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Extraction of Essential Oil from *Lavandula angustifolia* Flowers Preceded by Enzymatic Pre-Treatment and Investigate its Activity Against Free Radicals

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Abstract **–The aim of this study was to evaluate the effect of enzymatic pre-treatment using cellulase on the extraction efficiency of essential oil (EO) from *Lavandula angustifolia* flower (La.f). Clevenger apparatus was used to isolate the *L. angustifolia* essential oil (La.f-EO). The morphological structures of the cell wall of plant were observed using scanning electron microscopy (SEM). The volatile compound were analyses using GC-FID/GC-MS and the linalool was the main compound in this profile. The free radicle scavenging activity of La.f-EO was investigated using DPPH˙ scavenging and ABTS•$^{+}$assays. The results of this work showed that the enzymatic pre-treatment using cellulase demonstrated a high release calculated as yield% (0.82%) compare to untreated sample (0.63%). Similarity, the treated sample obtained the highest ratio of linalool (27.91%) than control sample (25.31%).The micrographs showed significant damage on the external surface of the enzymatic pre-treatment compared to soft body and untreated sample. In the other hand, the enzymatic pre-treatment achieved the highest radicle scavenging whether in DPPH˙(63.31%) or ABTS•$^{+}$(0.017 mM Trolox) assays. The enzymatic pre-treatment seems to cause the rupture of the cells and the glands of La.f more rapidly than in conventional hydrodistillation. This led to enhance the yield% and efficiency of bioactive components from La.f-EO.**

Keywords **– *Lavandula Angustifolia*, Essential Oil, Enzymatic Pre-Treatment, Free Radicle Scavenging, Lipid Peroxidation.**

I. INTRODUCTION

Essential oils are one of the most important resource that been gained a renewed interest in several applications. It can be used in the food [1] and pharmaceutical industries [2], therapeutic activities [3], parasites [4], fragrance and cosmetic applications [5-8], free radicle scavenging [9-12], antimicrobial [9,10,12].

The genus *Lavandula* (lavender) of Lamiaceae family consists of about 30 species [3].*L. angustifolia* Mill. (La) is an herbaceous plant belonging to the Lamiaceae. It is one of the most widely essential oil crops in the world. There is a renewed interest in cultivating La.f for non-food applications such as cosmetics and personal care products, and in food applications as food additives. The literature indicates that the primary components of *L.angustifolia* are linalool, linalyl acetate and β-linalyl [3, 7, 13, 14,].

Enzyme-assisted isolation can be used to enhance the yield of bioactive components from biological materials [9, 15-19]. Hydrolytic enzymes, including cellulase, pectinase and beta glucosidase are commonly used for the extraction of secondary metabolites. It has high advantages of high efficiency, compatibility with environmental, and easy operation processing [19].

There are few studies available which addressed the enhancing of essential oil release from *L. angustifolia*. Therefore, this study has been focused on a method which can promote the yield of essential oil from this plant by using the enzymatic pre-treatment before starting the isolation of essential oil procedures.

II. MATERIAL AND METHODS

A. Plant materials and chemicals

Aerial parts of La.f were purchased from the SILUYUAN Co., Ltd (Xinjiang, China) supported with identification certificate. Cellulase from Aspergillus niger and 6-Hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox) were purchased from Sigma-Aldrich (USA). 2, 2-azino-bis-(3-ethylbenzothioline-6-sulphonicacid)-di-ammonium salt (ABTS), 1, 1-diphenyl-2-picrylhydrazyl(DPPH), 2-(1, 1-Dimethylethyl)-1, 4-benzenediol(TBHQ), 2, 6-Bis(1, 1-dimethylethyl)-4-methylphenol(BHT) were purchased from TCI EUROPE N.V. Belgium. The other reagents used were of analytical grade.

