 2, the L* value of the samples ranged from 74.59 to 89.38 and decreased with infusion time on both the flower and the stalk. Although the highest L* values (79.00 and 89.38 respectively) in lavender flower and stalk infusions were observed in 3 minutes, the lowest L* values (74.59 and 88.53 respectively) were observed in 12 minutes. Depending on infusion duration, the L* value decreased. This reduction in the value of L* was statistically significant ($p < .05$) and observed that over time, infusions were darker in colour. The a * / - a * and b * / - b * signifyredness / greenness and yellowness / blueness, respectively.

During infusion time, the a* and b* values of the extracts were positively significant ($p <.05$). As can see Table 2, the color of lavender stalk infusion is green, and depending on the time of infusion, a* value was increased. It is assumed that when exposed to high temperatures and long infusion periods, phenolics undergo degradation, epimerization and oligomerization (Li et al., 2011; Wu & Sun, 2013). The increase in the b * value of the extracts depending on the infusion times can be associated with the density of flavonols formed as a result of enzymatic oxidation of phenolic compounds, and flavonols are in the yellow-orange pigment group (Kelebek, 2016). The findings demonstrate that through infusion time, color parameters enhance and achieve the greatest value at 12 min.

*3.2. pH and Total Acidity of Lavender Infusions*

pH and titratable acid are determined analytically in separate ways, and these have an impact on food quality (Flores-Martínez et al., 2018). Given the Table 2, depending on the infusion time, the pH showing the active acidity of the sample is between 5.54 and 5.65 in the flower of *Lavandula* x *intermedia* cv. 'SUPER' , while it is between 5.47 and 5,62 in the stalk, and the samples show slightly acidic properties. The pH values of the samples were not statistically significantly different (p>.05). In their study, Karabagias et al. (2019), calculated the pH value of *Lavandula stoechas* as 5.74 and reported that the extracts had slightly acidic characteristics.

Total acidity was within the range of 0.43-1.08 g/100 ml in the dried flower and stalk of *Lavandula* x *intermedia* cv. 'SUPER' and no statistically significant difference was determined ($p > .05$). Data on the physicochemical parameters of

Lavander infusions is scarce. Therefore, comparisons with literature data cannot be provided.

### *3.3. Fatty Acid Compositions of Lavender*

Considering that seed ethereal extracts consist mainly of fatty acid triesters bound to glycerol molecules, along with fat-soluble vitamins, carotenes, waxes, resins and sterols in greater or lesser quanti-ties, the absolute concentration of all fatty acids in this lipid fraction must be measured in terms of productivity and functional properties (Quílez et al., 2020). Based on this, the fatty acid composition of *Lavandula* x intermedia cv. 'SUPER' was shown in Table 1. Also, the GC chromatogram of Lavender was presented in Figure 1. As can be seen in the table, a total of 7 fatty acids are determined in sample. The main fatty acids were oleic acid (C18:1- cis ω9 ), linoleic acid (C18:2 ω6), while oleic acid had the highest concentration (%61.61) in sample. With a reaction catalyzed by desaturase enzymes present in plants, oleic acid transforms into linoleic acid (Harwood, 1988; Polari et al., 2019). As a source of important bioactive components, the interest in the ω-3 and ω-6 PUFA stems from the effect on the proper functioning of the metabolic pathways of a sufficient intake of these ω-3 acids (linolenic and stearidonic) and ω-6 acids (linoleic and ϒ-linolenic) (Masoodi et al., 2008).

*Table 1. Fatty acid compositions (% of total fatty acids) of Lavender*

| Fatty acid composition | % Peak Area |
|---|---|
| *C12:0* | 0.73 |
| *C16:0* | 8.76 |
| *C16:1* | 1.16 |
| *C18:0* | 3.69 |
| *C18:1-trans ω9* | 0.91 |
| *C18:1- cis ω9* | 61.61 |
| *C18:2 ω6* | 14.95 |

**C12:0 lauric acid; C16:0 palmitic acid; C16:1 palmitoleic acid; C18:0 stearic acid; C18:1-trans ω9 elaidic acid; C18:1- cis ω9 oleic acid; C18:2 ω6 linoleic acid*

Oleic and linoleic acid concentrations were 19% and 27%, respectively, in the analysis of fatty acids in the *Lavandula angustifolia* extracts obtained using various extraction methods (Radu et al., 2020). While the major fatty acid was defined as α-linolenic acid in studies with different species, it was found as oleic acid in our study. The main fatty acid was detected as α-linolenic acid in all species in another study with various spieces of lavender, while the quantities of oleic and linoleic acid was stated to range between 8.6-14.2% and 9.5-16.5%, respectively (Urwin, & Mailer, 2008). Furthermore, factors such as growing conditions, seasons, or various stages of processing are thought to alter the oil content of lavender plants (Urwin, & Mailer, 2008).

