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Research Article

Chemical Composition and Biological Activities of *Lavandula coronopifolia* Poir Extracts: A Comparison between Callus Culture and Native Plant

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Nowadays, according to the development and high functions of in vitro cultivation, one of the questions that researchers are looking for is whether the characteristics and compositions of the obtained product meet the needs of the society or not. Therefore, this research was done aimed to compare the bioactive characteristics of callus and native plant aqueous extracts of *Lavandula coronopifolia* Poir. Thus, two different culture media were utilized for calluses' growth. Extracts from these calluses and native plants were obtained using the sequential ultrasound-microwave assisted extraction (SUMAE) method. The extraction conditions were a constant temperature of 70°C ultrasound in 20 min at 250 W and 8 minutes at 800 W for microwave at 85°C. At first, results revealed that callus cultures were not able to produce any significant amounts of essential oil. Therefore, aqueous extracts obtained from calluses as well as the natural plant were examined in terms of antioxidant activity, total phenolic content, total flavonoid content, and antimicrobial activity. Moreover, their volatile components were detected with GC-MS. Both DPPH and FRAP techniques demonstrated that the callus on MS + Auxin had stronger antioxidant activity than the other callus and native plant. It also had the greatest total phenolic content. In terms of total flavonoid content, the native plant has the highest value. Generally, it can be concluded that the extract of callus on MS + Auxin has shown significant activity against the tested microorganisms than the other samples.

1. Introduction

Lavandula species are medicinal plants belonging to the *Lamiaceae* family, commonly known as lavenders [1]. All species of this medical plant are extensively scattered in the tropical and subtropical regions of the world [2]. This herb contains varied chemical compounds which impart different types of biological and pharmacological activities [2–5]. The genus *Lavandula coronopifolia* Poir is an everlasting wood that grows into a large shrub with 80 cm annual stems that are green to gray-green in color [1].

In order to obtain the essential oil and other beneficial chemical components from this plant, hydrodistillation and steam distillation are the two main traditional methods. These processes require prolonged heating, which can lead to the breakdown of the thermolabile compounds present in the starting plant material and a deterioration in odor. Additionally, with rising energy costs and the need to cut CO_2 emissions, universities and businesses are under pressure to develop novel technologies that can cut energy use, comply with emission-related legal requirements, and result in cost savings and better quality [6–8]. A variety of promising applications in various branches of science and technology have been created as a result of recent developments in the field of bioactive compounds extracted from natural sources. Ultrasonic-microwave-assisted extraction, which has been widely used for extraction from medicinal plants, combines the benefits of both microwave

and ultrasonic extraction methods [9]. There are a lot of studies on extraction methods of essential oils in order to examine their antioxidant and antimicrobial properties. In addition, the aqueous and alcoholic extracts of lavender have been shown in many studies to be effective. There have been reports of anticonvulsant, antispasmodic, and sedative effects, antifungal, antioxidant, antihyperglycemic activities, and anti-inflammatory activity. Moreover, Soheili and Salami conducted a study on in vitro method and determined that the *Lavandula angustifolia* extract has a considerable radical scavenging activity [10].

In vitro cultures are promising approaches for the bioprospection of natural products [11, 12]. In the search for alternatives to the biosynthesis of valuable secondary metabolites from plants, biotechnological techniques, particularly plant tissue cultures, have been found to have potential as a companion to conventional agriculture in the industrial production of phytochemical compounds [13]. By improving secondary metabolite content, tissue culture technology has a substantial impact on the conservation of medicinal plants as well as their therapeutic potential [14]. Micropropagation and secondary metabolite processing through callus culture are alternative methods for producing disease-free plantlets and biochemicals on a large scale. Therefore, laboratories all over the world are trying to produce secondary metabolites from plant tissue cultures. In addition to determining the optimal culture method, the challenge here is if the cell culture plant can suit our therapeutic needs. Rajaram et al. [15] investigated the antioxidant and antiproliferative capabilities of callus culture from *Tephrosia tinctoria*. Their findings revealed that this callus culture can produce more bioactive chemicals than the wild plant and could potentially be employed as a cancer treatment. Dakah et al. [16] obtained the aqueous and methanol extracts from *Ziziphora tenuior* L. and compared the antioxidant activities between in vitro propagated and wild plants. To the best of our knowledge, there is no report about the comparison of extract properties of a species of the lavender plant and its two callus cultures.

According to Karabagias et al. [17] results, *Lavandula stoechas* has a significant antioxidant activity, especially in its aqueous extract. Man et al. [18] demonstrated that *Lavandula angustifolia* extract had antibacterial activity. Canli et al. [19] evaluated the antimicrobial activity of *Lavandula stoechas* ethanol extract against 22 bacteria and 1 yeast, and the results confirmed the lavender's antimicrobial activity. Recently, Sharifzadeh et al. [20] reported that *Lavandula coronopifolia* Poir, whose extract was studied in the present work, has considerable antioxidant and antibacterial activities.

