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Research Article

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Total Phenolics, Flavonoids, Tannin Contents and Antioxidant Properties of *Pleurotus ostreatus* Cultivated on Different Wastes and Sawdust

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Abstract: In this study, the usage possibilities of some agro-industrial wastes such as; peanut wastes, potatoes farm wastes, walnut and orange tree sawdust in *Pleurotus ostreatus* cultivation were investigated and total phenolic, flavonoid, condensed tannin content and antioxidant properties of these methanolic mushroom extracts were examined. For the determination of the total phenolic contents, the Folin-Ciocalteau procedure was used. The content of total flavonoid present in the methanolic extracts was measured using a spectrophotometric assay. Condensed tannins were determined according to the method by Julkunen-Tıtto. The antioxidant capacity was determined using ferric reducing antioxidant power (FRAP) and free radical scavenging activity of DPPH. The highest total phenolic content (2.672 ± 0.003 mg GAE/g) was found in mushroom cultivated on walnut sawdust. The highest condensed tannin (1.011 ± 0.088 CE mg/g) and ferric reducing antioxidant power (FRAP) (12.332 ± 0.017 µmol $FeSO4.7H_2O$/g) were observed in the same mushroom extract. The highest total flavonoid and free radical scavenging activity of DPPH were found in extract of mushroom cultivated on potatoes handle. Bioactive properties of *P. ostreatus* cultivated on walnut tree sawdust were generally exhibited remarkable results.

Keywords: Agro-Industrial Wastes, Antioxidant, Flavonoids, Phenolics, *Pleurotus ostreatus*

1. Introduction

As an edible white-rot fungus; *Pleurotus ostreatus* falls under the category of non- timber forest products (NTFP) and *Pleurotus* genus contains about 40 species [1]. Thanks to their enzyme systems; they can utilize lignocellulosic materials such as agricultural wastes [2]. *Pleurotus* mushroom is the third most cultivated edible mushroom worldwide after *Agaricus bisporus* [3]. Because of easy growing techniques and broad adaptability, *P. ostreatus* have an important role in using of recycling organic wastes [4]. Some industrial and agricultural wastes such as soybean, sorghum, peanut and wheat straw [5] leaves of hazelnut, waste paper [6] cotton straw, lentil straw, rice bran [7] etc. can be used as substrate for cultivation.

Mushrooms accumulate some metabolites such as terpenes and steroids, phenolic compounds, polyketides [8]. These metabolites influence odor, taste, appearance and oxidative stability of nutrients [9]. It was reported that some of them can have some of pharmacological and biochemical properties such as antioxidant, antimicrobial, antimutagenic, antithrombotic and anticarcinogenic activities [10-12]. These kind of natural

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bioactive compounds such as phenols and flavonoids become more important day to day since it has been announced carcinogenesis risk of synthetic antioxidants [13]. The phenolic configuration in mushrooms can be affected some factors such as composition of growth media for in vitro cultured species, mushroom strain/species, content of the substrates etc. [14]. Production of orange, peanut, walnut and potatoes are made in Turkey. Potatoes, orange, walnut and peanut production areas and amounts of production in 2015 are given Table 1 [15].

Table 1. Potatoes, orange, walnut and peanut production area and amount of production in 2015, Turkey

	Production area (decare)	Amount of production (ton)
Potatoes	1.540.801	4.763.060
Orange	542.984	1.816.798
Walnut	718.196	190.000
Peanut	377.729	147.537

In our country; sawdust of orange and walnut trees are generally used as firewood without re-cycled. Potatoes farm wastes and peanut wastes are used for fire, too. In this study, the possibility of using these wastes for *P. ostreatus* cultivation was investigated since this mushroom has high saprophytic ability and most of cellulosic wastes can grow its on [16].

The main objectives of the study were to investigate the usage possibilities of some agro-industrial wastes such as; peanut wastes, potatoes farm wastes, walnut and orange tree sawdust in *P. ostreatus* cultivation and to determinate the total phenolic, flavonoid, condensed tannin content and antioxidant properties of these mushrooms' methanolic extracts and compare them with each other.

2. Material and Methods

P. ostreatus spawn was purchased from a commercial firm located in Denizli province. Peanut wastes were obtained from one of peanut manufacturing in Osmaniye, orange tree sawdust from orange garden in Adana, potatoes farm wastes from a potatoes farm in Trabzon and walnut sawdust from workshop of Forest Industry Engineering, Karadeniz Technical University.

2.1. Mushroom cultivation

Peanut wastes, potatoes farm wastes, walnut and orange tree sawdust moistened with water until 70-80% and sterilized in an autoclave at 121˚C for 1.5 h. After cooling the substrates to 20˚C, they were placed in nylon bags of 1 kg and inoculated by spreading spawn on the surface of the substrate with a weight percentage of about 3% of the wet weight of compost. Substrate condition was carried out in four replications. Each nylon bags were inoculated in mushroom growing laboratory (at 15-25˚C, 70-80% relative humidity). Harvesting was started in fifth week and the fruit bodies' stipe and cap were calculated and weighed.

2.2. Yield and biological efficiency

Mushroom yield was calculated as total fresh weight of mushrooms obtained from 3 or 4 flushes in the harvest period [17]. Biological efficiencies were defined as the percentage ratio of the fresh weight of harvested mushrooms over the dry weight of substrates [18].

2.3. Preparation of the extract

Approximately 5 g of mushroom samples in were placed into a falcon tube 50 mL 99% with additional methanol. The mixture was stirred continuously with a shaker (Heidolph Promax 2020, Schwabach, Germany) at room temperature for a total of 24 hours. Particles were removed using filter paper. The final volume of the solution was adjusted by the level of methanol.

2.4. Determination of polyphenolic contents

The polyphenolic contents of the methanolic samples were evaluated three different ways; total phenolic contents (TPC), total flavonoids (TF) and total tannin (TT).

For the determination of the total phenolic contents, the Folin-Ciocalteau procedure was employed and gallic acid was used as standard [19]. Shortly, 20 µL of various concentrations of gallic acid and samples, 400 µL of 0.5 N Folin-Ciocalteu reagent and 680 µL of distilled water were mixed and vortexed. After 3 min incubation, 400 µL of Na_2CO_3 (10%) solution was added and vortexed. Then the mixture was incubated for 2 h at 20 °C with interrupted shaking. Absorbance measurement was carried out at 760 nm at the end of the incubation period. A standard curve was prepared using gallic acid as a standard with different concentrations of gallic acid, and the results were expressed as mg (GAE) per g methanolic extracts.

The concentration of total flavonoid present in the methanolic extracts was measured using a spectrophotometric assay. Briefly, 0.5 mL samples, 0.1 mL of 10% $Al(NO_3)_3$ and 0.1 mL of 1 M $NH_4.CH_3COO$ were added to a test tube and incubated at room temperature for 40 min. Then the absorbance was measured against a blank at 415 nm. Quercetin was used for the standard calibration curve. The total flavonoid concentration was expressed as mg of quercetin equivalents per g sample [20]

Condensed tannins were determined according to the method by Julkunen-Tıtto [21]. For each sample, various concentrations of 25 µL mushroom extracts were mixed with 750 µl of 4% vanillin (prepared with MeOH) and then 375 µL of concentrated HCl was added. The well-mixed solution was incubated at room temperature in darkness for 20 min. The absorbance against the blank read at 500 nm. (+)-Catechin was used to make the standard curve (0.05–1 mg/ml). The results were expressed as mg catechin equivalent to (CE)/g sample.

2.5. Determination of antioxidant capacity

The antioxidant capacity was determined using ferric reducing antioxidant power and free radical scavenging activity of DPPH•.

2.5.1. Ferric reducing antioxidant assay (FRAP)

FRAP assay was tested to determine the total antioxidant capacity of the samples. This method is based on the reduction of tripyridyltriazine complex (Fe (TPTZ) $^{3+}$) to blue colored $Fe(TPTZ)^{2+}$ by antioxidants in acidic medium [22]. The preparation of working FRAP reagent was carried out by mixing 25 mL of 0.3 M acetate buffer pH 3.6 with 2.5 mL of 10 mM 2,4,6-tripyridylstriazine (TPTZ) solution in 40 mM HCl and 2.5 mL of 20 mM $FeCl_3.6H_2O$ solution. The reaction mixture consisting of 1mL of the sample and 3 mL of freshly prepared FRAP reagent was incubated at 37 °C for 4 min. Then, the absorbance was determined at 593 nm against blank prepared with distilled water. A calibration curve prepared with an aqueous solution of ferrous sulfate $FeSO_4.7H_2O$ in the range of 100-1000 µM was used. Trolox was also tested under the same conditions as a standard antioxidant compound. FRAP values were expressed in wet weight of the samples as µmol of ferrous equivalent Fe (II) per g sample.

2.5.2. Scavenging of free radical (DPPH) assay

The DPPH assay was applied [23] to determine the radical scavenging capacity of the methanolic extracts of the mushroom. The simple method is based on scavenging the DPPH radicals with an antioxidant substance of the investigated solution. For each sample, six different concentrations of 0.75 mL of the extracts of the samples were mixed with 0.75 mL of 0.1 mM of DPPH in methanol, and the absorbance was read at 517 nm. The values were expressed as SC_{50} (mg sample per mL), the concentration of the samples causing 50% scavenging DPPH radicals.

2.6. Statistical analysis

All assays were performed in triplicate. The data were recorded as means ± standard deviations and analyzed by using Statistical Package for Social Sciences (SPSS version 23.0). The obtained data were analyzed by ANOVA and tests of significance were carried out using Duncan's multiple range tests.

3. Results and Discussion

3.1. Yield and biological efficiency

P. ostreatus was cultivated on four different materials namely peanut wastes, potatoes farm wastes, walnut and orange tree sawdust. Yield (g/100g) and biological efficiency (%) are presented in Table 2.

Table 2. Yield (g/100g) and biological efficiency (%) of cultivated mushroom

Material	Yield (g/100g)	Biological Efficiency (%)
	$\bar{X}$ ± SD	$\bar{X}$ ± SD
Potatoes farm wastes	11.4 ± 0.8[a]	40.8 ± 2.9[a]
Orange tree sawdust	16.9 ± 1.7[b]	60.1 ± 6.2[bc]
Walnut tree sawdust	18.4 ± 2.1[b]	65.2 ± 7.5[c]
Peanut wastes	14.7 ± 1.1[c]	52.4 ± 4.6[b]

[a] Means having the same superscript letter(s) are not significantly different ($p>0.05$) by Duncan's multiple range test.

Total yield (g/100g substrates) was calculated after harvest period and substrates, walnut tree sawdust produced highest yield (18.4 ± 2.1 g/100g substrates), whereas potatoes farm wastes produced the lowest (11.4 ± 0.8 g/100g substrates) and our results are comparable with other *P. ostreatus* cultivation studies with 2-41 g/100g substrates [24-26]. In previous studies, yield of *P. ostreatus* cultivated on different composts was reported from and biological efficiency was reported from 0-61% [27] to 48.9- 90.5% [28]. Differences of yield and biological efficiency can be results of different compost components [27, 29]. All of materials used in this study can be evaluated for *P. ostreatus* cultivation.