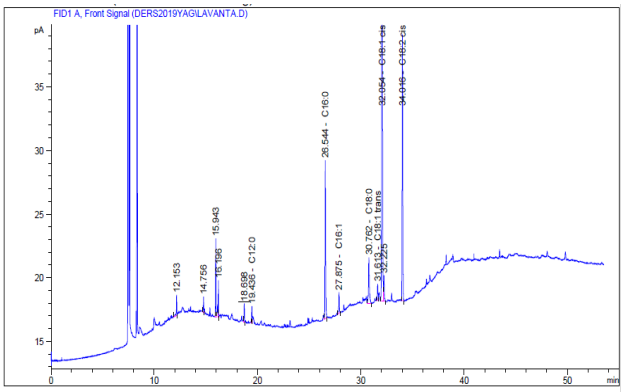


*Figure 1. Chromatograms of fatty acids determined in Lavandula x intermedia cv. 'SUPER'*

### *3.4. Antioxidant Capacities, and Total Phenolic Contents of Lavender Infusions*

The effect of infusion time on free radical scavenging ability was determined by DPPH and ABTS tests and both were found to increase with infusion time. DPPH is as a stable free radical that can become a stable diamagnetic molecule by accepting an electron or hydrogen radical from the antioxidant agents (Blažeković et al., 2010). With increasing infusion time, water penetration into the plant matrix may be promoted by increased mass transfer and thus increased solubility. DPPH radical-scavenging of the extract obtained from the dried lavender flower and stalk at various infusion times ranged from 85920.63 to 149365.08 µmol Trolox /L (Table 2). Statistical analysis revealed that there was no significant difference ($p > .05$) between infusions of lavender stalk at 3 and 6 minutes, while in the others there was a significant difference ($p < .05$) in antioxidant activity. The highest antioxidant activity was obtained in 12

minutes with 149365.08 μmol Trolox/L of lavender flower (Table 2). In the lavender flower, DPPH radical scavenging activity is higher than in the stalk. It is known that phenolic compounds account for a large portion of the antioxidant potential in many plants (Yogesh et al., 2014; Ghasemi Pirbalouti et al., 2014). In a study, free radical scavenging activities of L. x *intermedia* 'Budrovka' and L. angustifolia extracts were determined between 15.06-45.25 μg/mL and 10.62-33.95 μg/mL, respectively and the free radical scavenging effectiveness of L. x *intermedia* 'Budrovka' and L. angustifolia extracts decreased in order as leaf > flower > inflorescence stalk (Blažeković et al., 2010). In a study conducted with different plant species, DPPH findings expressed as mM Trolox equivalents of *Lavandula angustifolia* have been reported to vary between 0.459 mM TE/g and 0.495 mM TE/g (Duda et al., 2015).

Due to the presence of phenolic compounds that have an effect on antioxidant properties, total phenolic content was obtained in correlation with antioxidant capacity results. As can see Table 2, although no statistically significant difference ($p$ >.05) was observed in the total phenolic content of the lavender stalk during the infusion time, it was observed that the total phenolic content of the lavender flower increased with the increase in the infusion time ($p$ <.05). The lowest total phenolic content was obtained in 12 minutes with 14757.78±554.58 mg GA / L of lavender stalk.

Bajalan et al., (2016) determined the highest total phenolic content of *Lavandula × intermedia* extracts collected from various regions as 105.39 mg GAE / 100g DW. Polyphenolic content of *L. angustifolia* (lavender) harvested in two different phenological periods were determined by Duda et al., (2015) as 12.44-18.16 mg GAE/g DW.