This paper describes the volatile organic compounds obtained from two calluses derived in vitro conditions of *Lavandula coronopifolia* Poir. The plant volatiles were extracted using a sequential ultrasound-microwave assisted extraction approach, a novel technique that has been employed in different studies involving volatile compound analysis [21–23], including the authors' prior work on *Lavandula coronopifolia* Poir [20]. Herein, we focused on the preparation and characterization of callus extracts from *Lavandula coronopifolia* Poir and assessed their antioxidant activities using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging activity and FRAP. Following that, we looked at the overall amount of phenols and flavonoids in callus cultures and wild plants. The following describes the technique of work and the obtained results of the comparison.

2. Material and Methods

2.1. Plant Materials. Native lavender (*L. coronopifolia* Poir) was selected from a mountainous area in Niakan village, Kuhrang county's central district, Chaharmahal and Bakhtiari provinces in the southwest of Iran (altitude is 2383 m height above mean sea level). The lavender was harvested in July 2021. The plant's aerial parts were carefully picked, dried in the shade, and stored in a dry, cool place until needed for extraction. Fresh parts (stem and leaf) of the lavender were also used for in vitro culture. This ensured that the natural plant and calluses were of the same species. The plant parts were surface sterilized with 0.1% NaOCl and then treated with 0.05% $HgCl_2$. They were then cleaned three times with sterile distilled water before being implanted in Murashige and Skoog culture medium (MS).

2.2. Chemicals. 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), quercetin, Folin–Ciocalteu's reagent, hydrochloric acid, gallic acid, aluminum chloride ($AlCl_3$), sodium carbonate (Na_2CO_3), cyclohexan, sodium persulfate ($K_2S_2O_8$), iron (II) chloride ($FeCl_2$), and 2,4,6-tri-(2-pyridyl)-1,3,5-triazine (TPTZ) have been bought from Sigma-Aldrich (USA). Sodium sulfate (Na_2SO_4), sodium acetate, dimethyl sulfoxide (DMSO), ascorbic acid (Vitamin C), Mueller–Hinton broth, and Muller–Hinton agar have been acquired from Merck (Germany). Other chemical substances and solvents have been analytical or chromatographic grade. Ultrapure water became used in the course of the experiments.

2.3. In-Vitro Growth Conditions. Under aseptic settings, the lavender explants were transplanted to sterile Petri plates using sterile forceps. The baseline medium consisted of inorganic salts and MS vitamins and auxin. In this study, for the growth of explants, two types of culture medium were used. The first was MS culture medium that had not been supplemented with any substance, and the second was MS culture medium that had been supplemented with the hormone auxin. In this way, two types of calluses were obtained: callus on MS and callus on MS + Auxin.

The pH was adjusted to 5.8 before autoclaving, and the carbon source was 30 g/L sucrose. BAP, NAA, and IAA were used as plant growth regulators. The media was solidified and autoclaved for 15 minutes at 121°C using 0.8 percent agar. For three weeks, the cultures were incubated at a 16-hour photoperiod with a light intensity of 3000 lux and a temperature of 25 ± 2°C using fluorescent tube lights [24].

Three explants in a single flask were utilized for each treatment. After three successive subcultures into

proliferation media, the plantlets were removed from the media and washed in distilled water. Finally, the collected calluses were dried in the shade and taken to a laboratory for extraction. The final cultivated calluses before extraction are shown in Figure 1.

2.4. Preparation of Extracts for Biochemical Analyses. Before the extraction operation, the plant material was only slightly chopped and not grinded into powder. In the preliminary tests, it was found that when the samples were ground and powdered, a lot of foam was produced, and the extraction process was difficult. The sequential ultrasound-microwave assisted extraction (SUMAE) approach was used to extract samples from two calluses and the plant. The SUMAE was introduced as a green and environmental friendly method for extraction [20, 23]. The procedure was based on the method described by Karimi et al. [23], with minor modifications. Ten grams of dried samples of plant and each callus were weighed and then mixed with 100 mL of distilled water. The mixture was transferred into an ultrasonic homogenizer (Topsonic, UHP-400, Iran) and homogenized at a fixed temperature of 70°C for 20 min at 250 watts. The mixture was then microwaved for 8 minutes at 800 watts. The extraction temperature was set to 85°C. After extraction, the extract was filtered through muslin cloth, followed by Whatman filter paper No. 1 and then freeze-dried (Dena Vacuum, FD-5005-HS-BT, Iran). The schematic of the extraction steps is summarized in Figure 2. The final extracts were kept in a refrigerator.