3.2. Polyphenolic contents

The total polyphenols (mg GAE/g), total flavonoids (mg QE/g) and condensed tannin contents (CE mg/g) of *P. ostreatus* cultivated on different medium are presented in Table 3.

In this study, the highest total phenolic content (2.672 ± 0.003 mg GAE/g) was determined in mushroom cultivated on walnut sawdust and the lowest one (1.073 ± 0.028 mg GAE/g) in mushroom cultivated on peanut wastes. These values are higher than some vegetables consumed frequently in Turkey such as *Chicory* and *Lepidium sativum* (1.091 and 1.261 mg GAE/g, respectively) [30] and higher than other wild mushroom's content such as

Pleurotus eryngii (0.634 ± 0.004 mg GAE/g) and *Cyttaria gunnii* (0.761 ± 0.004 mg GAE/g) [31].

High level of phenolic compounds in mushrooms have been attributed to antioxidant activity and they were recorded as natural substrates of oxidative enzymes in the literature [32, 33].

Table 3. Total polyphenols (mg GAE/g), total flavonoids (mg QE/g) and condensed tannin contents (CE mg/g) of *P. ostreatus* cultivated on different medium

Mushroom	Total Polyphenols (mg GAE/g)	Total Flavonoid (mg QE/g)	Condensed Tannin (CE mg/g)
	$\bar{X}$ ± SD	$\bar{X}$ ± SD	$\bar{X}$ ± SD
P. ostreatus cultivated on potatoes farm wastes	1.389 ± 0.007[a]	0.134 ± 0.001[a]	0.694 ± 0.004[a]
P. ostreatus cultivated on orange tree sawdust	1.777 ± 0.024[b]	-	0.422 ± 0.018[b]
P. ostreatus cultivated on walnut tree sawdust	2.672 ± 0.003[c]	0.130 ± 0.006[a]	1.011 ± 0.088[c]
P. ostreatus cultivated on peanut wastes	1.073 ± 0.028[d]	-	0.447 ± 0.003[b]

[a] Means having the same superscript letter(s) are not significantly different (p>0.05) by Duncan's multiple range test.

The capacity of flavonoids to act as antioxidants depends upon their molecular structure. The arrangement of hydroxyl groups and the other features characteristic in the chemical configuration of flavonoids are important for their free radical scavenging activities and antioxidant properties [34]. In this study, the highest total flavonoid content (0.134 ± 0.001 mg QE/g) was found in mushroom obtained from potatoes farm wastes and the lowest one (0.130 ± 0.006 mg QE/g) from walnut sawdust. These values are lower than some wild mushrooms such as *Pleurotus florida* (0.17 ± 0.02 mg QE/g), *Flammulina velutipes* (0.20 ± 0.05 mg QE/g) [35]. The flavonoid content in mushroom cultivated on peanut wastes and orange tree wastes couldn't determine.

As it is known that tannins are polyphenolic compounds responsible for several bioactivities such as antitumor, antimicrobial and antioxidative activities [36]. In this study; the highest condensed tannin content (1.011 ± 0.088 CE mg/g) was seen in mushroom cultivated on walnut sawdust. The lowest condensed tannin content was observed (0.422 ± 0.018 CE mg/g) in mushroom cultivated on orange tree wastes. Our values are higher than some reported wild mushrooms such as *Lentinus ciliatus* (0.343 ± 0.030 CE mg/g), *Schizophyllum commune* (0.280 ± 0.024 CE mg/g), *Hygrocybe conica* (0.251 ± 0.011 CE mg/g) and *Pleurotus ostreatus* (cultivated) (0.326 ± 0.025 CE mg/g) [37]. The antioxidant activity of *P. ostreatus* cultivated on different medium is presented in Table 4.

Table 4. The antioxidant activity of P. *ostreatus* cultivated on different medium

Mushroom	FRAP (μmol $FeSO_4.7H_2O$/g)	DPPH-SC_{50} (mg/mL)
	$\bar{X}$ ± SD	$\bar{X}$ ± SD
P. ostreatus cultivated on potatoes farm wastes	4.826 ± 0.001[a]	15.473 ± 0.001[a]
P. ostreatus cultivated on orange tree sawdust	6.976 ± 0.012[b]	7.641 ± 0.499[b]
P. ostreatus cultivated on walnut tree sawdust	12.332 ± 0.017[c]	4.937 ± 0.001[c]
P. ostreatus cultivated on peanut wastes	4.096 ± 0.037[d]	8.596 ± 0.002[d]

[a] Means having the same superscript letter(s) are not significantly different (p>0.05) by Duncan's multiple range test.

The FRAP assay actually measures the ability of antioxidants to reduce ferric iron [38]. According to the Table 4; the highest FRAP activity (12.332 ± 0.017 μmol $FeSO_4.7H_2O$/g) was found in mushroom cultivated on walnut sawdust and the lowest (4.826 ± 0.001 μmol $FeSO_4.7H_2O$/g) one in mushroom cultivated on potatoes farm wastes. The FRAP activities of *P. ostreatus* was expressed as 2.385,71(μmol/g) by Keleş et al [39].

DPPH method characterizes the antioxidant capacity of extracts against oxidation caused by free radicals. [40]. In this study, the highest DPPH activity (15.473 ± 0.001 mg/mL) was found in mushroom obtained from potatoes farm wastes. The lowest one (4.937 ± 0.001mg/mL) obtained from walnut sawdust. DPPH activity of studied mushrooms' methanolic extracts are is generally higher than *Lactarius deterrimus* (5.85 ± 0.51 mg/mL) and lower than *Boletus edulis* (21.90 ± 0.92 mg/mL) and *Xerocomus chrysenteron* (27.42 ± 1.23 mg/mL) [41].

4. Conclusion

In this study the usage possibilities of some agro-industrial wastes such as; peanut wastes, potatoes farm wastes, walnut and orange tree sawdust in *Pleurotus ostretatus* cultivation were investigated and the total phenolic, flavonoid, condensed tannin contents and antioxidant properties of these mushrooms' methanolic extracts were examined.

The results of this study indicated that peanut wastes, potatoes handle, walnut sawdust and orange tree sawdust can be used as substrate for *Pleurotus ostreatus* cultivation. In many respects; bioactive properties of *P. ostreatus* cultivated on especially walnut tree sawdust were generally exhibited remarkable results (the highest yield and biological efficiency, the highest total phenolic content, the highest condensed tannin and the highest FRAP activity) compared to the bioactive properties of mushrooms cultivated on the other wastes types. So; walnut tree sawdust and its habitat can be investigated by further analysis. On the other hand; it has not been determined flavonoid content in mushroom cultivated on peanut wastes and orange tree wastes.

Total phenolic, flavonoid and antioxidant properties of mushrooms cultivated on different medium were found significantly different ($p<0.05$) by Duncan's multiple range test. Bioactive properties of mushrooms highly depend on mushroom species, growing conditions, extraction process and substrate medium. To obtain better results from mushroom extracts, different substrate types and different extraction methods can be tested.

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Modelling of Baker's Yeast Production

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Abstract: In the present work, parametric models for the control of bioreactor temperature have been applied. Various order discrete time model parameters were evaluated theoretically and experimentally. Two types of input signals were used as external force to determine Auto Regressive Moving Average with Exogenous (ARMAX) model parameters with Recursive Least Square (RLS) parameter estimation algorithm. The third order ARMAX model is utilized, and compared with the second order one. Ternary and square disturbances are given to the cooling water flow rate which can be chosen as manipulating variable in closed loop cases. System response is monitored continuously and the model parameters are calculated. The models with experimentally identified parameters are compared with ones that their parameters are identified theoretically.

Keywords: Baker's Yeast, System Identification, *Saccharomyces cerevisiae* as a second metabolite source

1. Introduction

As *S. cerevisiae* investigations continue in research and development of food and drug need improvement, as well as biotechnological and genetic purposes. The literature on microbial genomic sequences highlighted the necessity for the production of desired metabolites. The important compounds *S. cerevisiae* have importance commercially as one of the most studied model organisms with large scale bioreactor operation. The control performance can be enable a considerable increase in the industrial application of Baker's Yeast. Studies have described *S. cerevisiae* production that there is great emphasize on pharmaceutical usage, the investigations related with the production is then encouraged to explore the data in order to obtain a better understanding of it [1, 2].

Baker's yeast production can be achieved by means of batch or fed-batch operation under aerobic conditions. Time varying behavior of bioprocesses exhibits complex, nonlinear behavior of which the modelling is very difficult [1].

Examination of the internal structure of bio-systems illustrates a complete case with unknown factors and unmeasurable variables [3]. According to requirements, order and accuracy definitions may be changed for bioprocess model applications. The modelling with parameters identification is one of the effective procedures to define the systems. Therefore two main part of it include choosing the model structures and evaluation of any certain model parameters. An acceptable approximation of the system should be achieved by utilizing the

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best estimates of model degree and all the unknown variables of operation. Considerable computation time must be also spent for parameter estimation procedure [4].

For process control applications, proposed system models were usually written in discrete-time domain. The input and output variables were sampled to identify process parameters, although the process is implemented in continuous-time nature, and represented by differential equations. Svoronos et al., (1981) reported a bilinear model based upon minimum-variance self-tuning rule. Their model effectiveness was examined by using the simulations. Hapoglu et al. (2001) utilized a Controlled Auto Regressive Integrated Moving Average (CARIMA) model and its parameters were identified with Bierman computation procedure in which data obtained by enforcing the system with a pseudo random binary sequence (PRBS). Akay et al. (2003) investigated parametric and non-parametric models which include the relationship between dissolved oxygen concentration and air flow rate in *S. cerevisiae* production medium. These models theoretical and experimental identification were realized. During process control application, the manipulated variable was inlet air flow rate. Controlled Auto Regressive Moving Average (CARMA) model parameters were evaluated by disturbing the system with various types of input signals and using Recursive Least Square (RLS) parameter estimation algorithm. The aeration step response was obtained to maintain the dynamic matrix and the non-parametric model [7].

The actual part of the system can be modelled with approximated structure and estimated parameters of it. During the control, the closed loop performance of the system highly depends on the real process and its model mismatch. Many models relate input-output in a non-linear and linear fashion, such as, Hammerstein type (Zhu and Seborg, 1994), Wiener type (Norquay et al., 1996), Non-linear state predictor (NSP) form (Wang et al., 2004), subspace-based linear type (Sotomayor et al.,2003), linear multivariable discrete-time model [12]. Akay et al. (2011) designed a batch bioprocess parametric model and optimized the model order with experimental transient behavior analysis. Experimental DO control of the medium was realized and compared with theoretical results [1].

In the present investigation, the models of Baker's yeast production in a batch process are obtained experimentally and theoretically. Various ARMAX type models are utilized and tested.