B. Extraction of La.f-EO

For enzymatic pre-treatment, 10mg of cellulase was dispersed in 500ml deionized distilled water to obtain a homogenized solution. 100g of La.f was added to the enzyme solution. An individual treatment without cellulase was prepared as a control. Both of treated and untreated sample were incubated in a shaking water bath at 40°C for 120 min. After incubation, the samples were subjected to Clevenger-type apparatus for hydrodistillation process according to the method recommended in the [20]. La.f-EO was separated and dried using anhydrous sodium sulphate (Na_2SO_4). The isolated La.f-EOs kept into sealed brown glass bottles at 4ºC. Eq.(1) andEq. (2) were used to calculate the %yield andtheincreaserate of La.f-EO, respectively.

$$\text{La. EO } Yield\% = [\text{EO obtained (g)} / \text{Initial wt. of Lp (g)}] \times 100 \quad (1)$$

$$IR\% = [(A_1/A_0) \times 100] - 100 \quad (2)$$

where IR% is the increase rate, A_1 is the EO obtained from enzymatic pre-treatment sample and A_0 is the EO obtained from untreated sample (Ctrl).

C. Gas chromatography-mass spectrometry (GC-MS)

La.f-EOs were analyzed to investigate the volatile compounds. The analysis was carried out using Varian 1200 with a CP-3800 GC (Varian) fitted with a splitless injector. A Bruker SCION 450-GC equipped.A capillary column DB-Wax (30m × 0.25mm (i.d), 0.25µm film thickness, J&W Scientific, Folsom, CA, USA) was used to separate the volatile compounds. Temperature was programmed from 40ºC for 3 min, then increased to 120 ºC at 8ºC/min and to 230ºC at 10ºC/min. Helium was used as a carrier gas at flow rate of 0.8mL/min. For Mass-spectrometric (MS), an electron Impact ionization (EI) mode with ionization energy voltage of 70 eV and emission current of 35mA were used. Interface temperature of 250ºC and ion source temperature of 200 ºC were programmed to perform a 1.0µL splitless injection. Injector(250ºC) and MS(200ºC) transfer line temperatures were adjusted to perform a 1.0µL splitless injection. The identification of the essential oil constituents was based on their MS data compared to the NIST 08 2005 (National Institute of Standards and Technology, Gaithersburg, MD, USA), Wiley 7(Wiley, New York, NY, USA) library and published mass spectra.

D. Scanning Electron Microscopy (SEM)

The samples with and without cellulase were scanned using A Hitachi High-Technologies Corp., SEM SU 1510 (Tokyo, Japan). The samples were fixed on adhesive to the sample holder with aluminum tape and sputtered with a thin layer of gold-palladium using an ion sputter coater. The samples were examined under high vacuum conditions, an accelerating beam voltage at 5.00kV, magnification coefficient at x10 and a working distance 8.2 mm.

E. DPPH• Scavenging Activity Assay

The free radical-scavenging activities of La.f-EOs were assessed using the stable radical DPPH according to [9]. 20µL of each La.f-EO was mixed with 3.5mL of DPPH• dissolved in pure methanol at concentration of 6×10^5Mol/L. TBHQ and BHT were used as positive references at 120mg/L. All treatments were carried out in triplicate. The reaction solutions were incubated in the dark at room temperature for 30 min. Then, the absorbance of the reaction solutions were measured at 517nm. The DPPH•scavenging activity (DPPH•-SA%) was calculated as follows:

$$DPPH^{\cdot} - SA\% = [(A_0 - A_1)/A_0] \times 100 \quad (3)$$

where A_0 and A_1 pointing to the absorbance at 517nm of the control and targeted treatment. A_0 value was measured at the initial and final point of interaction where the absorbance did not record observable decreases.

F. ABTS•$^+$ Scavenging Activity Assay

The ABTS•$^+$ scavenging activity assay was carried out based on the method of [21] with slight modifications. Briefly, ABTS radical cations (ABTS•$^+$) were produced by reacting 7 mmol/L of ABTS solution with 2.45 mmol/L of potassium persulfate in the dark at ambient temperature for 14-16 h before use. The ABTS•$^+$ solution were diluted with pure ethanol to an absorbance of 0.70±0.02 at 734 nm. The target sample (50 µL) was mixed with 3450 µL of fresh ABTS•$^+$ solution. The reaction mixture was allowed to stand at ambient temperature for 10 min and the absorbance was immediately recorded at 734 nm. All treatments were carried out in triplicate. The calibration curve of Trolox was used to explain the free radical scavenging activity of La.f-EO in the term of mM Trolox.