2.5. GC Analysis. Gas chromatography–mass spectrometry (GC-MS) identification technique was used to determine the chemical content of the extracts. Before injection, the freeze-dried samples were extracted with a mixture of solvents (toluene, chloroform, ethanol, and ethyl acetate), and 1 μL of each extract was injected into a GC-MS, and analyses were performed using Agilent Technologies 7890 series coupled with an Agilent HP-5 ms GC column (with 30 m length and 0.25 m internal diameter) and mass selective detector (MSD), 5975 series. It was applied using the following condition: carrier gas of He; constant flow rate of 0.8 mL/min; injection volume of 1 μL; injection temperature of 290°C; and ion-source temperature of 280°C. The following was the stepwise temperature program: initial temperature of the oven started at 50°C and hold for 5 min, followed by a gradient rate of 3°C/min until it reaches to 240°C, and then with an increase of 15°C/min to 280°C held for 10 min. Total running time for the GC-MS process was 75 min.

Compound identification was done by comparing retention times and mass spectra to published data, as well as computer matching with Wiley 7n and National Institute of Standards and Technology (NIST5.0) libraries, as well as Adams book, in the Analytical Chemistry Laboratory of Agriculture and Natural Resources University of Khuzestan. The unknown component's spectrum was compared to the known component's spectra recorded in the libraries.

2.6. Antioxidant Assay. Antioxidant activity was measured with a small modification of the DPPH [25] and FRAP [26] assays.

2.6.1. 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Activity Assay. Extract solution samples were dissolved in ethanol to reach different concentrations. To 3 μL of DPPH solution, 0.25 mL of each concentration was added. After 20 minutes of room temperature incubation, the absorbance was measured at 517 nm using a UV–VIS spectrophotometer (UNICO, UV/Vis 2100). As a blank sample, the equal amount of ethanol and DPPH solution was used without any sample. All tests were performed in triplicate, and the results were averaged. Following the same process, relative activities were estimated from the calibration curve of ascorbic acid standard solutions. The calibration curve was used to calculate antioxidant activity. The proportion of scavenging activity, IC%, was calculated using the following formula:

$$\mathrm{IC\%} = 100 - \left[\frac{\left(A_{\mathrm{sample}} - A_{\mathrm{blank}}\right)100}{A_{\mathrm{control}}}\right], \tag{1}$$

where A_{control} is the absorbance of the control, A_{blank} is the absorbance obtained in the absence of sample, and A_{sample} is the absorbance of the sample reaction. Free radical scavenging capacity was analyzed as their IC50 values which represented the essential oil concentration caused 50% inhabitation. The IC50 was determined by linear regression analysis from the obtained IC% values.

2.6.2. Ferric-Reducing Antioxidant Power (FRAP) Assay. To determine the reducing power, 900 μL of FRAP reagent (2.5 mL of TPTZ + 2.5 mL $FeCl_3$ + 25 mL of acetate buffer with a pH of 3.6), which is a labile solution and should be prepared just before the experiment, was added to 90 μL of distilled water and 30 μL of extract solution sample. After 30 minutes of incubation at 37°C in the dark, the rising absorbance of reaction mixtures was measured at 593 nm using the FRAP solution as a blank. To assess the antioxidant capacity of extracts, a standard curve was created using the $FeSO_4H_2O$ linear regression. Afterward, the results were represented as micromoles of Fe(II) equivalents per milligram of test material (mole eq $FeSO_4.7H_2O$/mg).

2.7. Determination of Total Phenolic Content. The total phenolic contents of aqueous extracts of wild plants and in-vitro-derived calluses were determined using the Folin–Ciocalteu reagent (FC), which was modified slightly from Hosu's et al. [25] technique. Briefly, the extract samples (250 μL of different dilutions) were mixed with 750 μL of Folin–Ciocalteu reagent solution (0.2 mol/L) for 5 min. Then, 250 μL of sodium carbonate solution (0.7 mol/L) was added; the mixture was stirred and allowed to stand in the dark at room temperature for 120 min. After the reaction



(a) (b)

Figure 1: Before extraction, calluses were cultivated; (a) the callus on MS, (b) the callus on MS + auxin.