1.1. Discrete-time System Models

An ARMAX model in discrete-time domain for the single input single output system representation is utilized as following:

$$A(z^{-1})y(t) = z^{-k}B(z^{-1})u(t) + C(z^{-1})e(t) \tag{1}$$

Where k is system velocity lag, y(t) is the response of the system, u(t) represents the most effective input variable, A, B and C are polynomials.

$$A(z^{-1}) = 1 + a_1 z^{-1} + a_2 z^{-2} + \ldots + a_{na} z^{-na} \tag{2}$$

$$B(z^{-1}) = b_0 + b_1 z^{-1} + b_2 z^{-2} + \ldots + b_{nb} z^{-nb} \tag{3}$$

$$C(z^{-1}) = 1 + c_1 z^{-1} + c_2 z^{-2} + \ldots + c_{nc} z^{-nc} \tag{4}$$

By using matrix notation, the system model can be rearranged as below:

$$\mathrm{y(t)} = \mathrm{X^T(t)\theta(t)} + \varepsilon(t) \tag{5}$$

Where X and θ vectors consist of the data and parameters.

$$X^T(t)=[y(t-1),....,y(t-na),u(t-1),....,u(t-nb-1),1,e(t-1),....e(t-nc)] \quad (6)$$

$$\theta(t)=\lfloor a_1,a_2,...,a_{na},b_0,b_1,..,b_{nb},c_1,...,c_{nc}\rfloor \quad (7)$$

1.2. Recursive Least Square Identification

The predicted and measured system responses differences are evaluated and sum of the squares of these is minimized as parameter identification criteria. This system identification technique (Soderström and Stoica, 1998) describe error prediction (see Eq.8) by means of model in Eq.5.

$$\varepsilon(t+1)=y(t+1)-X^T(t+1)\theta(t) \quad (8)$$

Recursive evaluation of Eq. 9 and Eq. 10 are realized until the stopping criterion is satisfied.

$$P(t+1)=P(t)-\frac{P(t)X(t+1)X^T(t+1)P(t)}{1+X^T(t+1)P(t)X(t+1)} \quad (9)$$

$$\theta(t+1)=\theta(t)+P(t+1)X(t+1)\varepsilon(t+1) \quad (10)$$

In the cases studied, the model testing was achieved by using the integral of absolute error (IAE) and the integral square of error (ISE) criteria (see Eq. 11 and Eq. 12) and parameter estimation error norm (PEEN) criteria which is given in Eq. 13.

$$IAE=\sum_{t=1}^{N}\left|\varepsilon(t)\right| \quad (11)$$

$$ISE=\sum_{t=1}^{N}\left|\varepsilon(t)^2\right| \quad (12)$$

$$PEEN=\frac{\sum_{i=1}^{n}\left|y(t)-X^T(t)\theta(t-1)\right|}{\sum_{i=1}^{n}\left|y(t)\right|}*100 \quad (13)$$

2. Materials and Methods

The Northern Regional Research Centre, ARS Culture Collection (Peoria, IL, USA) microorganism NRRL-Y-567 was used. The growth medium and scaling details are same as the previous works [1, 7].

A 2 L jacketed bioreactor was used and equipped with the following: an oxygen sensor, a pH sensor, a thermocouple, a 4 bladed turbine type impeller, an immersed heater, air supplier, a pump for cooling water, a circulator, a V/I converter, a rotameter, an I/P transducer, a microbiological filter and an on-line computer control system. Experiments were carried out at the optimum growth conditions of temperature 32°C and pH 5. Agitation rate was maintained at 600 rpm and cooling water was passed through the jacket at 21°C. Foam

formation was prevented by adding antifoam bioreactor at certain time intervals and microorganism growth was observed by using UV spectrophotometer analyses. An on-line computer with an I/O module receives DO, pH and temperature signals at every sampling instants. The on-line computer with VISIDAQ data acquisition and programming package in which cooling water flow rate was adjusted continuously in the jacket was used. The model identification was achieved in MATLAB.

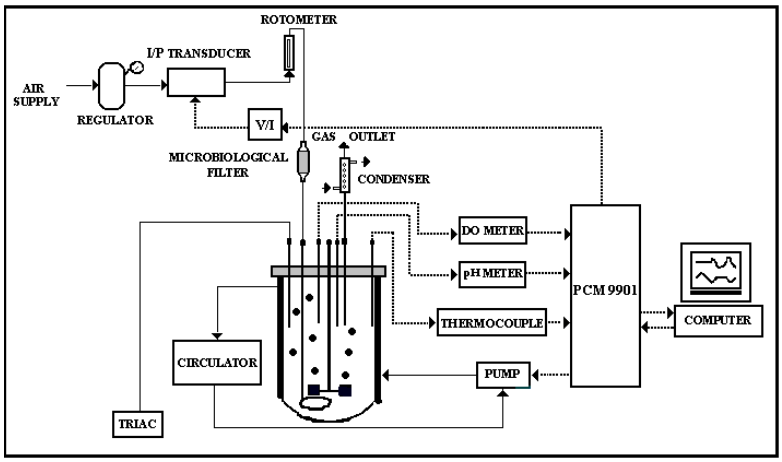

Figure 1. Experimental system

3. Results and Discussion

The discrete-time modelling is very user friendly and the estimation algorithms can be easily implemented utilizing MATLAB. In addition the discrete-time noise processes can be handled in the on-line bioprocess. For bioprocesses, if physics is not well understood although some knowledge about processes is always available, a black-box approach can be utilized. This will lead to some iterative procedure. Then further refinements can be achieved using system identification methods.

The system model in discrete-time domain, ARMAX simulates the system transient behavior for control purposes. The model Eq. 1 relates cooling water flow rate and bioreactor temperature. For using experimental and theoretical square wave input and ternary wave input signals for identification, the bioprocess open loop response is obtained. The periodic square input change is applied as 25-35 magnitude of pump signal. The ternary input change with three values of 25-35-50 also is given as periodic pseudo disturbance. In the face of different periodical effects, temperature transients of bioprocess medium were examined theoretically and experimentally (Figures 2, 3, 4 and 5).

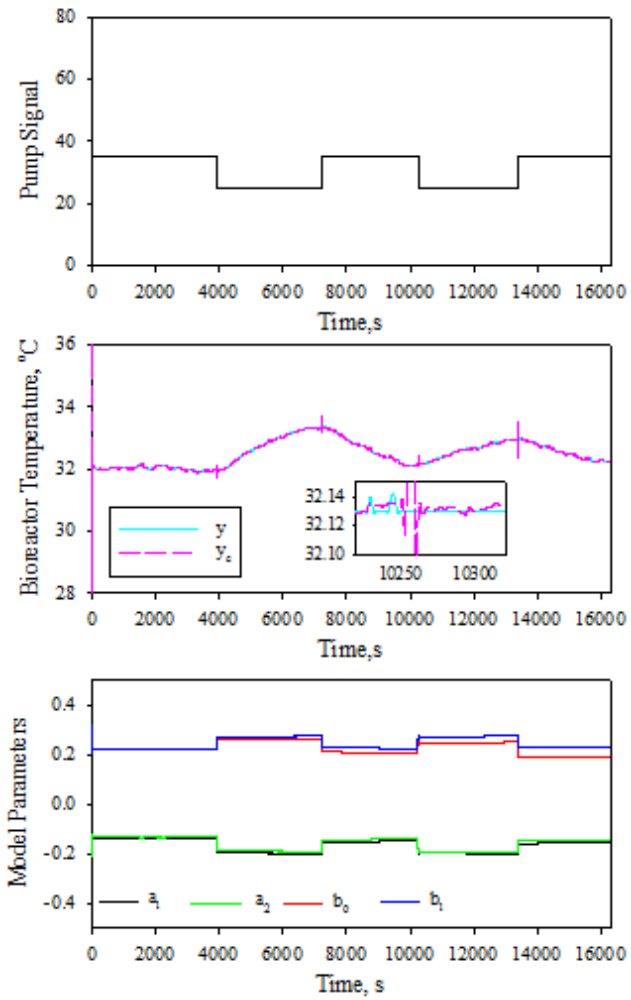

Figure 2. Identification of second order model with square wave input (experimental)

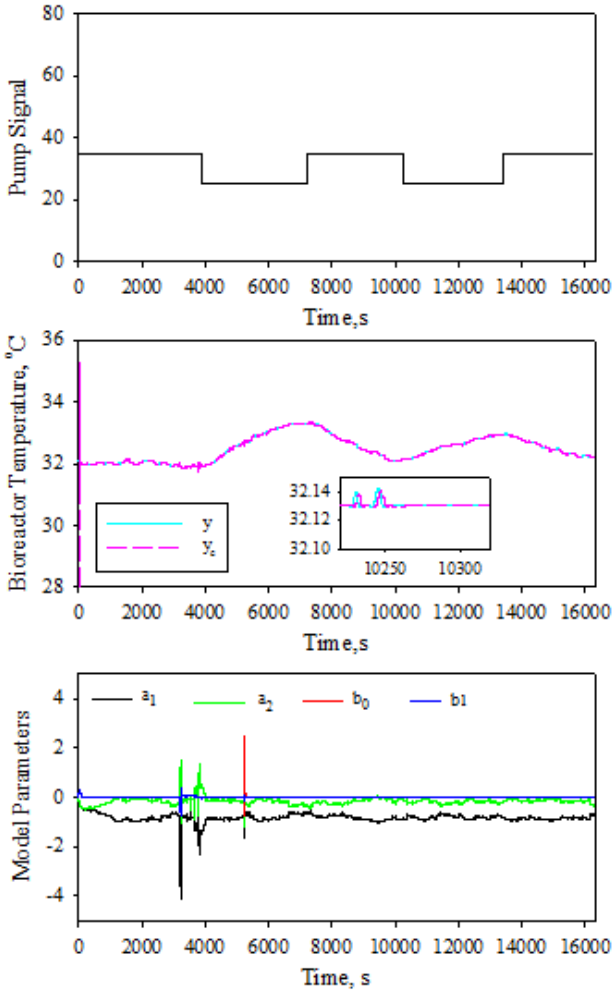

Figure 3. Identification of second order model with square wave input (theoretical)

A RLS technique was utilized in MATLAB package for estimation of various order ARMAX model parameters. These models and theirs estimated parameters are shown in Table 1. Performance of the second order system models were illustrated in the face of square and ternary external force dynamic behavior in Figure 2, 3 and 4.

Different model order can be viable but over-parameterization cause problems. The third order model for this study can identify the transient behavior of bioprocess medium which is shown in Figure 5.

By means of the recursive least square method, ARMAX model parameter changes were found in the face of the certain pump signal disturbance. The calculated values of ARMAX model parameters are shown in Table 1.