I. Statistical Analysis

Data were expressed as a mean ± standard deviation. Significant differences between means were analyzed statistically using One-way analysis of variance (ANOVA) according to Duncan new multiple-range test through SPSS 17.0 (SPSS Inc., Chicago, USA). Differences were considered significant when $p < 0.05$.

III. Results and Discussions

A. Extraction of La.f-EO

The effect of enzymatic pre-treatment on the glandular structure of the plant cells was assessed to obtain the maximum yield % essential oil from La.f. The results has shown in (Table 1). The yield of CEase treatment was 0.82% with increase rate (IR%) reached to 30.10% compared to the yield% of the control sample (0.63%). These results illustrated that the cellulase could promote the release of EO extracted from La.f. From this sense, the destruction of cell walls with the help of hydrolytic enzymes was useful for releasing of La.f-EO [9, 10, 15, 17]. Jiao et al. [16] found that the cellulase had the highest extraction efficiency of oil release from pumpkin seeds than control sample using microwave-assisted aqueous enzymatic extraction of oil from pumpkin seeds and same finding was reported by [18] using dispersive Liquid-Liquid Microextraction (DLLME).

Table 1. Yield%, DPPH•-SA (%) and ABTS•$^+$ (mM as Trolox) of La.f-EO with increase rate (IR%) compare to control

Treatment system	Yield (%)		DPPH•-SA (%)		ABTS•$^+$ (mM as Trolox)	
	Result	IR[a]	Result	IR	Result	IR
Control	0.63 ± 0.04	0	44.44 ± 0.54	0	0.014 ± 0.001	00.0
CEase	0.82 ± 0.05	30.1	63.31 ± 0.53	42.5	0.017 ± 0.001	21.4
TBHQ	nt[b]	nt	77.33 ± 1.05	74.0	0.026 ± 0.001	85.7
BHT	nt	nt	64.21 ± 0.46	44.5	0.017 ± 0.002	21.4

[a]IR = The increase rate (%).[b] nt = not test

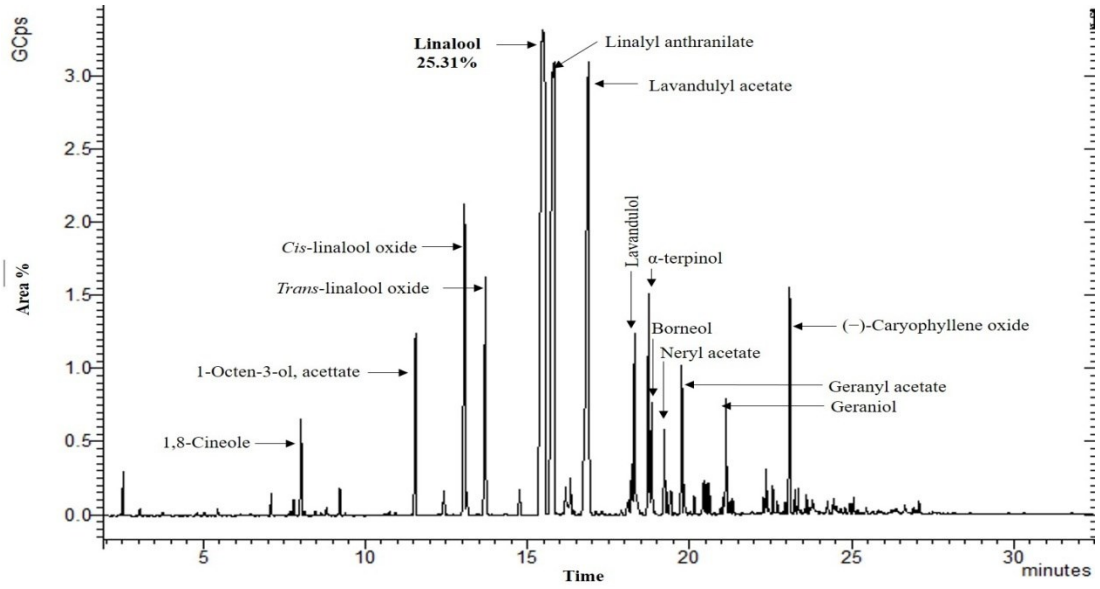

Fig. 1. The GC chromatogram of L. angustifolia essential oil (Control) includes the main components.