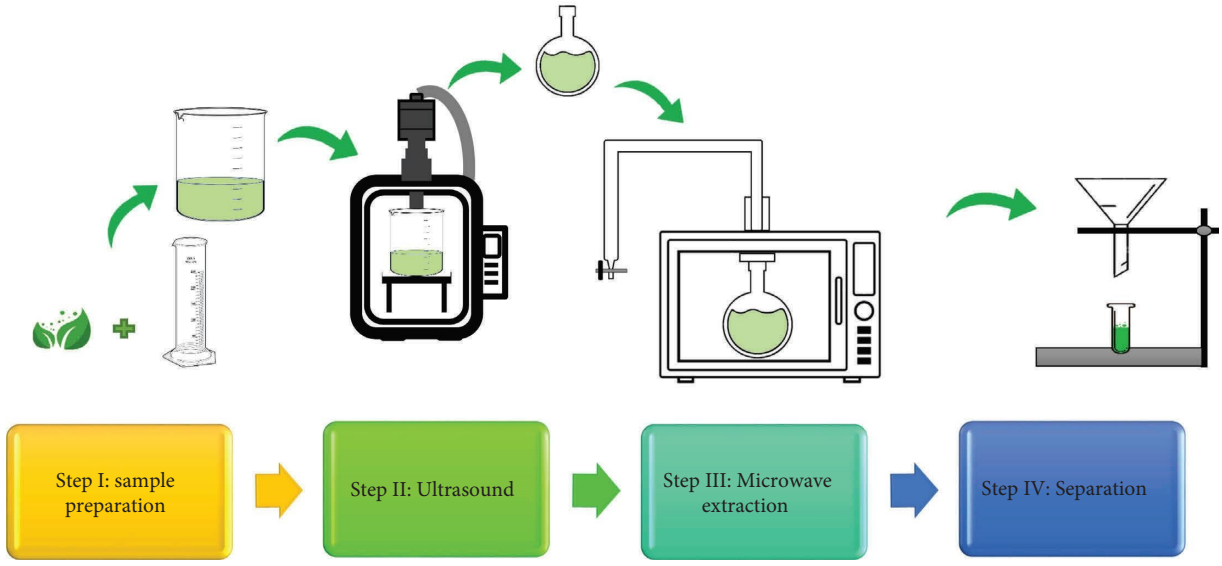


Figure 2: Extraction steps in the present work schematically.

time, the absorbance at 760 nm was measured (UV–VIS spectrophotometer (UNICO, UV/Vis 2100)). Tests were run in triplicate, and the results are presented as mg of gallic acid equivalents per 100 g of raw material (mg GAE/100 g).

2.8. Determination of Total Flavonoid Content. The total flavonoid contents of aqueous extracts of wild plants and in-vitro-derived calluses were determined using the Folin–Ciocalteu reagent (FC), with a minor modification from Hosu's et al. technique. Briefly, 200 μL sample of each extract solution (20 mg/mL) was mixed with 1.5 mL of 95% w/w methanol, 125 μL of 2% w/w aluminum chloride, 500 μL of 1 M sodium acetate, and 2.8 mL of water. The mixture was stirred and incubated for 15 minutes at room temperature. After the reaction time, the absorbance was measured at 430 nm (UV–VIS spectrophotometer (UNICO, UV/Vis 2100)). The total flavonoid content results are presented as mg of quercetin per 1 g of raw material (mg quer/g), calculated using a quercetin standard curve. All measurements were carried out in triplicate.

2.9. Antimicrobial Activity in Lavandula Leaves and Calluses

2.9.1. Microorganisms. Gram-positive bacteria (*Staphylococcus aureus* ATCC 1652) and Gram-negative bacteria (Escherichia coli ATCC 1885) were examined for

antibacterial activity. The examined microorganisms were supplied by the Razi Research and Development Center's Microbiology Laboratory in Karaj, Iran.

2.9.2. Determination of Minimum Inhibitory Concentration. Minimum inhibitory concentrations (MICs) of extract samples were evaluated using a broth microdilution technique in a 96-well plate, as reported by Cockerill et al. [27]. Extract samples with a concentration of 20 mg/mL were dissolved in 5 percent dimethyl sulfoxide (DMSO) and Muller–Hinton broth culture and then diluted to different quantities using the same combination. 75 μL of bacterial suspension (10^5–10^6 CFU/mL) and 75 μL of extract at different concentrations were placed in sterile 96-well microplates. Each well had a total capacity of 150 liters. The wells, which contained both culture and bacterial suspension, were employed as a positive growth controls. The target organisms were inoculated into the medium without extracts as a negative control. Then, the plates were sealed with sterile plastic lids and incubated for 24 hours at 37°C. The MIC of an antibiotic was defined as the lowest concentration at which observable growth was suppressed (absence of turbidity). All tests were performed in triplicate.

2.10. SEM. To investigate the effect of the extraction procedure on the surface morphology of calluses and plant cells, their dried lavender leaves were scanned using an SEM system (MIRA III, TESCAN, Czech). Samples were mounted on an aluminum holder and then sputtered with gold before being evaluated under high-vacuum conditions with an acceleration tension of 20 kV. The SEM images were captured at a magnification of 800.

2.11. Statistical Analysis. All antioxidant assays (DPPH, FRAP, total phenolic content, and total flavonoid content) and antimicrobial activity experiments were performed in triplicate. The obtained data were analyzed by analysis of variance (ANOVA) using Minitab v.16 (Minitab, State College, USA). Means were compared at the confidence level of 95% ($P< 0.05$) by Tukey's comparison test.