Table 1. Model identification results in the face of the ternary disturbance

ARMAX model	Evaluation with experimental data	Evaluation with theoretical data
$y(t)=b_0u(t-1)+b_1u(t-2)$ $-a_1y(t-1)-a_2y(t-2)$	a_1=-0.142 b_0= 0.183 a_2=-0.164 b_1=0.228	a_1=-0.886 b_0=3.26x10^{-7} a_2=-0.114 b_1=-8.27x10^{-5}
$y(t)=b_0u(t-1)+b_1u(t-2)+b_2u(t-3)$ $-a_1y(t-1)-a_2y(t-2)-a_3y(t-3)$	a_1= -0.187 b_0= 0.186 a_2= -0.135 b_1= 0.214 a_3= -0.185 b_2= 0.242	a_1=-0.893 b_0=-8.97x10^{-7} a_2=-0.171 b_1=-2.47x10^{-4} a_3=0.064 b_2=1.69x10^{-4}

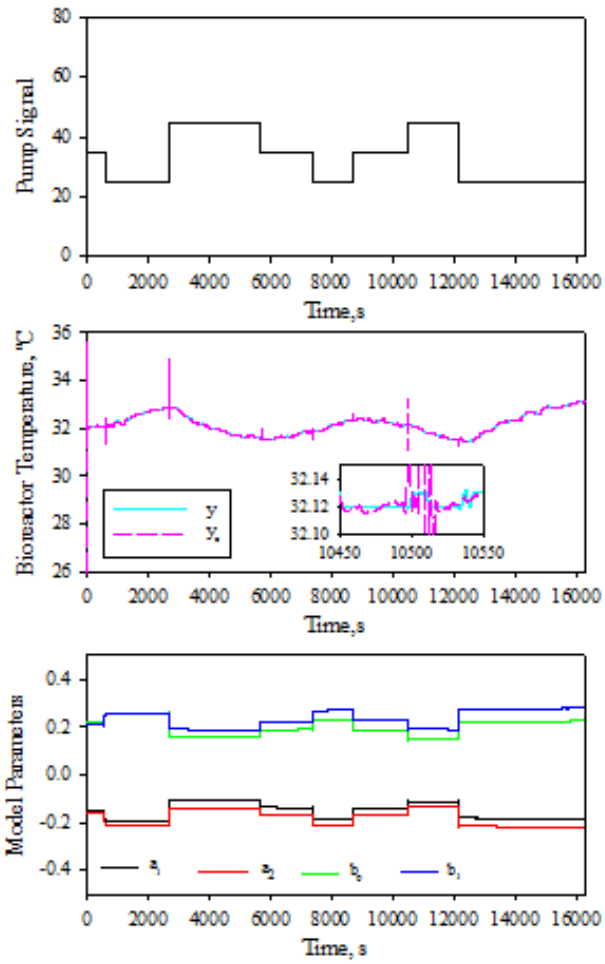

Figure 4. Identification of second order model with ternary wave input (experimental)

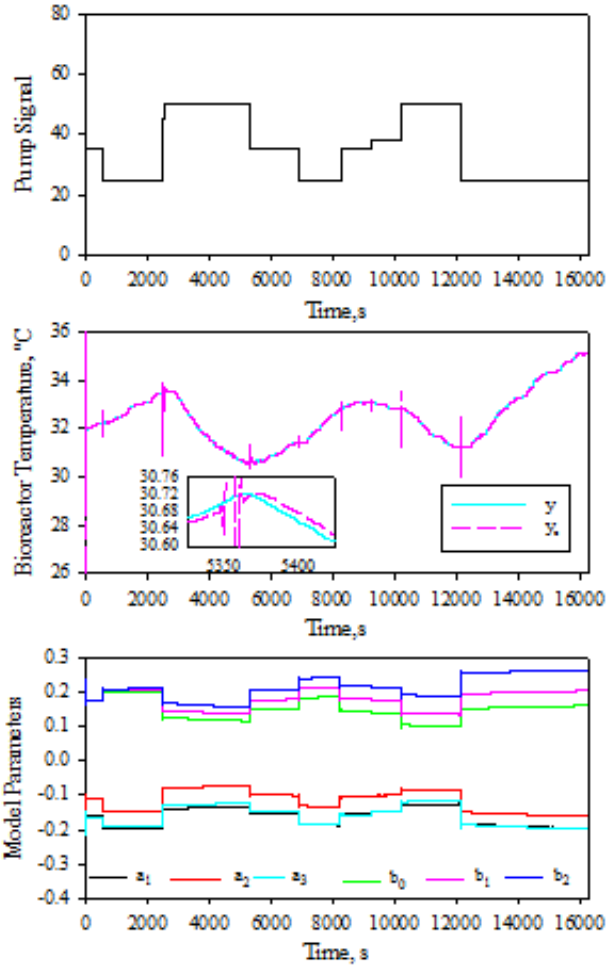

Figure 5. Identification of third order model with ternary wave input (experimental)

4. Conclusion

The identified models given in Table 2 can be utilized in discrete time advanced control algorithm. The various order ARMAX models are tested with evaluation of IAE, ISE and PEEN criteria.

As the model order increases to the true value, the IAE, ISE and PEEN values will decrease. Then a better fit can be obtained by using theoretical data obtained from bioprocess simulation program. In this theoretical study, use of ternary input signal type disturbance

gives better results than square input signal application to obtain system response in second order model identification according to three performance criteria mentioned, (see Table 2). It is noted that considerable experimental errors occur in bioreactor temperature response compare to simulation result as it is expected in experimental cases studied (see Figure 4 and Figure 5). These errors have high impact on three performance criteria calculated in Table 2. Thus, in experimental cases, second and third order model performance should be compared by using estimated and real time bioreactor temperature changes with time in Figure 4 and Figure 5.

Table 2. Performance criteria for ARMAX model with two different orders.

Input Signal Type	Model Order	EXPERIMENTAL			THEORETICAL		
		IAE	ISE	PEEN	IAE	ISE	PEEN
Square	Second	23.672	1.3876	0.0091	20.995	0.3173	0.0081
Ternary	Second	30.102	8.977	0.0112	18.865	0.133	0.0073
	Third	94.410	18.497	0.0361	12.140	0.125	0.0047

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Quantification of total phenols, flavonoides and tannins from Ziziphus jujuba (mill.) and Ziziphus lotus (l.) (Desf). Leaf extracts and their effects on antioxidant and antibacterial activities

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Abstract: This work was performed to determine the biochemical composition, antioxidant and antimicrobial activities of leaf extracts collected from four different provenances: Mahdia and Mahres (*Ziziphus jujuba*); Kairouan and Rouhia (*Ziziphus lotus*). Total phenols, flavonoids, tannins contents and antioxidant activity were evaluated using the Folin ciocalteux, Aluminum trichloride, vanillin and scavenging activity on 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radicals methods, respectively. The antimicrobial activity was evaluated against three bacterial strains (*Escherichia coli*, *Staphylococcus aureus* and *klebsiella pneumoniea*) and three fungal strains (*Fusarium culmorum*, *Fusarium solani* and *Botrytis cinerea*), according to well Agar diffusion method. Total phenols and flavonoids were present at levels of 21.98 mg GAE /g DW and 7.80 mg ER/g DW; respectively in *Ziziphus lotus*. These levels did not exceeded 13.70 mg GAE /g DW and 6.73 mg ER/g DW for *Ziziphus jujuba*. The tannin contents were present in equal levels (7.9 mg EC/g DW) in two species. The high antioxidant activity (0.01 µg/ml) was noted in Rouhia provenance. The *Ziziphus lotus* leaf extracts showed promising efficiency against all tested microorganisms with a zone of inhibition ranging between 22 and 23.5 mm. This study could validate the medicinal potential of *Ziziphus* specie and explain why tunisian people traditionally use it in medicine to treat several pathologies. *Ziziphus* leaf extracts may be suggested in foods and pharmaceutical industries. Leaf extracts proved also to be effective against tested microorganisms. So, an adequate toxicological study must be carried out to verify the possibility of using these plants for fighting microorganisms.

Keywords: Baker's Yeast, System Identification, *Saccharomyces cerevisiae* as a second metabolite source

1. Introduction

Nowadays, there is an upsurge of interest on medicinal plants that have attracted more and more attention due to their therapeutic properties and pharmacological activity [1]. Polyphenols were one class of secondary metabolites that were used in plant adaptation against unfavorable conditions (salinity drought, temperature…), but also in organism prevention

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against some illness such as cancer, inflammatory and cardiovascular disease [2]. Flavonoids, known as vitamin P, were mostly used in plants to produce yellow and other pigments, which play an important role in the colors of plants.

The jujube species, a medicinal tree menaced, were present, in Tunisia, in three species: *Ziziphus lotus* (l.), *Ziziphus jujuba* (mill.) and *Ziziphus spina-christi* (l.). *Z. jujuba* was widely studied for its therapeutic and alimentary uses [2-4]. In fact, according to these authors, tunisian *Z. jujuba* extracts (leaves, seeds or pulps) were rich on fatty acids (linolenic, palmitic, oleic, linoleic acids), sterols (sitosterol, stigmasterol…) and flavonoïds (rutin and apigenin). This richness improved the use of these organs in cosmetics and in pharmacology. *Zizyphus spina-christi* and *Ziziphus lotus* had been used in folk medicine as a demulcent, depurative, anodyne, emollient, stomachic, These characteristics make those species with valuable multi-purpose shrubs for semi-arid to arid ecological areas [2, 5].

In order to validate the medicinal potential of this specie and to explain why tunisian people traditionally use it to treat several pathologies, this study was carried out to evaluate the phytochemical constituents and antioxidant potential of the leaf extracts of four *Ziziphus* provenances: Mahres and Kairouan (*Z. lotus*), Rouhia and Mahdia (Z. *jujuba*).

2. MATERIAL AND METHODS

2.1. Plant material

Leaves and were sampled in summer 2014. The identification of the plant material was done by Professor Mohamed Boussaid and a voucher specimen of the plant was deposited at the Herbarium of INRGREF (Tunisia). The leaves were dried at room temperature during a half month in a dry and airy environment. Dried leaves were grounded by a mill equipped with a grid whose holes were 1.00 mm in diameter and stocked in plastic bags in the dark until chemical analysis. Leaf powders (1 g) were submitted to maceration with 10 ml of pure methanol for 30 min. The extracts, filtered through Whatman N°1 filter paper, were pooled and concentrated under vacuum.

2.2. Chemical Reagents

Folin-ciocalteu, phenol, DPPH, gallic acid, catechin sodium carbonates, hydrochloric acid and methanol were purchased from Sigma-Aldrich (St. Louis, MO). All solvents and reagents used were of the highest purity.

2.3. Total phenol and flavonoid contents

The total phenolic content was estimated flowing the Folin-Ciocalteu method as described by Singleton et al., (1965) with slight modifications. From each sample, 0.5 ml of methanolic solution was added to 2.5 ml of Folin- Ciocalteu reagent and 2 ml of sodium carbonate (75 g/l) solution. The reading of the absorbance was done at 765 nm using a Shimadzu 1600-UV spectrophotometer after incubation during 30 min. The same procedure was repeated for gallic acid used as standard. Total phenols of each fraction, expressed into mg GAE/g DW, were measured using the regression equation of a calibration curve $y = 0.0114x + 0.518$, $R^2 = 0.9932$.