In another study conducted with eight different lavender species, the highest total phenolic content was determined as 97.4 μg GAE / mg in L. intermedia 'Impress purple' (Lee et al., 2011). In the study conducted by Spiridon et al., (2011) in different Romanian plants, the total phenolic content of *L. angustifolia* was determined as 50.6 mg GA/g. In another study on *Lavandula angustifolia* L. flowers, the total phenolic content was determined as 2860-3520 mg GAE / 100g DW (Stanciu et al., 2019). Higher amounts of phenolic are obtained since all metabolic pathways during flowering are adjusted to produce volatile compounds. This may be correlated with the high quantity of phenolic compounds in flowers (Baser et al., 2000; Oniga et al., 2010; Zhu et al., 2012; Hassiotis et al., 2014; Carrasco et al., 2015). With increasing infusion time, water penetration into the plant matrix may be promoted by increased mass transfer and thus increased solubility (Rodino, & Butu, 2019). As the permeability of cell walls increases with heat treatment, high temperatures improve the extraction efficiency of components. The solubility of phenolic compounds increases over time and total phenolic content can be at maximum (İlyasoğlu et al., 2017).

The black tea was brewed for 3, 6 and 10 min and the highest total phenolic content was obtained at the highest infusion time (Kelebek 2016). Herbal extracts are a major source of biologically active compounds with a key role in human health. There are bioactive or phytochemical compounds in herbal teas and beverages that have been reported to have beneficial effects on the prevention of metabolic diseases such as diabetes, glucose intolerance, and obesity (Rodino, & Butu, 2019). Provides the optimal environment for the release of phytochemicals that are water-soluble, such as hot, liquid herbal infusions, phenols and flavonoids (Periche et al.,2014). Herbal drink consumption is gaining popularity due to the fact that many are rich sources of natural bioactive compounds, such as alkaloids, carotenoids, coumarins, flavonoids, polyacetylenes and terpenoids (Chandrasekara, & Shahidi, 2018).

### *3.5. Phenolic Compounds by LC/ESI-MS/MS of Lavender Infusions*

Phenolic acids, which contribute to nutritional and organoleptic properties, are essential phenolic

*Table 2. The effects of infusion time on color, pH, antioxidant capacity, total phenolic contents of lavender flower and stalk*

| Analysis | Lavender flower | | | Lavender stalk | | |
|---|---|---|---|---|---|---|
| | Infusion time | | | Infusion time | | |
| | 3 min | 6 min | 12 min | 3 min | 6 min | 12 min |
| General Analysis | | | | | | |
| L* | 79.00±0$^{c}$ | 77.98±0$^{b}$ | 74.59±0$^{a}$ | 89.38±0$^{f}$ | 89.33±0$^{e}$ | 88.53±0$^{d}$ |
| a* | 5.63±0$^{d}$ | 6.42±0$^{e}$ | 9.16±0$^{f}$ | -1.47±0$^{b}$ | -1.60±0$^{a}$ | -1.35±0$^{c}$ |
| b* | 44.74±0$^{d}$ | 46.76±0$^{e}$ | 51.97±0$^{f}$ | 26.41±0$^{a}$ | 26.96±0$^{b}$ | 29.27±0$^{c}$ |
| c* | 45.10±0$^{d}$ | 47.20±0$^{e}$ | 52.77±0$^{f}$ | 26.45±0$^{a}$ | 27.01±0$^{b}$ | 29.30±0$^{c}$ |
| H | 82.82±0$^{c}$ | 82.18±0.01$^{b}$ | 80±0$^{a}$ | 93.20±0.01$^{e}$ | 93.40±0$^{f}$ | 92.64±0$^{d}$ |
| pH | 5.65±0$^{a}$ | 5.55±0$^{a}$ | 5.54±0$^{a}$ | 5.62±0$^{a}$ | 5.57±0$^{a}$ | 5.47±0$^{a}$ |
| Total acidity (g/100ml) | 0.65±0$^{a}$ | 0.65±0$^{a}$ | 1.08±0$^{a}$ | 0.43±0$^{a}$ | 0.65±0$^{a}$ | 0.43±0$^{a}$ |
| Total phenolic content (mg/L) | 22577$^{b}$ | 24688$^{c}$ | 27278$^{d}$ | 15057$^{a}$ | 15792$^{a}$ | 14757$^{a}$ |
| DPPH (µmol Trolox/L) | 122539$^{c}$ | 135523$^{d}$ | 149365$^{e}$ | 85920$^{a}$ | 86000$^{a}$ | 98063$^{b}$ |
| ABTS (µmol Trolox/L) | 122500$^{c}$ | 131122$^{d}$ | 145900$^{e}$ | 79655$^{a}$ | 87688$^{b}$ | 86866$^{ab}$ |

$^{a-f}$ *Different letters in the rows represent statistically significant differences ($p < 0.05$).*

compounds widespread in foods. The bioavailability of these compounds depends on its free or conjugated existence in food matrices, that is also influenced by food preparation processes and its contributes to taste, color and nutritional properties (Bento-Silva et al., 2020). Phenolic profiles of lavender extracts were identified by LC-DAD-ESI-MS/ MS analysis. Table 3 showed the phenolic compounds of the lavender flower and stalk extracts collected at various infusion times, and Table 4 showed the concentration of these phenolic compounds. LC-ESI-MS/ MS chromatogram of the identified phenolic compounds was displayed in Figure 2.