3. Results and Discussion

3.1. Gas Chromatography–Mass Spectrum Analysis. Since the native plant contains essential oil, during this analysis, we tend to examine two callus cultures through an equivalent method to obtain a considerable quantity of essential oil as well. After extraction, the results indicated that these callus cultures cannot produce a measurable amount of essential oil. Thus, we analyzed the obtained volatiles to the GC-MS to own a comparison in terms of components to native plants. As previously stated, in the present study, calluses were developed in two different culture media (MS and MS + Auxin). Table 1 shows the detected chemicals in calluses and native plants obtained by the GC-MS technique. The existence of 13 chemicals was discovered by GC-MS analysis of each aqueous extract (data for all compounds prepared here are available as supplementary information) (available here). For callus on MS, dodecamethylpentasiloxane (32.56%) was the most abundant, followed by dodecamethylcyclohexasiloxane (18.82%) and benzoic acid, 2,5 bis(trimethylsiloxy)-, trimethylsilyl ester (17.43%). Major components for callus on MS + Auxin were the same, and they were dodecamethylpentasiloxane (30.66%), benzoic acid, 2,5 bis(trimethylsiloxy)-, trimethylsilyl ester (17.00%), and dodecamethylcyclohexasiloxane (14.29%). On the other hand, major components for native plant were linoleic acid (40.05%), 2-myristynoyl-glycinamide (7.96%), dodecamethylpentasiloxane (6.41%), and tetracosamethylcyclododecasiloxane (6.06%). The aqueous extracts of calluses have revealed the presence of many high potential compounds which were absent in the native plant. Callus cultures extracts showed the presence of borneol, thymol, benzoic acid, 2,5 bis(trimethylsiloxy)-, trimethylsilyl ester, heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-, octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-, 4H-Dibenz[de,g]isoquinoline,5,6,6a,7-tetrahydro-1,2,9,10-tetramethoxy-5-methyl, cyclotrisiloxane, hexamethyl-, N-cyano-N′,N′,N″,N″-tetramethyl-1,3,5-triazinetriamine, acetic acid, [4-(1,1-dimethylethyl)phenoxy]-, methyl ester, hexamethylcyclotrisiloxane, hydromethylsiloxane, and silane, 1,4-phenylenebis[trimethyl]-. Based on previous literature, 4H dibenz[de,g]isoquinoline,5,6,6a,7-tetrahydro-1,2,9,10-tetramethoxy-5-methyl- [28], hydromethylsiloxane [29], and silane, 1,4-phenylenebis [trimethyl] [30] have antioxidant activities; heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl- [31] has antimicrobial activities, and amongst these beneficial compounds, borneol [32], thymol [33], benzoic acid, 2,5 bis(-trimethylsiloxy)-, trimethylsilyl ester [34], octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl- [35], cyclotrisiloxane, hexamethyl- [36], N-cyano-N′,N′,N″,N″-tetramethyl-1,3,5-triazinetriamine [37], acetic acid, [4-(1,1-dimethylethyl)phenoxy]-, methyl ester [38], and hexamethylcyclotrisiloxane [39] showed antioxidant and antimicrobial activities. In addition to this, the existence of various bioactive chemicals such as dodecamethylcyclohexasiloxane and dodecamethylpentasiloxane, with positive uses in the cosmetic sector, such as personal care products [40, 41], was also discovered by GC-MS analysis of aqueous extracts of calluses. Medicinal plants have rich biodiversity and can produce valuable compounds, even though terrestrial biodiversity forms the foundation of the pharmaceutical industry. By comparison with those from other studies, our findings confirmed that calluses of Lavandula coronopifolia Poir contain a wide variety of biological elements.

3.2. Evaluation of Antioxidant Activities

3.2.1. DPPH Test. Table 2 shows the DPPH radical scavenging activities of extracts from callus cultures and native plants. The callus derived from the plant that was established on the MS and applied Auxin hormones (callus on MS + Auxin) showed relatively the highest radical scavenging activity with IC50 value of 0.102003 mg/ml. This activity was higher than that of the aqueous extract of the native plant, which had an IC50 of 0.2261 mg/ml. On the

Table 1: Chemical composition of native plant, callus developed in MS culture, and callus developed in MS + auxin culture.