B. Chemical Composition of La.f-EO

The chemical composition of La.f-EO demonstrated more than 40 components in both of untreated and treated samples. Figures 1 & 2 showed the main components. The main components were as follows: linalool (27-90 - 25.31%), linalyl anthranilate (18.35–17.86%), lavandulyl acetate (13.62 - 11.14%), (−)-Caryophyllene oxide (3.21 – 2.40%), Geranyl acetate (1.97–1.67%), geraniol (1.45 – 1.32%) and nerryl acetate (1.17–0.98%). Linalool was the main component with 25.31% and 27.90% for control and CEase treatments, respectively. This results were in agreements with the findings of Rashed et al. [10] Chrysargyris et al. [11]; Lafhal et al. [22] which focused on *Lavandula angustifolia* essential oil isolated using ultrasonic-microwave assisted and hydrodistillation Clevenger-apparatus, respectively. The results of this work also showed that the enzymatic pre-treatment by cellulase would promote the yield% of essential oil and enhances the release of linalool (%). Same findings were reported by Boulila et al. [15] who studied the effect of cellulase, hemicellulase, xylanase and combined enzymes on the efficiency extraction of bioactive compounds from bay leaves (*Laurusnobilis* L.). Hosni et al. [17] found that the enzymatic-assisted extraction of thyme (*Thymus capitatus* L.) and rosemary (*Rosmarinus officinalis* L.) using cellulase and hemicellulase and combined of both enzymes have been achieved significant effect on the efficiency of yield% and chemical composition of the samples.

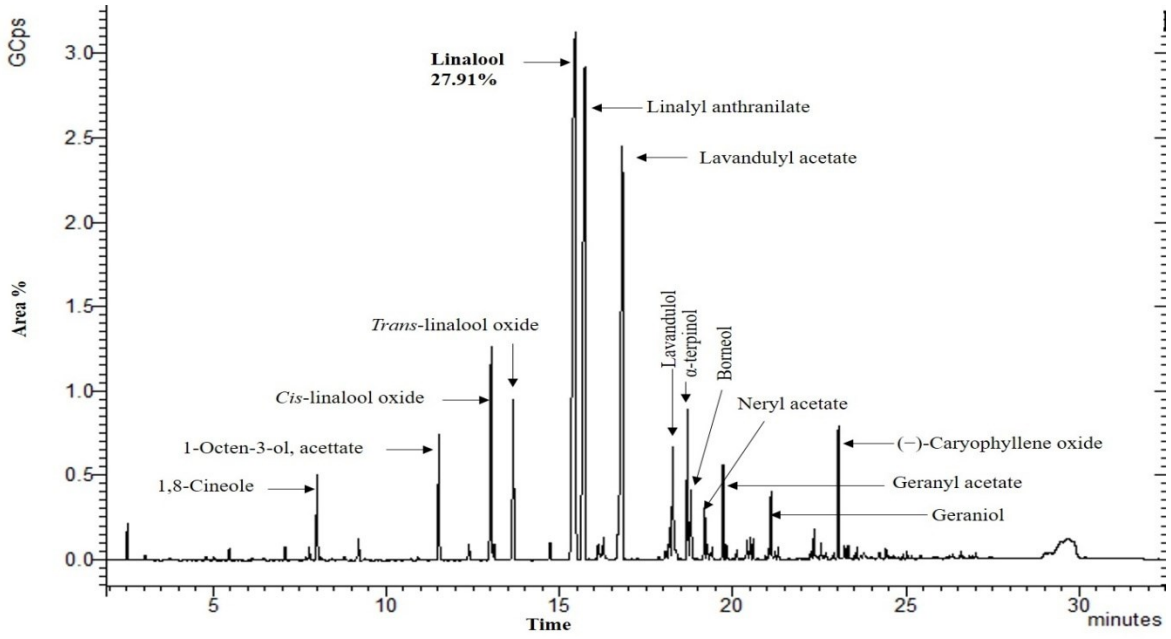

Fig. 2. The GC chromatogram of *L. angustifolia* essential oil (Enzymatic pre-treatment by cellulase) includes the main components.