An ANOVA analysis using the concentration from Table 4 was applied to determine the effect of infusion time on the phenolic compounds. The quantification of individual compounds was calculated with calibration curves of the standard compounds. The curves were obtained using the commercial standards of the concentrations normally present in lavender extracts (approximately 1–400 mg/L). The total amount of the identified phenolic compounds in lavender flower depending on infusion time (3 min, 6 min, 12 min) increased from 236.06 to 522.17 mg/L and also in lavender stalk increased from 149.0 to 186.25 mg/L. Significant statistical differences were determined between phenolic compounds ($p$ <.05). Furthermore, luteolin-*O*-glucuronide and salvianolic acid derivative 2, 3, and 4 were not determined in lavender stalk. The highest ratio of these compounds was observed in infusions obtained at lavender flower for 12 min. As heat is applied, the amount of polyphenols and flavonoids increases due to the deterioration and breakdown of cellular structures at high temperatures and the release of polyphenols and flavonoids from the plant material matrix (Ferracane et al.,2018). In lavender flower and stalk extracts, rosmarinic acid derivatives and rosmarinic acid have been identified as phenolic acids. Depending on the increased infusion time, the concentration of rosmarinic acid derivatives and rosmarinic acid changed significantly ($p$ < .05). The predicted molecular ion at m/z 359 [M-H]- and fragment ions

*Table 3. Retention time, mass spectral characteristics, and identity of phenolic compounds present in lavander flower and stalk infusions*

| Peak No | Rt | λmax | Compounds | $[M-H]^-$ | $MS^2(m/z)$ |
|---|---|---|---|---|---|
| 1 | 16.20 | 280 | Rosmarinic acid derivates | 359 | 197 |
| 2 | 17.27 | 280 | 3,4-dihydroxyphenyllactic acid | 197 | 179-135 |
| 3 | 22.60 | 280 | Undetermined | 501 | 395-343-173-113 |
| 4 | 24.83 | 280,320,360 | Caffeoyl tartaric acid | 311 | 179-149-113 |
| 5 | 28.24 | 280,320 | Coumaric acid-O-glucoside 1 | 325 | 119-163 |
| 6 | 35.23 | 280,320,360 | Ferulic acid hexoside | 355 | 193-149 |
| 7 | 38.34 | | Hydroxycinnamic acid glucoside | 327 | 165-121 |
| 8 | 41.96 | 280,320,360 | Coumaric acid-O-glucoside 2 | 325 | 119-163 |
| 9 | 42.54 | 280,320,360 | Salvianolic acid derivative 1 | 493 | 295-179 |
| 10 | 46.12 | 280,320,360 | Ferulic acid hexoside 2 | 355 | 193-149 |
| 11 | 46.96 | 280,320,360 | Salvianolic acid derivate 2 | 537 | 493-373-173-129 |
| 12 | 48.55 | 280,320,360 | Salvianolic acid derivate 3 | 537 | 493-373-173-129 |
| 13 | 49.51 | 280,320,360 | Luteolin-O-glucuronide | 461 | 285-169 |
| 14 | 50.10 | 280,320 | Yunnaneic acid derivates | 539 | 297 |
| 16 | 52.68 | 280,320,360 | 6-Caffeoylsucrose | 503 | 179-161 |
| 17 | 55.01 | 280,320,360 | Sagerinic acid | 719 | 135 |
| 18 | 56.21 | 280,320,360 | Rosmarinic acid | 359 | 197-179-161 |
| 19 | 58.67 | 280,320,360 | Salvianolic acid derivative 4 | 717 | 537- 519- 493 |
| 20 | 60.35 | 280 | Salvianolic acid derivative 5 | 717 | 539-537-517-197 |