The total flavonoid contents were assayed using the Aluminum trichloride method (Earp et al. 1981). 1 ml of $AlCl_3$ was added to 1 ml of plant extract. The volume was adjusted to 25 ml with methanol and thoroughly mixed. The absorption was measured after 40 min by a Shimadzu UV-1600 (Tokyo, Japon) spectrophotometer at 420 nm. Flavonoid contents were expressed as mg quercetin equivalent /g DW. The calibration curve range was 0-50 µg/mL ($R^2 = 0.981$). All samples were analyzed in three replications.

2.4. Condensed tannin contents

Condensed tannins were determined by colorimetric analysis [7]. A mixture of vanillin (1 ml) and 4 ml of HCl were added to 200 µl of leaf extracts and incubated 20 min in obscurity.

Catechin was used as a standard (0- 1250 µg/ml). All measurements were performed in triplicate. After agitation, absorbance's were read at 500 nm using a Jenway 6100 spectrophotometer. The results were expressed as microgram catechin equivalent per gram dry weight (µg CE/g DW)

2.5. Antioxidant activity

The working solution was prepared by mixing the two stock solutions in equal proportions and allowing them to react for 12-16 hours. This solution was stored in a dark place at room temperature. Before use, the solution was diluted with ethanol to obtain absorbance between 0.800 and 1.000 nm. This solution was mixed with sample (2.5 to 50 µg mL^{-1}) or standard solutions. A control containing methanol and DDPH solution was also realized. The absorbance was read at 734 nm after 30 min of incubation at 25°C.

In test tubes, 2.36 mg of DPPH previously dissolved in 100 ml of ethanol, mixed and incubated in obscurity. The leaf extracts were diluted with methanol (0.75 to 0.125 µg/ml) before use. The absorbance was read at 490 nm after 30 min incubation in dark place. Measurements for each experiment were done in triplicate. Antioxidant activity, expressed as inhibitory effect of the DPPH radical, was calculated using this formula:

$$\text{The percentage of inhibition} = [(A_0 - A_c) / A_0] \times 100$$

where A_0 was the absorbance of the control and A_c was the absorbance of the plant extract/ standard. The IC50 value, the concentration (in µg/ml) of the compound required to scavenge DPPH radical by 50, was determined graphically by the linear regression [8].

2.6. Antimicrobial activity

The test microorganisms used in this study were: *Escherichia coli* (ATCC10536), *Staphylococcus aureus* (ATCC 6538) and *klebsiella pneumoniea* (ATCC 10031). These species were generously provided by Laboratory of Ecology, Technology and Microbiology (INSAT). The *Fusarium culmorum*, *Fusarium solani* and *Botrytis cinerea* were obtained from the culture collection of the Tunisian National Institute of Agronomic Research (INRAT). The bacterial and fungal stock cultures adjusted to suspension of 10^6 cells were incubated for 24 hours at 37°C on potato dextrose agar (PDA) medium and were refrigerated at 4°C. Antibacterial tests were evaluated using well Agar diffusion method under strict aseptic conditions. So 100µl of suspension had been placed in 5 ml of melted cooled test agar [9, 10]. It was inoculated by flooding on Petri dishes containing agar culture medium BTCS. After agar solidification, three wells (10 mm in diameter) were bored using a sterile cork borer. Three concentrations of each leaf extracts (5; 60 and 100 mg/mL) were prepared and dripped directly into the first, the 2nd and 3rd well, respectively with micropipette. Sterilized distilled water was used as a negative control which was introduced into the 4th well. After 24h of incubation at 37°C, the diameter of inhibition zone surrounding each well was measured.

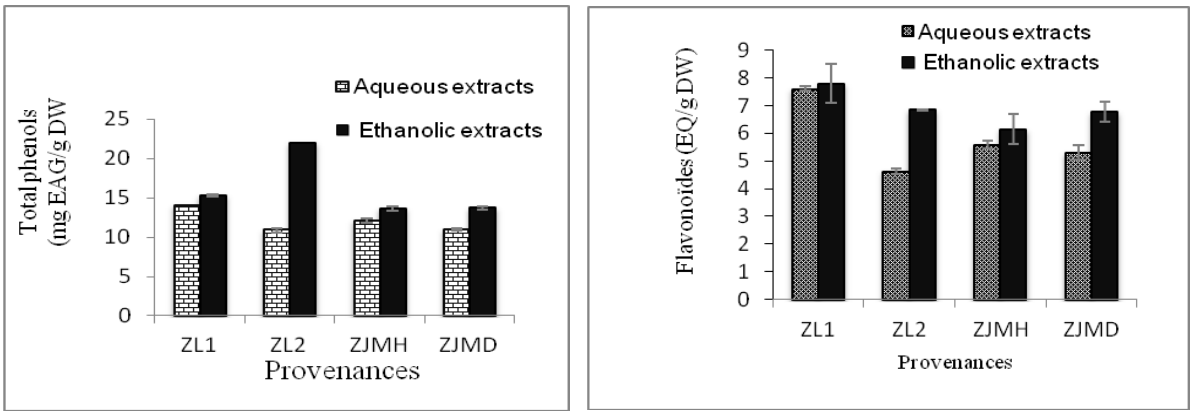
2.7. Statistical analysis

Results were statistically evaluated using STATISTICA. Data from three samples was reported as means ± standard deviation. Differences were tested with the ANOVA procedure using the Duncan test with a significance level of $p < 0.05$.

3. RESULTS AND DISCUSSION

3.1. Total phenol and flavonoid contents

The results indicated that 1 g DW of *Ziziphus* leaves contained between 13.62 and 21.98 mg GAE of phenolic contents. The $EEZL_2$ was the richest one. The methanol was found to be the efficient solvent to extract *Ziziphus* leaves. This idea was also confirmed by Medini et al. (2014). The relative amounts of phenols in *Z. jujuba* leaves found in this study were greater more important than those done (6.04 mg EAG/g DW) by Elaloui et al. (2016). This variability could be explained by many factors including the origin, the period of harvest, the age and the stage of plant development. Other environmental factors (temperature, altitude, sunshine, animal aggression and diseases) could also influenced this variability. The physiological stage could also the polyphenol contents and biological activity [12].



a **b**

Figure 1. Total phenolic (a) and flavonoid (b) contents of four provenances of *Ziziphus* leaf extracts, **ZL1:** *Z. lotus* (Kairouan), **ZL2:** *Z. lotus* (Rouhia), **ZJMH:** *Z. jujuba* (Mahres), **ZJMD:** *Z. jujuba* (Mahdia).

1. The data are the mean values of three measurements ± SD (standard deviation)
2. The confidence intervals were calculated at the threshold of 5%.

As noted in figure, the total flavonoids contents varied between study sites and provenances of collection. It ranged between 5.30 - 6.73 and 4.63 - 7.80 for *Z. Lotus* and *Z. jujuba* respectively. It has been proved that the levels of total phenols and flavonoids were high when the environment conditions of the plant were not adequate. In this case, the plant promoted the synthesis of secondary metabolites in order to adapt and survive [13].

3.2. Condensed tannin levels

Results proved that condensed tannin levels oscilled between 4.38 and 6 mg EC/g DW for aqueous extracts (figure 2). Level were higher than ethanolic extracts that attempt levels between 7.05 and 8 mg EC/g DW.

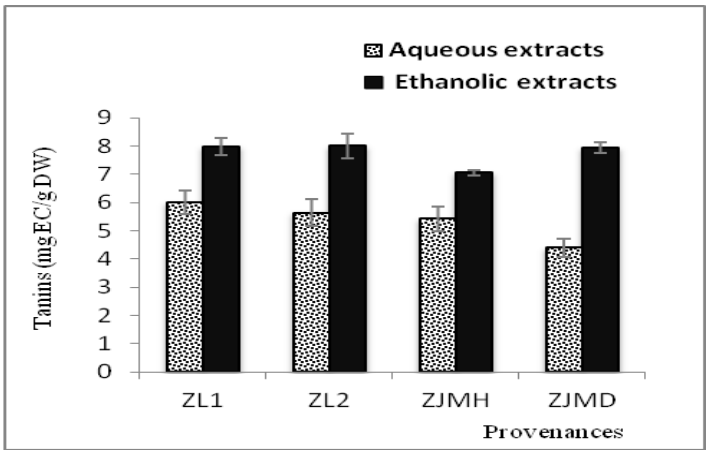


Figure 2. Condensed tannin contents of four provenances of *Ziziphus* leaf extracts, **ZL1:** *Z. lotus* (Kairouan), **ZL2:** *Z. lotus* (Rouhia), **ZJMH:** *Z. jujuba* (Mahres), **ZJMD:** *Z. jujuba* (Mahdia).

1. The data are the mean values of three measurements ± SD (standard deviation)
2. The confidence intervals were calculated at the threshold of 5%.

As shown in this figure, the effect of solvent in tannin solubility showed the same classification as phenolic and flavonoid contents.

3.3. Antioxidant activity

The leaf of *Z. lotus* of Rouhia provenance registered the highest activity (0.01 mg/ml). The EAZJMD showed high antioxidant activity (CI_{50} =0.61 mg/ml).

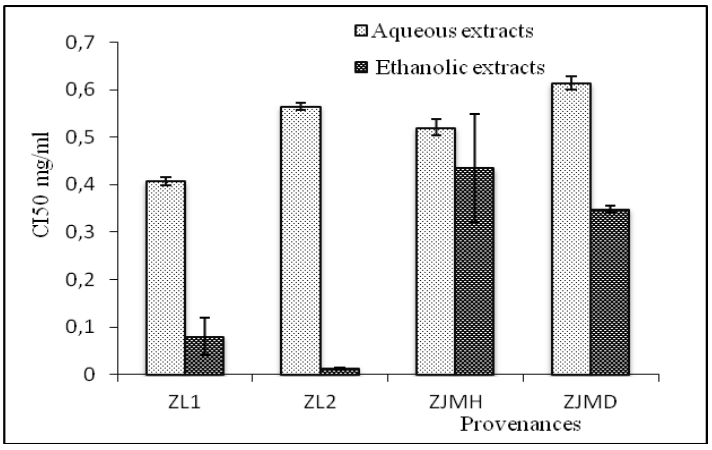


Figure 3. Inhibitrice concentration of four provenances of *Ziziphus* leaf extracts, **ZL1:** *Z. lotus* (Kairouan), **ZL2:** *Z. lotus* (Rouhia), **ZJMH:** *Z. jujuba* (Mahres), **ZJMD:** *Z. jujuba* (Mahdia

1. The data are the mean values of three measurements ± SD (standard deviation)
2. The confidence intervals were calculated at the threshold of 5%.

In fact, a linear correlation ($R^2 = 0.795$) had been observed between the CI_{50} of Z. leaf extracts and their phenolic levels (Figure 4). In fact, phenolic levels and antioxidant activity varied in the same way. Our results corroborate by Al-Jassabi and Abdullah et al. (2013). The antioxidant capacity levels were maximum at the flowering stage [11].