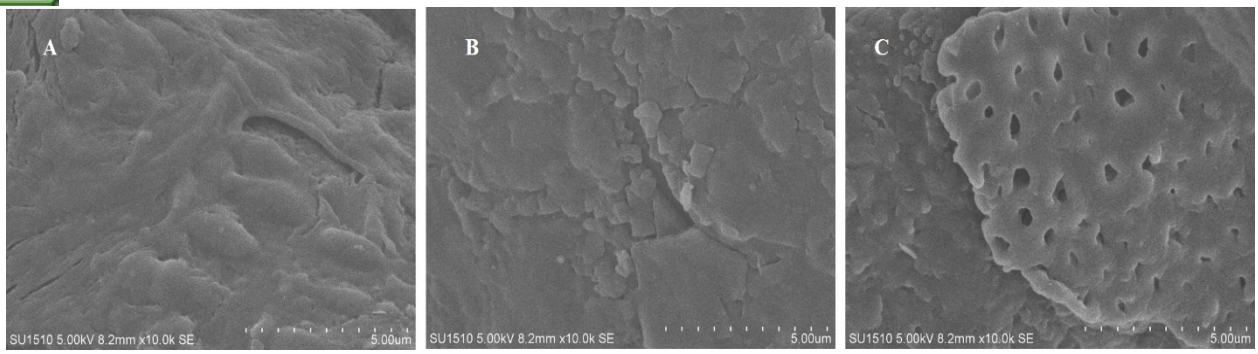

Fig. 3. SEM of *L. angustifolia* surface structures changes: soft body (A), (B) untreated sample (control) and (C) enzymatic pre-treatment by cellulase.

C. Morphological Observations

The structural changes of La.f for soft body, control sample and enzymatic pre-treatment sample were examined using SEM and the micrographs have been shown in Fig. 3. The soft body structure before extraction process presented in Fig. 3A. The surface of the soft body showed slight ruptures with a partial destruction after extraction of La.f-EO using Clevenger apparatus without any enzymatic pre-treatment (Fig. 3B). In the other hand, significantly morphological structural changes in enzymatic pre-treatment micrograph can be seen, where the most of cell walls have been ruptured. These results have been demonstrated that the destruction of cell walls when using hydrolytic enzymes such as cellulase can enhance the release of La.f-EO from the glands in the plant cells. This is due to the ability of cellulase to hydrolyze the cellulose, which constitutes the highest composition in the plant cell wall [9, 15-17]. According to this phenomenon, Nagendra chari et al. [19] found that the enzymatic pre-treatment of ginger using a-amylase, viscozyme, cellulase, protease and pectinase enzymes had a significant influence on the yield of oleoresin, 6-gingerol and the total polyphenols. This enhances the ability of the solvents to penetrate the cell walls and easy access to the glands where essential oil is stored according to Rashed et al. [10] who reported that the enzymatic pre-treatment with ultrasonic-microwave assisted was a useful method where led to facilitate access of the solvent through the cell wall and reach the mitochondria.

D. DPPH˙ Scavenging Activity (DPPH˙-SA%)

Table 1 showed the values of DPPH˙-SA%for control, CEase, TBHQ and BHT. The CEase treatment achieved higher DPPH˙-SA% (63.31%) than control sample (44.44%) with increase rate IR% of 30.1%. TBHQ obtained the highest DPPH˙-SA% (77.33%) among all treatments with no significance in DPPH˙-SA% between BHT (64.21%) and CEase.

E. ABTS•$^{+}$ Scavenging Activity

By observing the results presented in Table 1, the ABTS•$^{+}$ scavenging activity ABTS•$^{+}$ -SA of La.f-EOs were 0.014, 0.017 (mM Trolox) for control and CEase treatments, respectively, with IR% reached to 21.4% compared to control sample. In the other hand, the ABTS•$^{+}$-SA of TBHQ and BHT samples were 0.026 and 0.017 (mM Trolox), respectively, with IR% reached to 85.7% for TBHQ and 21.7% for BHT. No significant difference in ABTS•$^{+}$-SA between CEase and BHT samples.The results of free radical-scavenging using ABTS•+ assay were in agreement with those which achieved using the DPPH• − SA assay.