at m/z 197-179-161 were produced by the mass spectrum of rosmarinic acid. Rosmarinic acid had the fifth highest concentration in all samples. Rosmarinic acid is an ester of caffeic acid and 3,4 dihydroxyphenylactic acid is a naturally occurring flavonoid polyphenol. It is by trapping free radicals, functions as an effective antioxidant (Brewer, 2011) and has antioxidant, anti-inflammatory, anti-allergic, anti-depression, antimicrobial, and anti-hyperglycemic impact (Bulgakov et al., 2012; Swamy et al., 2018). Rosmarinic acid, with its antioxidant and anti-inflammatory effects, suppresses skin tumors and may have significant therapeutic effects on various types of cancer such as prostate, skin and breast cancer and inhibits the growth and spread of cancer cells in several ways (Ramanauskiene et al., 2016 ; Xu et al., 2010 ; Lee et al., 2006; Swamy et al., 2018). A phenolic acid of the hydroxycinnamic acid family, coumaric acid-*o*-glucoside (*p*-coumaric acid) plays a central role in secondary metabolism as it can be transformed into phenolic acids (e.g. caffeic acid, ferulic acid, chlorogenic acid, and sinapic acid), flavonoids, precursors of lignin, and other secondary metabolites (El-Seedi et al., 2012).

The flavonoids found in lavender flower and stalk extracts was coumaric acid-*O*-glucoside and ferulic acid hexoside. A mass spectrum of coumaric acid-*O*-glucoside formed the predicted molecular ion at m/z 325 [M-H]- and fragment ions at m/z 119-163 and for ferulic acid hexoside formed the predicted molecular ion at m/z 355 [M-H]- and fragment ions at m/z 193-149. Among the phenolic compounds, coumaric acid-*O*-glucoside was the most dominant and was followed by salvianolic acid derivative 3 and rosmarinic acid. The most important increase was detected in this dominant compound. The concentration of the compound increased from 64.73 to 120.55 mg/L in lavender flower depending on infusion time and increased from 46.97 to 58.72mg/L in lavender stalk.

Previous studies have shown that coumaric acid and its derivatives exhibit a variety of bioactivity, including anti-oxidant, anti-diabetes, anti-inflammatory, anti-mutagenic, anti-ulcer and anti-cancer activities (Pragasam et al., 2013; Espinosa et al., 2015; Ou et al., 2009). Coumaric acid has a higher bioavailability than chlorogenic acid, rosmarinic acid, caffeic acid and ferulic acid. The phenyl hydroxyl group of coumaric acid is

responsible for its antioxidant activity (Pei et al., 2016). Ferulic acid is a secondary metabolite typically found in plant tissues and has different chemical structures and biological properties (Mattila et al., 2002; Bezerra et al., 2017). It exhibits a wide variety of biological properties such as antioxidant, anti-allergic, anti-carcinogenic, anti-inflammatory, anti-microbial, anti-viral (Tee-ngam et al., 2013; Bezerra et al., 2017; Cota Arriola et al., 2017; Moldovan et al., 2017). Salvianolic acids and derivatives have been reported to have potent antioxidant properties due to their polyphenolic structure. Because of their polyphenolic composition, salvianolic acids are considered to be free radical scavengers. The predicted molecular ion at m/z 493 [M-H]- and fragment ions at m/z 295-179 were produced by a mass spectrum of Salvianolic acid derivative 1.

The concentration of the compound increased from 1.41 to 12.30 mg/ L in lavender flower depending on infusion time and increased from 5.40 to 6.76 mg/ L in lavender stalk. A mass spectrum of salvianolic acid derivative 2 and 3 formed the predicted molecular ion at m/z 537 [M-H]- and fragment ions at m/z 493-373-173-129. The concentration of the compound 2 and 3 increased from 16.81 to 53.84 and 29.33 to 70.26 mg/ L in lavender flower depending on infusion time, respectively and not determined in lavender stalk. In addition, the predicted molecular ion at m/z 717 [M-H]- and fragment ions at m/z 539-537-517-197 were produced by a mass spectrum of salvianolic acid derivative 4 and 5. The concentration of the compound 4 increased from 4.31 to 8.36 mg/ L in lavender flower depending on infusion time and not determined in lavender stalk and, also salvianolic acid derivative 5 increased from 5.64 to 10.19 mg/ L in lavender stalk. It has not only been emphasized to have antioxidant function in vitro, but has also been reported to serve as in vivo cardiovascular protectors (Ho& Hong, 2011). The protective effects of salvianolic acid derivatives against oxidative injuries have been determined. It has been reported to exhibit greater scavenging activities than vitamin C against free hydroxyl radicals, superoxide anion radicals, DPPH radicals and 2,2'-azino-bis radicals (Zhao et al., 2008).