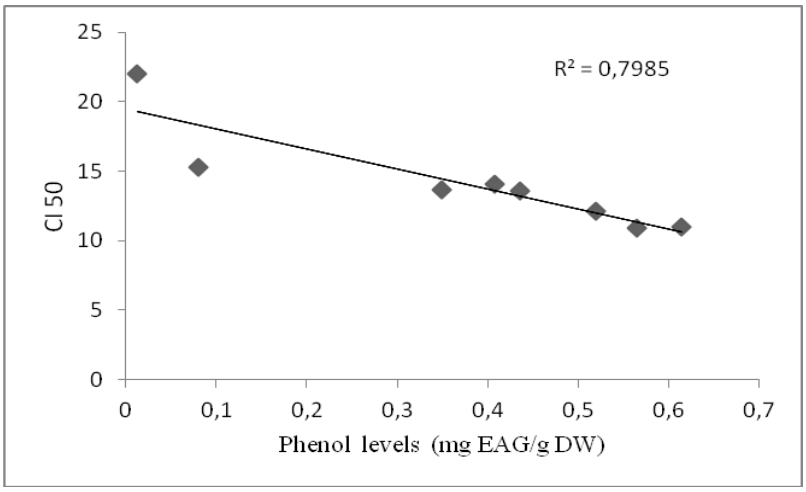
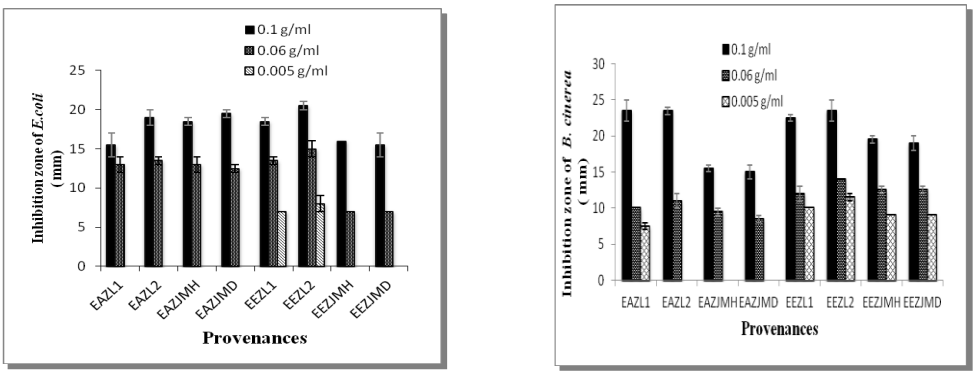


Figure 4. Linear correlation ($R^2 = 0.798$) between phenols levels and antioxydant activity of *Ziziphus lotus* and *Ziziphus jujuba* leaf extracts

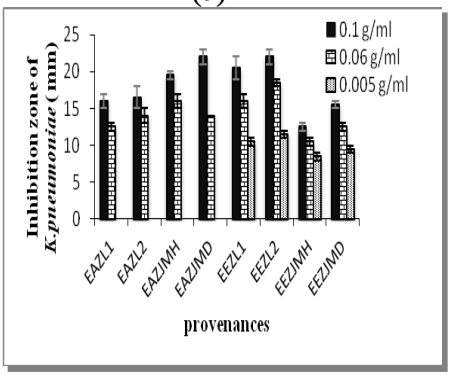
3.4. Comparative study on the effects of *Ziziphus* extracts on growth of bacterial pathogens

3.4.1. Antibacterial activity

Both aquoeus or ethanolic *Ziziphus* leaf extracts had an antibacterial activity on *E. coli* used at 0.1 et 0.06 g/ml concentrations (Figure 5a).



(a) (b)



(c)

Figure 5. Antibacterial activity of *Ziziphus* leaf extracts against *E. coli* (a), *S. aureus* (b) and *K. pneumoniae* (c) croissance's.

EEZL1: ethanolic extracts of *Z. lotus* (Kairouan), **EEZL2:** ethanolic extracts of *Z. lotus* (Rouhia) **EEZJMH:** ethanolic extracts of *Z. jujuba* (Mahres); **EEZJMD:** ethanolic extracts of *Z. jujuba* (Mahdia) **EAZL1:** aqueous extract of *Z. lotus* (Kairouan), **EAZL2:** aqueous extract *Z. lotus* (Rouhia) **EAZJMH:** aqueous extract *Z. jujuba* (Mahres), **EAZJMD:** aqueous extract *Z. jujuba* (Mahdia).

The ethanolic leaf extracts of *Z. lotus* collected from Rouhia provenance (EEZL2) had shown the highest antibacterial activity for *E. coli* with Zone of inhibition more than 20 mm (0.1 g/ml) and 15 mm (0.06 g/ml), while the 0.05 g/ml did not possess significant antibacterial activity against *E. coli* exception done for EEZL. The same tendency was observed for *S. aureus*, with inhibition zone diameter ranged between 21.5 mm (EEZL2) and 23 mm (EAZJMD) used at 0.1 g/ml concentration.

This zone of inhibition attempted the diameter of 22 mm for *K. pneumoniae* treated by EEZL2 and EAZJMD at the concentration of 0.1 g/ml. These results could confirm that the EEZL2 extract appeared to be efficient against bacterial strains. This could justify its richness on secondary metabolites especially on tannins contents. For *S. aureus*, the leaf extracts had greater inhibitor zone (23 mm) than oil fruits (11 mm). This idea was confirmed by Bukar et al. (2015). This could be explained by the richness of leaf extracts on oxygenated compounds compared to oil fruits [15].

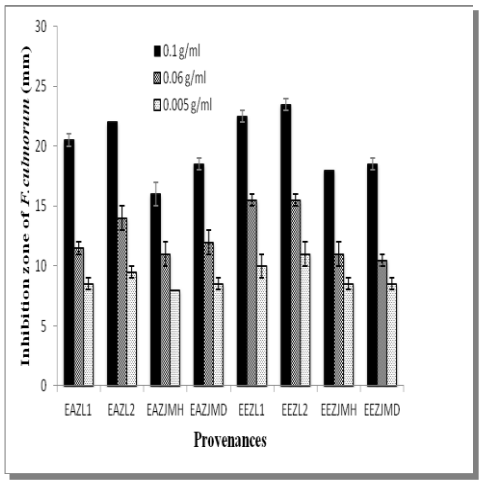
Our results were similar to these obtained by Bashir et al. (2011) who confirmed the antifungal activity of *Z. jujuba* methanolic leaf extracts. The essential oil of *Ziziphus spina-christi* fruits have been reported to have a zone of inhibition against *Escherichia coli* (10 mm). This idea was confirmed by Bukar et al. (2015). Ethanol extracts were found to be the most effective, while aqueous extracts had the moderate activity. This was idea was also identified by Medini et al. (2014).

3.4.2. Antifungal activity

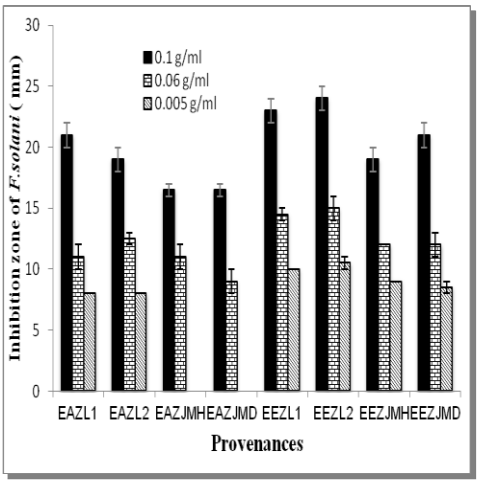
The concentration 0.1 g/ml of EEZL1 and EAZL2 showed the highest antifungal activity with the zones of inhibition of 23.5 mm, 22.5 mm and 22 mm, respectively (Figure 6).

The *F. Solani* was inhibited by all *Ziziphus* extracts used at 0.1 et 0.06 g/ml concentrations, while the EAZJMH and EAZJMD had not any antifungal activity if they were used at 0.005 g/ml concentration. The zone of inhibition varied from 16.5 to 24 mm; 9 to 15 mm and 8 to 10.5 mm for 0.1; 0.06 and 0.005 g/ml concentrations, respectively. This could be explained by the richness of theses organs on secondary metabolite especially phenols which had known by its antimicrobial activities [17]. This activity was related to the high rate of not only by the monoterpene hydrocarbons [15], but also by the tannins which bind cell walls of ruminal bacteria [1].

The aqueous and ethanolic leaf extracts of two studied *Ziziphus*, used at 0.1 g/ml concentration, were found the most efficient against *B. cinerea* with inhibition zones ranging from 15 mm (EAZJMD) to 23.5 mm (EEZL2, EAZL1 et EAZL2). Ethanolic leaf extracts of *Z. jujuba* showed a high antifungal activity (25 mm) on *Trichophyton rubrum* compared to aqueous extracts (19 mm) used at concentration of 10 mg/ml [18].



(a)



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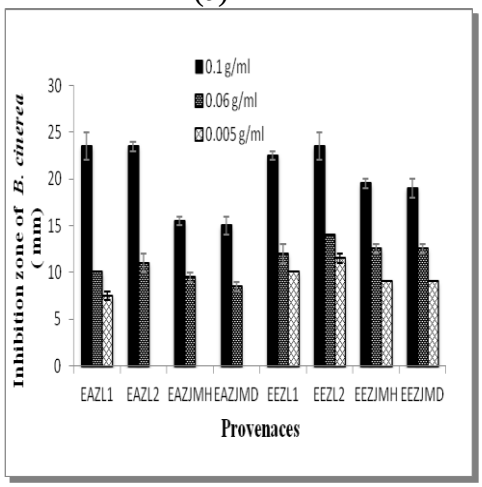

(c)

Figure 6. Antifungal activity of Ziziphus jujuba et Ziziphus lotus leaf extracts on F. culmorum, (a) F. Solani (b) and B. cinerea (c) crouissance's

EEZL1: ethanolic extracts of *Z. lotus* (Kairouan), **EEZL2:** ethanolic extracts of *Z. lotus* (Rouhia) **EEZJMH :** ethanolic extracts of *Z. jujuba* (Mahres) **EEZJMD:** ethanolic extracts of *Z. jujuba* (Mahdia) **EAZL1:** aqueous extract of *Z. lotus* (Kairouan), **EAZL2:** aqueous extract *Z. lotus* (Rouhia) **EAZJMH:** aqueous extract *Z. jujuba* (Mahres), **EAZJMD:** aqueous extract *Z. jujuba* (Mahdia).

4. CONCLUSION

These *in vitro* results were a first step in the search for biologically active substances of natural origin. So, others studies of the effects of aqueous and ethanol extracts of *Ziziphus* leaves would be desirable to better promote these products instead of chemicals with high degrees of toxicity to preserve the environment and improve agricultural production. An adequate toxicological study must be also carried out to verify the possibility of using these plants for fighting microorganisms.