The effect of La.Eo as DPPH˙-scavenging and ABTS•+ scavenging can be attributed to the linalool and other bioactive compounds which found at a high level in both of control and CEase treatments. The results of DPPH•-SA and ABTS•+ (mM Trolox) were in a compatibility with the chemical components results (Table 3) and the observations of SEM (Fig 3). The DPPH• values (µmol Trolox/g) of *L. angustifolia*, *J.sambac* and *R. officinalis* EOs were 184.73, 64.54 and 174.50, respectively, while the ABTS•$^{+}$ value (µmol Trolox/g) were 261.18, 97.43 and 273.75, respectively according to Lafhal et al. [21] who analyzed the essential oil of *L. angustifolia* and lavandin. While the DPPH values (as IC_{50}) of EOs which obtained from different thymus species were ranged between 273.36–693.75µg/mL [23].

IV. Conclusion

This work describes the effect of pre-enzymatic treatment using cellulase on the yield% of *L. angustifolia* essential oilfor the free radicles scavenging capability. The findings of this work demonstrated that cellulase has ability to decompose the plant cell wall and this would be enhanced the efficiency of essential oil isolation. Both of *angustifolia* essential oil from control sample or pre-enzymatic treatment sample showed high abilities as free radicles scavenging activity. The use of pre-enzymatic treatment would help to improve the isolation efficiency of essential oil and save time and cost and effort of isolation. The finding of the current work shows to the ability of use the La.EO as food additives and as a natural alternative of synthetic compounds as antioxidants. The natural bioactive can be safer and easier for the food, nutrition supplements and antibiotics industries.

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REFERENCES

[1] Danh L.T., Han L. Triet N.D. A., Zhao J., Mammucari R., Foster N. (2013). Comparison of Chemical Composition, Antioxidant and Antimicrobial Activity of Lavender (*Lavandula angustifolia* L.) Essential Oils Extracted by Supercritical CO_2, Hexane and Hydrodistillation. Food Bioprocess Technol, 6, 3481–3489.

[2] El Asbahani A., Miladi K., Badri W., Sala M., Addi E.H.A., Casabianca H., El Mousadik, A., Hartmann D., Jilale A., Renaud F.N.R., Elaissari A. (2015). Essential oils: From extraction to encapsulation. International Journal of Pharmaceutics 483, 220–243.

[3] Aburjai T., Hudiab M. (2005). Chemical Composition of the Essential Oil from Different Aerial Parts of Lavender (*Lavandula coronopofolia* Poiert) (Lamiaceae) Grown in Jordan. J. Essent. Oil Res., 17, 49-51.

[4] Yazdani E., Sendi J.J., Aliakbar A., Senthil-Nathan S. (2013). Effect of *Lavandula angustifolia* essential oil against lesser mulberry pyralid *Glyphodespyloalis* Walker (Lep: Pyralidae) and identification of its major derivatives. Pesticide Biochemistry and Physiology, 107, 250–257.

[5] Bikmoradi A.,Seifi Z., Poorolajal J., Araghchian M., Safiaryan R., Oshvand K. (2015). Effect of inhalation aromatherapy with lavender essential oil on stress and vital signs in patients undergoing coronary artery bypass surgery: A single-blinded randomized clinical trial. Complementary Therapies in Medicine, 23 (3), 331–338.

[6] Ali B., Al-Wabel N. A., Shams S., Ahamad A., Khan S. A., Anwar F. 2015. Essential oils used in aromatherapy: A systemic review. Asian Pac J Trop Biomed, 5(8), 601–611.

[7] Prusinowska R., Smigielski K.B. (2015). Hydrosols from Lavender (*Lavandula angustifolia*)-Determination of the Chemical Composition Using Dispersive Liquid-Liquid Microextraction (DLLME). TEOP, 18 (3), 519 – 528.

[8] Prusinowska R., Śmigielski K.B. (2014). Composition, biological properties and therapeutic effects of lavender (*Lavandula angustifolia* L.). A review. Herba Polonica, 60 (2), 56 – 66.