Luteolin and luteolin-O-glucoside derivatives have anti-oxidative and anti-inflammatory activities. The predicted molecular ion at m/z 461[M-H]- and fragment ions at m/z 285 and 169 were produced by a mass spectrum of luteolin-O-glucoside. The concentration of the compound increased from 1.41 to 8.78 mg/ L in lavender flower depending on infusion time and not determined in lavender stalk. Type 2 diabetes is one of the major non-communicable and fastest growing public health problems in the world, it is a chronic disease (Asif, 2014, Seelinger et al., 2008). Luteolin and luteolin-O-glucoside derivatives have been reported to be effective in improving diabetes and to have anti-diabetic effects in previous studies (Asif, 2014; Seeinger , et al., 2008; Zang, et al., 2016).

Yunnaneic acid is one of rosmarinic acid derivatives. The predicted molecular ion at m/z 539 [M-H]- and fragment ions at m/z 297 were produced by a mass spectrum of Yunnaneic acid derivates. The concentration of the compound increased from 3.14 to 20.10 mg/L in lavender flower depending on infusion time and increased from 17.95 to 22.44 mg/L in lavender stalk. Rosmarinic acid and its chemically derived compounds, including lithospermic acid, salvianolic acid, melitric acid and yunnaneic acid, have pharmacological importance due to antitumor, anti-inflammatory, antioxidant and antimicrobial effects. In order to improve people's health and treat Alzheimer's disease, cardiovascular disease, atopic dermatitis and allergies, they are therefore included in the diet (Sanbongi et al., 2004; Karthik et al., 2011; Jang et al., 2011; Vladimir-Knezevic et al., 2014 ; Kim et al., 2015; Ramanauskiene et al., 2016; Cao et al., 2016).

The predicted molecular ion at m/z 503 [M-H]- and fragment ions at m/z 179-161 and m/z 719 [M-H]- and fragment ions at m/z 135 were produced by a mass spectrum of 6-Caffeoyl sucrose and sagerinic acid, respectively.

*Table 4. Concentration of phenolic compounds (mg/L ± standard deviation) in lavender flower and stalk infusions*