Acknowledgements
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Biodiversity of Bacteria Isolated from Different Soils

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Abstract: The aim of this study was to determine the biodiversity of PHB producing bacteria isolated from soils where fruit and vegetable are cultivated (onion, grape, olive, mulberry and plum) in Aydın providence. Morphological, cultural, biochemical, and molecular methods were used for bacteria identification. These isolated bacteria were identified by 16S rRNA sequencing and using BLAST. The following bacteria *Bacillus thuringiensis* (6), B*acillus cereus* (8), *Bacillus anthrachis* (1), *Bacillus circulans* (1), *Bacillus weihenstephanensis* (1), *Pseudomonas putida* (1), *Azotobacter chroococcum* (1), *Brevibacterium frigoritolerans* (1), *Burkholderia sp.* (1), *Staphylococcus epidermidis* (1), *Streptomyces exfoliatus* (1), *Variovorax paradoxus* (1) were found. The Maximum Likelihood method was used to produce a molecular phylogenetic analysis and a phylogenetic tree was constructed. These bacteria can produce polyhydroxybutyrate (PHB) which is an organic polymer with commercial potential as a biodegradable thermoplastic. PHB can be used instead of petrol derivated non-degradable plastics. For this reason, PHB producing microorganisms are substantial in industry.

Keywords: PHB; bacteria; 16S rRNA; biodiversity; soil

1. Introduction

Plastics products are indispensable from automobiles to medicine in our lives. We use plastics and synthetic polymers produced from petrochemicals. As synthetic plastics are persistent in the environment, plastic materials are a substantial source of environmental pollution and damage natural habitats. Several 100,000 tons of plastic are disposing of marine environments every year and accumulate in certain oceanic regions. In this case, approximately 1.000.000 sea creatures are dies every year [1]. With the advancement of biotechnological research, the production of environmentally friendly plastic has been on the rise. These plastics are produced by microorganisms. Polyhydroxyalkanoates (PHA) are bacterial plastics. PHA's are synthesized by many prokaryotic and eukaryotic microorganisms in the appropriate growth conditions. Polyhydroxybutyrates (PHB) is the most significant member of polyhydroxyalkanoates. PHB was first described by Lemoigne in *Bacillus megaterium* [2]. PHB is an energy and carbon reserve material in microorganisms. PHB is accumulated as intracellular granules by bacteria such as *Bacillus spp*., *Azotobacter spp*., *Pseudomonas spp*. PHB is synthesized (when nutritional elements such as N, P, S, O, or Mg are deficient in the presence excess carbon source [3]. PHB granules are insoluble in water, relatively resistant to

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hydrolytic degradation, biocompatible and nontoxic. It has good oxygen permeability and ultra-violet resistance. It also has a Melting point of 175ºC and glass transition temperature 2ºC [4]. PHB has many obvious applications in the manufacture of packaging containers, bottles, wrapping films, bags and the like. Some important medical uses are serving a surgical pins, stables, wound dressing, bone replacements and plates, and biodegradable carriers for the long-term release of medicines [5].

Isolation and identification of PHB producing bacteria from environments such as soil, sea water, aerobic and anaerobic sewage is important since these bacteria (*Bacillus sp*, *Alcaligenes sp*., *Pseudomonas sp*., *Streptomyces sp*., *Azotobacter sp*., *Burkholderia sp*. *Brevibacterium sp*.) are used in industry [6]. İdentification of PHB producing bacteria using classical taxonomic methods is unreliable and time consuming. As a result, most researchers use molecular methods as 16s rRNA-PCR, ERIC -PCR, REP-PCR for bacterial identification. Sujatha et al. [7] identified *Pseudomona ssp*. LDC-5 using 16S rDNA gene sequence and researched PHB production. López-Cortés et al. [8] isolated PHB-producing bacteria in a polluted marine microbial mat and identified using 16S rDNA gene sequence. As result, they found *Bacillus*, *Staphylococcus*, *Paracoccus*, *Micrococcus*, *Rhodococcus* and *Methylobacterium*. Mauti et al. [9] carried out molecular identification of soil bacteria by 16S rDNA sequence and identified as *Burkholderia cenocepacia*.

In this study, the biodiversity of PHB producing bacteria isolated from soils where fruit and vegetable are cultivated (onion, grape, olive, mulberry and plum) in Aydın providence was investigated.

2. Experiment

2.1. Collection of samples soils

Various soil samples were collected from nine different gardens and lawn soil of Aydin providence in Turkey. The garden soil samples used in this study were collected from 0-15 cm layer.

2.2. Isolation of microorganisms

One gram of each sample was suspended in 99 mL of sterile distilled water and shaken. The samples were heated at 80ºC for 5 min in water bath for *Bacillus sp.* isolation from soil. Mannitol Agar Medium (10g mannitol, 0.5 g K_2HPO_4, 0.2 g $MgSO_4.7H_2O$, 0.2 g NaCl, 0.2 g $FeCl_3.6\ H_2O$, 0.005 g), Pseudomonas Selective Agar (Difco) and Nutrient Agar (Sigma) were used for isolation of *Azotobacter sp*., *Pseudomonas sp*. and *Bacillus sp*. respectively. Later, the liquid media were serially diluted in sterile 0.85 % physiological saline (NaCl) solution and the dilutions from 10^{-1} to 10^{-6} were plated on Agar Medium. Plates were incubated at 28-37 ºC for 24-48 h. After incubation different colony were isolated separately and stored in skim milk [10].

2.3. Identification of microorganisms

Morphological, cultural and biochemical identifications were made according to the Bergey's Manual of Systematic Bacteriology [11]. For molecular identification, DNA extraction was done using Green and Sambrook protocol [12]. DNA concentrations and purity was measured using a nanodrop spectrometer (Thermo Scientific). 16S rRNA PCR reactions were carried out at initial denaturation 95ºC 5min, denaturation 94ºC 40sec, annealing 50ºC 40 sec, extension 72ºC 40 sec with 35 cycles and final extension at 72ºC 10dk. Reagents used and their concentrations were 10X Taq Buffer, 0.5M dNTP mix, 10 pM from each primer, 7.5 mM $MgCl_2$ and 1U Taq polymerase with the final volume of 25 µl. PCR products were sent to the sequencing (GATC BioTech, Germany) after electrophoresis at 1.4% agarose jel at 90 V 40 min.

2.4. Phylogenetic analysis of isolates

Phylogenetic tree was constructed using the Maximum Likelihood method [13] .Using BLASTn software our sample sequences were referenced against sequences found in GENBank. Sequences were aligned with ClustalW program which is inside MEGA 7.0 software [14].

3. Results and Discussion

17 bacterial species were identified and the results were *Bacillus sp*., (1) *Pseudomonas sp*. (1), *Azotobacter sp*. (1), *Brevibacterium sp*. (1), *Burkholderia sp*., *Staphylococcus epidermidis*, (1) *Streptomyces exfoliates*, (1) *Variovorax paradoxus* (1) (Table 1).

Pseudomonas sp., *Azotobacter sp*., *Burkholderia sp*. and *Variovorax paradoxus* are Gr (-), rod shaped bacteria (Fig. 1a, b, c, d). *Bacillus sp*., *Brevibacterium sp*., *Streptomyces exfoliates* are Gr (+), rod shaped bacteria and *Staphylococcus epidermidis* is Gr (+), coc shaped bacteria (Fig. 2 a, b, c, d).

Table 1. Characterization and number of bacteria isolated from different soils.

Bacteria	Number of isolates	Characterizations
Bacillus sp.	17	Gr(+), rod shaped bacteria
Pseudomonas sp.	1	Gr(-), rod shaped bacteria
Azotobacter sp.	1	Gr(-), rod shaped bacteria
Brevibacterium sp.	1	Gr(+), rod shaped bacteria
Burkholderia sp.	1	Gr(-), rod shaped bacteria
Staphylococcus epidermidis	1	Gr(+), coc shaped bacteria
Streptomyces exfoliatus	1	Gr(+), rod shaped bacteria
Variovorax paradoxus	1	Gr(-), rod shaped bacteria

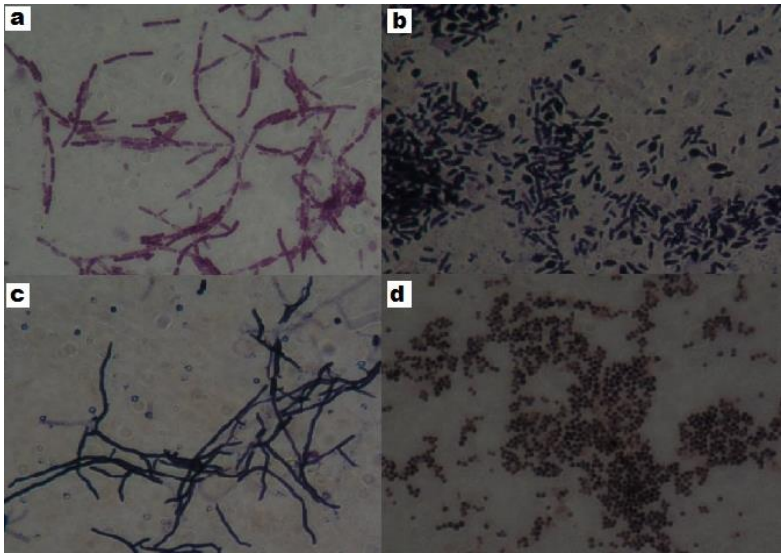

Figure 1. Image of bacteria under microscopic view on magnification x100.
a. *Bacillus sp*. ***b.*** *Brevibacterium sp*. ***c***. *Streptomyces exfoliates* ***d***. *Staphylococcus epidermidis*

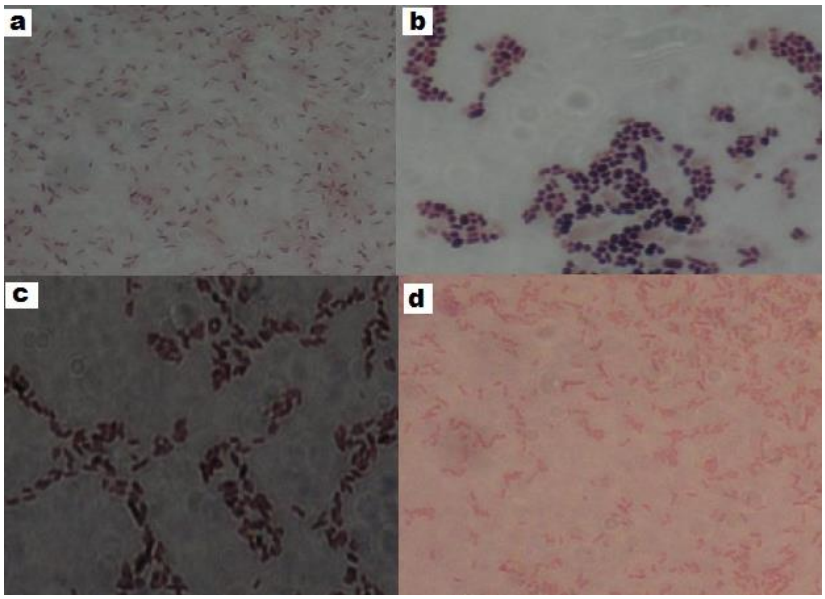

Figure 2. Microscopic view of bacteria magnification x100.
a. *Pseudomonas sp*. ***b.*** *Azotobacter sp*. ***c.*** *Burkholderia sp*. ***d.*** *Variovorax paradoxus*

PCR results of these samples were sent to GATC Biotech, Germany for sequencing. Amplification of the 16S rRNA gene showed that 61 isolates were PHB produced bacteria. Molecular identification was made by comparing sequence results with Gene bank using BLASTn software. The analysis of the soil samples of Aydın providences showed that there were twelve species with accession number (Table 2).