No	Names	RT[a]	Area%		
			Native plant	MS	MS + auxin
1	Borneol	20.930	—	—	2.55
2	Dodecamethylcyclohexasiloxane	**28.541**	0.59	**18.82**	**14.29**
3	Piperitenone oxide	30.192	2.12	—	—
4	Dodecamethylpentasiloxane	**35.782**	**6.41**	**32.56**	**30.66**
5	Tetracosamethylcyclododecasiloxane	**42.233**	**6.06**	—	—
6	Benzoic acid, 2,5 bis(trimethylsiloxy)-, trimethylsilyl ester	**42.233**	—	**17.43**	**17.00**
7	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	42.438	—	0.46	6.62
8	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	47.843	—	5.91	—
9	Palmitic acid	52.582	5.62	—	—
10	4H-dibenz[de,g]isoquinoline,5,6,6a,7-tetrahydro-1,2,9,10-tetramethoxy-5-methyl-	52.848	—	5.22	—
11	Capnellane-5α–ol	53.033	2.31	—	—
12	Cyclotrisiloxane, hexamethyl-	55.792	—	2.64	7.1
13	2-cis,cis-9,12-octadecadienyloxyethanol	55.782	1.54	—	—
14	5,8,11-heptadecatrien-1-OL	56.602	2.57	—	—
15	N-cyano-N′,N′,N″,N″-tetramethyl-1,3,5-triazinetriamine	57.453	—	2.16	9.11
16	Linoleic acid	**58.417**	**40.05**	—	—
17	Linolenyl alcohol	58.571	4.65	—	—
18	Acetic acid, [4-(1,1-dimethylethyl)phenoxy]-, methyl ester	65.225	—	—	3.40
19	Cyclobarbital	65.669	1.54	6.28	2.13
20	Methyl hexadecatrienoate	65.679	1.20	—	—
21	2-Methyl-7-phenylindole	66.130	1.79	—	—
22	Hexamethylcyclotrisiloxane	66.417	—	1.66	—
23	2-Myristynoyl-glycinamide	**66.879**	**7.96**	—	0.87
24	Hydromethylsiloxane	67.771	—	2.12	—
25	Silane, 1,4-phenylenebis[trimethyl]	67.853	—	1.08	—
26	Other components	—	11.98	0.29	1.95
	Identification (%)		96.39	96.63	95.68

[a]RT, retention time. The bold values are related to the prominent and interesting identified compounds in the native plant, callus developed in MS culture, and callus developed in MS + auxin culture.

Table 2: Antioxidant assay by DPPH and FRAP methods.

Aqueous extract sample	Assay method	
	IC50 DPPH (mg/m*l*)	FRAP (μmol $FeSo_4$ eq/1 g extract)
Callus on MS	0.45 ± 0.007^{A}	232.06 ± 19.10^{C}
Callus on MS + Auxin	0.10 ± 0.002^{C}	1024.68 ± 34.10^{A}
Native plant	0.26 ± 0.019^{B}	371.74 ± 29.1^{B}

Different letters represent significant differences at $P < 0.05$ probability level. Values represents average of triplicates ± standard deviation.

other hand, the lowest DPPH• scavenging was observed by the callus which was subcultured on MS medium (callus on MS) with an IC50 value of 0.45571 mg/ml. Here, the control was ascorbic acid, which had an IC50 of 9.35 mg/ml. Statistical analysis showed that the assay method had a significant effect ($P < 0.05$) on the results of DPPH test. Generally, in DPPH free radical scavenging assay, the extracts demonstrated strong antioxidant activity comparable to that of normal ascorbic acid. The results are consistent with those of El-Baz et al. [12], who found that the extract of *Citrullus colocynthis* callus had higher DPPH radical scavenging activity than the extract of field plant leaves.

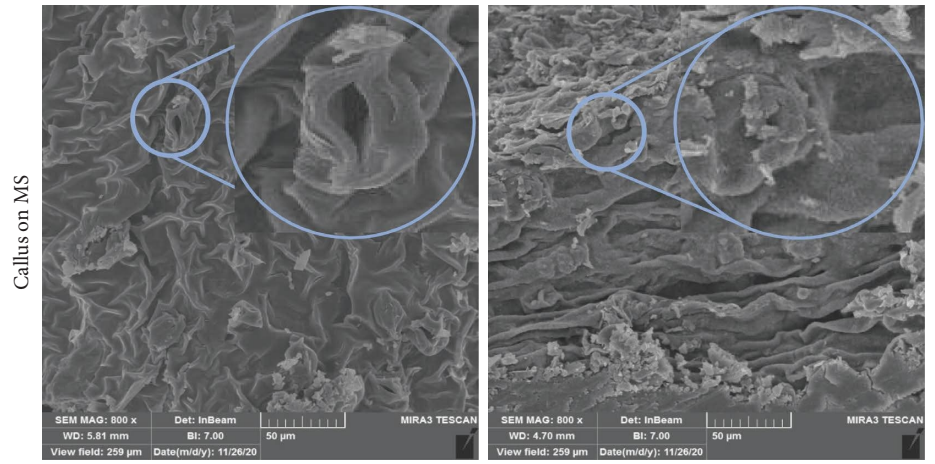
3.2.2. FRAP Test. Table 2 shows the results of the ferric-reducing capacities of the extracts. An antioxidant is able to donate an electron to the ferric-TPTZ (Fe (III)-TPTZ) complex, which causes the reduction of it into the blue TPTZ (Fe (II)-TPTZ) complex, which is strongly adsorbed at 593 nm. To compare the extracts, the callus on MS + Auxin had the highest ferric-reducing activity, whereas native plant extracts had the lowest. For callus on MS, the FRAP value was slightly lower. Here, as in the previous test, the statistical difference in the results is significant ($P < 0.05$). Similar results were observed for *Sericostoma pauciflorum*, according to Jaina et al. [42]. Their findings revealed that when studying plant cell or tissue cultures, the growth regulator plays a critical role in secondary metabolite accumulation.