| Compounds | Lavender flower | | | Lavender stalk | | |
|---|---|---|---|---|---|---|
| | Infusion time | | | Infusion time | | |
| | 3 min | 6 min | 12 min | 3 min | 6 min | 12 min |
| Rosmarinic acid derivate | 0.41±0.01$^{c}$ | 0.59±0.01$^{d}$ | 0.77±0.02$^{e}$ | 0.27±0.01$^{a}$ | 0.30±0.01$^{a}$ | 0.34±0.01$^{b}$ |
| 3,4-dihydroxyphenyllactic acid | 2.12±0.05$^{c}$ | 2.63±0.07$^{d}$ | 3.74±0.09$^{e}$ | 1.60±0.02$^{a}$ | 1.78±0.02$^{b}$ | 2.00±0.02$^{c}$ |
| Undetermined | 0.90±0.02$^{a}$ | 4.17±0.11$^{c}$ | 1.73±0.04$^{b}$ | n.d. | n.d. | n.d. |
| Caffeoyl tartaric acid | 4.60±0.22$^{a}$ | 5.70±0.17$^{c}$ | 6.64±0.09$^{d}$ | 4.50±0.11$^{a}$ | 5.01±0.13$^{b}$ | 5.63±0.14$^{c}$ |
| Coumaric acid-O-glucoside 1 | 64.73±1.64$^{d}$ | 90.83±0.87$^{e}$ | 120.55±1.62$^{f}$ | 46.97±1.19$^{a}$ | 52.28±1.33$^{b}$ | 58.72±1.49$^{c}$ |
| Ferulic acid hexoside | 23.46±0.60$^{a}$ | 30.53±0.77$^{c}$ | 40.48±1.03$^{d}$ | 25.05±0.64$^{a}$ | 27.89±0.71$^{b}$ | 31.32±0.79$^{c}$ |
| Hydroxycinnamic acid glucoside | 0.45±0.02$^{a}$ | 0.64±0.03$^{ab}$ | 0.85±0.03$^{b}$ | 9.76±0.14$^{c}$ | 10.87±0.16$^{d}$ | 12.20±0.18$^{e}$ |
| Coumaric acid-O-glucoside 2 | 29.75±0.76$^{c}$ | 36.61±0.93$^{d}$ | 49.59±1.26$^{e}$ | 13.02±0.33$^{a}$ | 14.49±0.37$^{ab}$ | 16.27±0.41$^{b}$ |
| Salvianolic acid derivative 1 | 1.41±0.04$^{a}$ | 5.46±0.14b | 12.30±0.31$^{e}$ | 5.40±0.14$^{b}$ | 6.02±0.15$^{c}$ | 6.76±0.17$^{d}$ |
| Ferulic acid hexoside 2 | 18.30±1.17$^{c}$ | 20.40±1.64$^{c}$ | 26.78±0.56$^{d}$ | 11.19±0.28$^{a}$ | 12.46±0.32$^{ab}$ | 14.09±0.21$^{b}$ |
| Salvianolic acid derivative 2 | 16.81±0.43$^{a}$ | 41.58±1.06$^{b}$ | 53.84±1.37$^{c}$ | n.d. | n.d. | n.d. |
| Salvianolic acid derivative 3 | 29.33±0.74$^{a}$ | 50.85±1.29$^{b}$ | 70.26±0.59$^{c}$ | n.d. | n.d. | n.d. |
| Luteolin-O-glucuronide | 1.41±0.04$^{a}$ | 1.62±0.04$^{a}$ | 8.78±0.22$^{b}$ | n.d. | n.d. | n.d. |
| Yunnaneic acid derivates | 3.14±0.08$^{a}$ | 4.98±0.13$^{b}$ | 20.10±0.51$^{d}$ | 17.95±0.46$^{c}$ | 19.98±0.51$^{d}$ | 22.44±0.57$^{e}$ |
| 6-Caffeoyl sucrose | 9.39±0.24$^{c}$ | 20.69±0.53$^{d}$ | 39.10±0.99$^{e}$ | 4.61±0.30$^{a}$ | 5.14±0.33$^{a}$ | 5.77±0.37$^{a}$ |
| Sagerinic acid | 9.03±0.23$^{b}$ | 8.80±0.22$^{b}$ | 21.08±0.54$^{c}$ | 2.61±0.07$^{a}$ | 2.90±0.07$^{a}$ | 3.26±0.08$^{a}$ |
| Rosmarinic acid | 10.87±0.28$^{c}$ | 12.81±0.33$^{d}$ | 27.04±0.69$^{e}$ | 3.59±0.09$^{a}$ | 3.99± 0.10$^{ab}$ | 4.49±0.11$^{b}$ |
| Salvianolic acid derivative 4 | 4.31±0.11$^{a}$ | 7.51±0.19$^{b}$ | 8.36±0.21$^{c}$ | n.d. | n.d. | n.d. |
| Salvianolic acid derivative 5 | 5.64±0.14$^{c}$ | 7.02±0.18$^{d}$ | 10.19±0.26$^{e}$ | 2.47±0.08$^{a}$ | 2.60±0.11$^{a}$ | 2.98±0.05$^{b}$ |
| **Total** | **236.06±6.82** | **353.41±8.70** | **522.17±10.26** | **149.00±3.81** | **165.70±4.03** | **186.25±4.47** |

$^{a-f}$*Different letters in the rows represent statistically significant differences (p < .05). n.d Not determined*

The concentration of the 6-Caffeoyl sucrose increased from 9.39 to 39.10 mg/ L in the lavender flower depending on infusion time and increased from 4.61 to 5.77 mg/ L in lavender stalk. The concentration of the sagerinic acid increased from 9.03 to 21.08 mg/ L in lavender flower depending on infusion time and increased from 2.61 to 3.26 mg/ L in the lavender stalk. There was no statistically significant difference ($p$ >.05) in 6-caffeoyl sucrose and sagerinic acid determined in lavender stem with the increase in infusion time.

Phenolics can bind or be free to cell membranes / walls in plants, and food processing procedures such as using temperature can induce the release of these compounds. Polyphenols bound to the wall may thus be released in this way (Minatel et al., 2017).

Heating can disturb the cell membrane, leading to the release of membrane-bound phytochemicals, which may lead to an increase in bioavailability (Minatel et al., 2017).