[9] Rashed M.A.R., Tong Q., Nagi A., Li J., Khan N., Chen L., Rotail A., Bakry A.M. (2017). Isolation of essential oil from *Lavandula angustifolia*by using ultrasonic microwave assisted method preceded by enzymolysis treatment, and assessment of its biological activities. Industrial Crops and Products. 100, 236–245.

[10] Rashed M.M.A. Rashed, Tong Q.,Abdelhai M.H., Gasmalla M.A.A., Ndayishimiye J.B., Chen L., Ren F. (2016). Effect of ultrasonic treatment on total phenolic extraction from *Lavandula pubescens* and its application in palm olein oil industry. Ultrasonics Sonochemistry 29, 39–47.

[11] Chrysargyris A., Panayiotou C., Tzortzakis N. (2016). Nitrogen and phosphorus levels affected plant growth, essential oil composition and antioxidant status of lavender plant (*Lavandula angustifolia* Mill.). Industrial Crops and Products, 83, 577–586.

[12] Martucci J.F., Gende L.B., Neira L.M., Ruseckaite R.A., (2015). Oregano and lavender essential oils as antioxidant and antimicrobial additives of biogenic gelatin films. Industrial Crops and Products, 71, 205–213.

[13] Dusková E., Dusek K., Indrák P., Smékalová K. (2016). Postharvest changes in essential oil content and quality of lavender flowers. Industrial Crops and Products, 79, 225–231.

[14] Adaszynska M., Swarcewicz M., Dzieeciol M., Dobrowolska A. (2013). Comparison of chemical composition and antibacterial activity of lavender varieties from Poland. Natural Product Research, 27 (16), 1497–1501.

[15] Boulila A., Hassen I., Haouari L., Mejri F., Ben Amor I.,Casabianca H., Hosni K. (2015). Enzyme-assisted extraction of bioactive compounds from bay leaves (*Laurusnobilis*L.). Industrial Crops and Products, 74, 485–493.

[16] Jiao J., Li Z., Gai Q., Li X., Wei F., Fu Y., Ma W. (2014). Microwave-assisted aqueous enzymatic extraction of oli from pumpkin seeds and evaluation of its physicochemical properties, fatty acid compositions and antioxidant activities. Food Chemistry 147, 17–24.

[17] Hosni K., Hassen I., Chaâbane H., Jemli M., Dallali S., Sebei H., Casabianca H. (2013). Enzyme-assisted extraction of essential oils from thyme (*Thymus capitatus* L.) and rosemary (*Rosmarinus officinalis* L.): Impact on yield, chemical composition and antimicrobial activity. Industrial Crops and Products 47, 291–299.

[19] Nagendra chari K.L., Manasa D., Srinivas P., Sowbhagya H.B. (2013). Enzyme-assisted extraction of bioactive compounds from ginger (*Zingiber officinale* Roscoe). Food Chemistry 139, 509–514.

[20] Wang, Y., Zu Y., Long, J., Fu, Y., Li, S., Zhang, D., Li, J., Wink, M., Efferth, T., (2011). Enzymatic water extraction of taxifolin from wood sawdust of *Larixgmelini* (*Rupr.*) Rupr. and evaluation of its antioxidant activity. Food Chemistry, 126, 1178–1185.

[21] British Pharmacopoeia, 2000. HSMO, London, UK.

[22] Chen G., Chen S., Xie Y., Chen F., Zhao Y., Luo C., Gao Y. (2015). Total phenolic, flavonoid and antioxidant activity of 23 edible flowers subjected to in vitro digestion. Journal of Functional Foods 17, 243–259.

[23] Lafhal S., Vanloot P., Bombarda I., Kister J., Dupuy N. (2016). Identification of metabolomic markers of lavender and lavandin essential oils using mid-infrared spectroscopy, Vibrational Spectroscopy, 85, 79–90.

[24] Tohidi B., Rahimmalek M., Arzani A. (2017). Essential oil composition, total phenolic, flavonoid contents, and antioxidant activity of Thymus species collected from different regions of Iran, Food Chemistry, 220, 153–161.

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