3.3. Total Phenolic Content. The total contents of phenols (TPC) and flavonoids were measured in order to assess the extract's antioxidant capacity. Table 3 shows the total phenolic content of extracts of calluses versus native plants

Table 3: Total phenolic content and total flavonoid content of extracts.

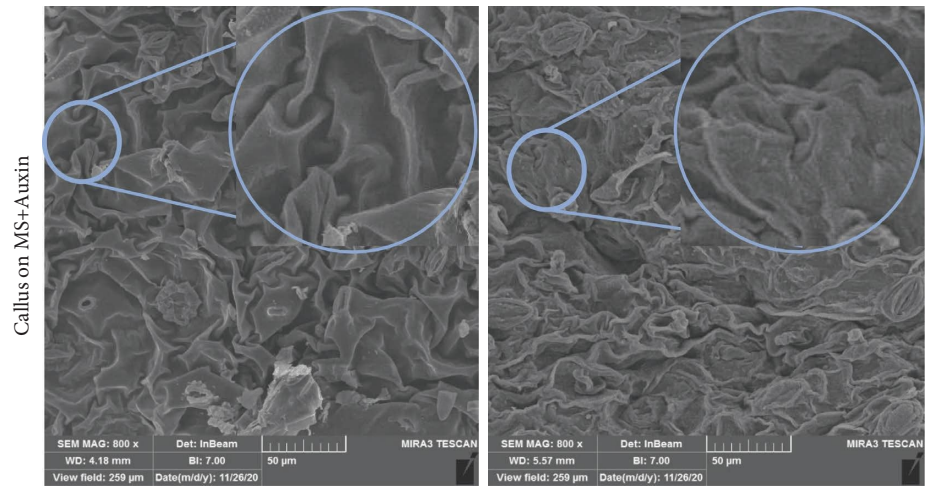
Aqueous extract sample	TPC (mg GAE/100 g)	TFC (mg QE/100 g)
Callus on MS	33.37 ± 1.96^{B}	2.19 ± 0.15^{C}
Callus on MS + auxin	64.73 ± 3.93^{A}	3.36 ± 0.17^{B}
Native plant	39.11 ± 0.59^{B}	7.45 ± 0.43^{A}

Different letters represent significant differences at $P < 0.05$ probability level. Values represents average of triplicates ± standard deviation.



Before extraction After extraction

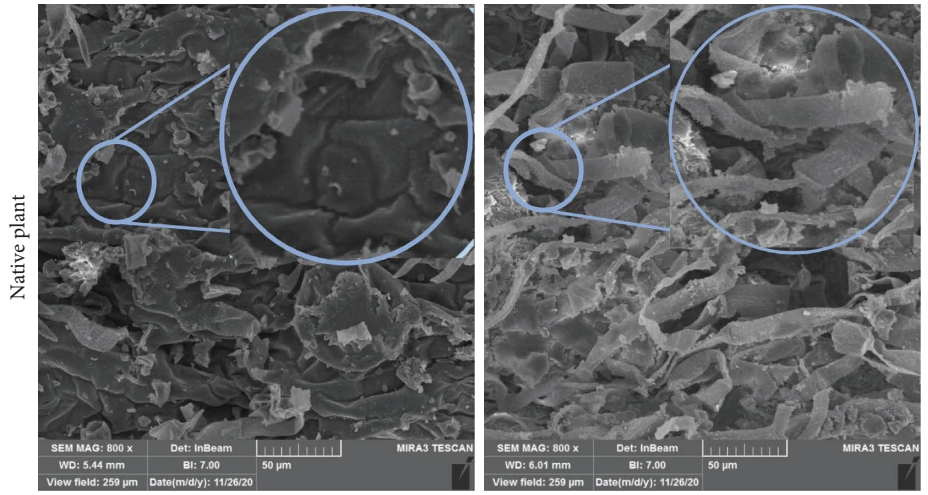
(a)



Before extraction After extraction

(b)

Figure 3: Continued.