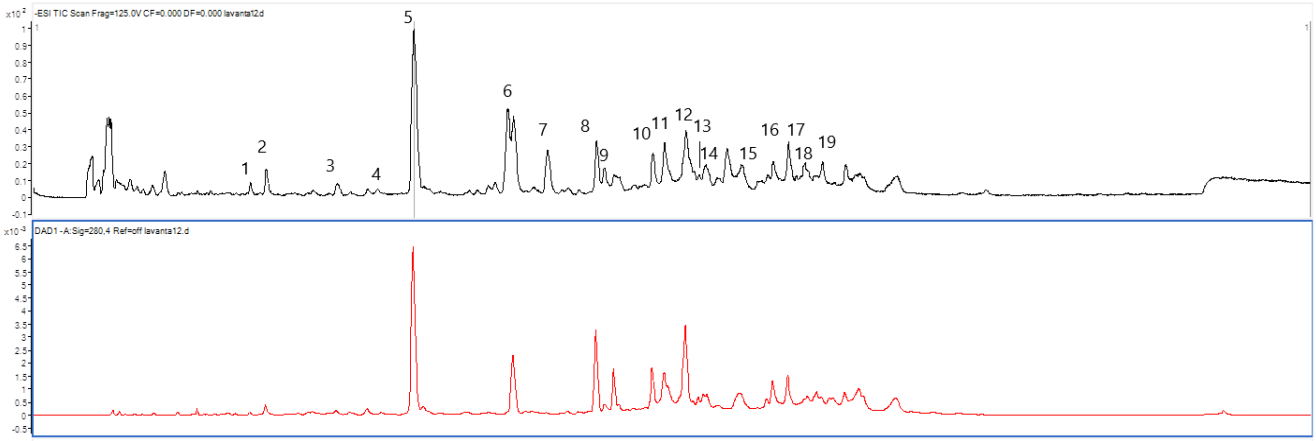


*Figure 2. LC-ESI-MS/MS chromatograms of phenolic compounds. Peaks correspond to compounds in Table 3.*
***Peak 1:*** *Rosmarinic acid derivates* ***2:*** *3,4-dihydroxyphenyllactic acid* ***3:*** *unidentified, peak* ***4:*** *caffeoyl tartaric acid* ***5:*** *coumaric acid-o-glucoside 1* ***6:*** *ferulic acid hexoside* ***7:*** *hydroxycinnamic acid glucoside* ***8:*** *coumaric acid-o-glucoside 2,* ***9:*** *salvianolic acid derivative 1* ***10:*** *ferulic acid hexoside 2* ***11:*** *salvianolic acid derivate 2* ***12:*** *Salvianolic acid derivate 3,* ***13:*** *luteolin-O-glucuronide* ***14:*** *yunnaneic acid derivates* ***15:*** *6-Caffeoylsucrose* ***16:*** *sagerinic acid* ***17:*** *rosmarinic acid* ***18:*** *salvianolic acid derivative 4* ***19:*** *salvianolic acid derivative 5*

Herbal infusions have been consumed as drinks for thousands of years (Fu et al., 2011). The use of it has increased worldwide due to the beneficial and protective impact on the human body (Kunle et al., 2012). Infusions of herbs include different forms of natural antioxidants and can be an excellent source of antioxidants (Kamara et al., 2003 ; Debnath et al., 2011).

## 4. CONCLUSIONS

In this analysis, dried lavender flower and stem were brewed at approximately 100°C for 3, 6 and 12 minutes, color, pH, total acidity, total phenolic content and antioxidant properties were evaluated. Increases in total phenolic content and antioxidant activity were observed in lavender flower with and increased infusion period. DPPH radical scavenging activity was found to be higher in lavender flowers compared to stems. Phenolic profiles of lavender infusions were determined by LC-DAD-ESI-MS / MS analysis. A total of 20 compounds were identified and in lavender infusions. Coumaric acid-*O*-glucoside 1, ferulic acid hexoside, coumaric acid-O-glucoside 2 were the most abundant phenolics in all lavender infusions. The lavender fatty acid composition was determined and a total of 7 fatty acids were detected in the sample. The main fatty acids were oleic acid (C18:1- cis $\omega$9), linoleic acid (C18:2 $\omega$6), while oleic acid had the highest concentration (61.61%) in the sample. In three different infusions (3 min, 6 min and 12 min) of lavender extracts, a massive increase was obtained in the concentrations of phenolic compounds and antioxidant activities with the extended infusion time. It was concluded that lavender extract be counted as a remarkable source of phenolic compounds and that the transition of these compounds towards the herbal tea is higher with the extended infusion time. Infusions of tea and herbs provide our diet with an essential source of phenolic compounds, and infusions of herbs can be an excellent source of antioxidants (Fu et al., 2011). Therefore, due to its high antioxidant content, lavender has an alternative herbal tea potential, in line with the results we have obtained.

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