(c)

Figure 3: Scanning electron microscopy (SEM) analysis of: (a) Callus on MS; (b) Callus on MS + auxin; (c) Native plant, before and after extraction.

Table 4: Antibacterial activities of calluses.

Microorganism	MIC ($\mu g/\mu l$)		Native plant
	Callus on MS	Callus on MS + auxin	
S. aureus	5 ± 0.05^{A}	2.50 ± 0.16^{B}	1.25 ± 0.07^{C}
E. coli	2.50 ± 0.03^{A}	1.25 ± 0.04^{B}	0.62 ± 0.01^{C}

Different letters represent significant differences at $P < 0.05$ probability level. Values represents average of triplicates ± standard deviation.

($P < 0.05$). The TPC values of the extracts were expressed as mg of gallic acid equivalents per 100 g of raw material (mg GAE/100 g). The highest TPC was observed in the callus on MS + Auxin, followed by the native plant, whereas the lower TPC values were observed in the callus on MS. In general, according to statistical analysis, the use of MS + Auxin medium in the growth of callus had a significant effect on TPC.

3.4. Total Flavonoid Content. Table 3 shows the TFC values expressed as mg of quercetin per 100 g of raw material (mg QE/100 g). The native plant yielded the highest total flavonoid production per gram extract (7.4467). This content was higher than callus on MS and callus on MS + Auxin content. Although the extract of the native plant was the highest, it is also noticeable that differences in values of the calluses demonstrate that growth regulators have an impact on flavonoid production. Here, the assay method presented a significant effect on the results ($P < 0.05$).

3.5. Surface Morphology. Figure 3 provides the SEM analysis results for callus on MS and callus on MS + Auxin. The surface of the calluses was smooth before the extraction process, as can be seen in the images. It can be seen that applying the SUMAE process has resulted in the opening of cavities containing essential oil, resulting in increased surface roughness.

3.6. Antimicrobial Activity. The broth microdilution technique was used to determine the antibacterial activities (MIC values) of aqueous extracts of calluses obtained against a Gram-positive bacterium (*Staphylococcus aureus*) and a Gram-negative bacterium (*Escherichia coli*). The results are shown in Table 4. In both cases, extracts from both calluses and native plants presented a significant effect on antimicrobial activity ($P < 0.05$). Against all of the bacteria examined, both extract samples showed varying degrees of antimicrobial activities. The results demonstrate that Gram-positive bacteria have a far higher sensitivity to the extract than Gram-negative bacteria. In fact, the foremost inclined bacteria for both aqueous extracts were *S. aureus*. Consequently, the antibacterial activity of the callus extract on MS + Auxin was higher than that of the callus extract on MS alone, which was most likely due to their chemical compositions. From our previous results, it was also noted that native plants showed significant antimicrobial activity compared to its callouses in the present study.

4. Conclusion

The aim of this study was to compare the properties of extracts obtained from cultured callus and native planet of *Lavandula coronopifolia* Poir. Thus, two callus cultures were generated: one on MS and another on MS + Auxin. Then, aqueous extracts were obtained using the optimized SUMAE technique. Investigation of the antioxidant activity of

extracts was done by the methods of DPPH and FRAP. Total phenolic and flavonoid contents have also been determined. Moreover, the antimicrobial activity of the aqueous extracts of samples was evaluated by the disc diffusion method and determined the minimum inhibitory concentration (MIC) values. Furthermore, GC-MS was used to identify the components in the extracts of callus cultures. SEM analysis also determined the effect of the extraction process on the samples. The results revealed that the aqueous extract of the callus on MS + Auxin generated from *Lavandula coronopifolia* Poir showed the highest amount of total phenolic content and better antioxidant activity than aqueous extracts for both native plant and the callus on MS, while the extract of the native plant contained the highest level of flavonoids. Moreover, the aqueous extract of the callus of MS + Auxin showed more antimicrobial activity on both Gram-negative and Gram-positive bacteria than other extracts. Additionally, dodecamethylpentasiloxane, dodecamethylcyclohexasiloxane, and benzoic acid, 2,5 bis(trimethylsiloxy)-, trimethylsilyl ester were the most representative compounds in both callus cultures extract samples. These constituents are used in the cosmetics industries. Based on these results, native plants and its callus cultures can be utilized as an easily accessible source of natural antioxidants, as a possible food supplement, or for pharmaceutical applications.

Data Availability

The data used to support the study are included within the article as supplementary data 1. Raw data can be acquired from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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Supplementary Materials

"Table 1: supplementary data" shows all chemical identified compounds of native plant, callus developed in MS culture, and callus developed in MS + Auxin culture while "Table 1" included in the paper shows only the interesting and prominent compounds. (*Supplementary Materials*)

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