Table 2. Molecular identification of the species isolated from soils where fruit and vegetable are cultivated (onion, grape, olive, mulberry and plum) in Aydın providence.

Name of The Species	Name of Samples	Number of Strains	Accession No
Bacillus thuringiensis	Plum, Onion,Grape,Mulberry	6	KU179338.1 FJ981909.1 JN590251.1 KT714039.1 KJ784474.1 JQ685228.1
Bacillus cereus	Olive, Onion,Grape, Mulberry	8	KX941838.1 KX301062.1 EF185296.1 GQ365209.1 KF831393.1 KT922033.1 FJ763651.1 KU179339.1
Bacillus anthrachis	Olive	1	CP008846.1
Bacillus circulans	Olive	1	KT983982.1
Bacillus weihenstephanensis	Mulberry	1	KT363050.1
Pseudomonas putida	Plum	1	KU977141.1
Azotobacter chroococcum	Plum	1	KX108861.1
Brevibacterium frigoritolerans	Olive	1	HQ202870.1
Burkholderia sp.	Onion	1	JQ917954.1
Staphylococcus epidermidis	Onion	1	KX019832.1
Streptomyces exfoliatus	Plum	1	LN774329.1
Variovorax paradoxus	Onion	1	AB622222.1

A neighbour-joining phylogenetic tree was constructed by MEGA 7.0 software from a partial 16S rDNA sequence of the bacterial isolates obtained in this study with selected sequences downloaded from GenBank, shown in Fig. 3. The ClustalW program in MEGA 7.0 was used to align the sequences. These isolates found were *B. thuringiensis* (6), *B. cereus* (8), *B. anthrachis* (1), *B. circulans* (1), *B. weihenstephanensis* (1), *P. putida* (1), *A. chroococcum* (1), *Brevibacterium frigoritolerans* (1), *Burkholderia sp.* (1), *Staphylococcus epidermidis* (1), *Streptomyces exfoliatus* (1), *Variovorax paradoxus* (1) (Fig. 3).

Many researchers have isolated and used molecular methods such as 16s rRNA-PCR, ERIC-PCR, REP-PCR in identifying PHB producing bacteria from different soil samples.

Nubia et al. [15] isolated PHB produced bacteria from soil and identified the isolates by partially sequencing the 16SrRNA gene. Singh and Palmar [16] carried out the biodiversity of PHB produced bacteria and found two novel species as *Rahnella aquatilis* and *Stenotrophomonas maltophilia*. Aarthi and Ramana [17] isolated PHB producing bacteria from garden soil and characterized based on their 16S rRNA gene sequences. They identified species as *Bacillus mycoides* DFC1, *Bacillus cereus* DC1, *Bacillus cereus* DC2, *Bacillus cereus* DC3 and *Bacillus cereus* DC4.PHB-producing bacterial strains were isolated from Antarctic soils and characterizated as *Pseudomonas spp*. and *Janthinobacterium* spp. by 16S rRNA gene sequence analysis by Goh and Tan [18]. Reji et al. [19] executed molecular identification using 16S r- RNA sequencing and the organism was identified as *Bacillus cereus*. Dul'tseva et al. [20] isolated bacteria of the genus *Variovorax* from the *Thioploca* mats of Lake Baikal and identified as *V. paradoxus*, *V. soli*, *V. ginsengisoli*, and *V. boronicumulans*, *V. dokdonensis* using 16S rRNA gene nucleotide sequences. Tonouchi et al. [21] isolated *Brevibacterium yomogidense sp.* from a soil sample conditioner made from poultry manure.Panigrahi and Badveli [22] researched the isolation and screening of soil bacteria PHB production and they observed that red soil was able to produce maximum yield of PHB. Ciesielski et al.[23] carried out molecular identification of polyhydroxyalkanoates-producing bacteria isolated from enriched microbial community and identified *Bacillus sp*.,*Microbacterium sp*., *Citrobacter sp*., *Aeromonas sp*., *Caulobacter sp*., *Sphingomonas sp*.Mazinani et al. [24] isolated Azotobacter sp. from soil samples and identified as *A. chroococcum*, *A. beijerinckii* and *A.vinelandii* using16S r- RNA sequencing. Chandani et al. [25] isolated *Bacillus sp*. from soil samples in Municipal Waste Areas and the isolate was characterized as *Bacillus tequilensis* NCS-3 based on 16S rRNA gene sequence. It was examined that Bacillus tequilensis NCS-3 produces PHB in different carbon and nitrogen sources, pH and temperatures. Prakash et al. [26] isolated a bacterium from soil of coastal region of India and identified as *Burkholderia pseudomallei* using 16S rRNA gene amplification. Agrawal et al. [27] reported that twenty four isolates of *Pseudomomas putida* isolated from soil samples and observed phenotypic characterization of these isolates. Osman et al. [28] isolated *Microbacterium* from soil sample and carried out molecular characterization as the 16s rRNA gene Biradar et al. [29] researched PHB producing *Bacillus* species isolated from agricultural soil and characterized using 16S r-RNA sequencing. Hall et al. [30] isolated *Burkholderia* species from soils in the Southern United States and diversed as *B. cenocepacia*, *B. cepacia*, *B. contaminans*, *B. diffusa*, *B. metallica*, *B. seminalis*, *B. vietnamiensis*. Kıran et al. [31] studied producing PHB from bacteria isolated from contaminated soils. Isolates were identified as *Bacillus anthracis* (IBB) and *Bacillus subtilis* with 16S rRNA gene amplification and phylogenetic relationship. Hoseinabadi et al. [32] carried out using16S rRNA sequencing identification of poly β-Hydroxybutyrate over-producing bacteria and identified as *Bacillus coagulans*. Hassan et al. [33] isolated *Bacillus sp*. from Egypt and examined production of PHB by *Bacillus* sp. N-2.

The aim of this study was to isolate and identify, using 16S rRNA sequencing methods, the PHB producing bacteria from garden soil samples. According to these; it has been showed that morphological methods are not always adequate and confidential for identification of species. Thus, both morphological and molecular methods for identification of bacteria were used. It can be seen that, in recent years, molecular identification gained more importance.

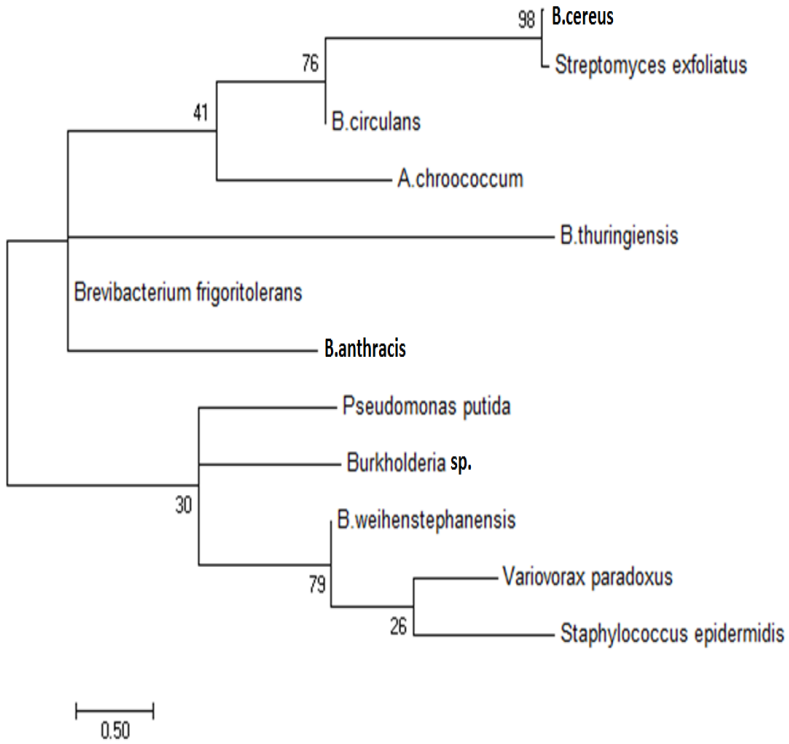

Figure 3. Molecular Phylogenetic analysis by Maximum Likelihood method.

Using the Tamura based model of the maximum likelihood method the evolutionary history was found and the as shown in the figure is the highest log likelihood. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree is drawn to scale, with branch lengths representıng the number of substitutions per site. The analysis involved 12 nucleotide sequences. Codon posıtıons were the usual trıplets wıth some non codıng nucleotıdes. All positions with less than 95% site coverage were eliminated. That is, fewer than 5% alignment gaps, missing data, and ambiguous bases were allowed at any position. There were a total of 166 positions in the final dataset. Evolutionary analyses were conducted in MEGA7.

4. Conclusion

In this study isolation of PHB producing bacteria from soils where fruit and vegetable are cultivated (onion, grape, olive, mulberry and plum) in Aydın providence were carried out. In addition, we obtained twelve various bacteria as *Bacillus thuringiensis*, B*acillus cereus, Bacillus anthrachis*, *Bacillus circulans*, *Bacillus weihenstephanensis*, *Pseudomonas putida*, *Azotobacter chroococcum*, *Brevibacterium frigoritolerans*, *Burkholderia sp.*, *Staphylococcus epidermidis*, *Streptomyces exfoliatus*, *Variovorax paradoxus* based on nucleotide homology and phylogenetic analysis. These bacteria can be used as PHB producers in industry.

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Review Article

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An Important Euroasian Genus: *Scutellaria* L.

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Abstract: *Scutellaria* is found on every continent except for Antarctica. Especially it is found in Eurasia which has rich areas with the most species. The objective of this study is to focus on the importance of this genus. *Scutellaria* species need to be set a value upon and evaluated. Because the genus *Scutellaria* is rich in flavonoids and diterpenoids and has a wide range of pharmacological effects such as antitumor, anti-angiogenesis, hepatoprotective, antioxidant, anticonvulsant, antibacterial, antiviral activities and cancer cell inhibition. Furthermore *Scutellaria* species have very beautiful blooms. Some countries such as the US and China achieved cultivation of this genus and they grow some species of *Scutellaria* as a crop production and ornamentals. But in Turkey, cultivation for crop production hasn't been worked on yet. *Scutellaria* species can be cultivated so as to gain economic and ecological benefits. Even though the wild populations of *Scutellaria* is widely distributed in the world, some of these species are becoming rare or threatened with population pressure, environmental pollution, and destruction of their natural habitat. Conservation by cultivation is an effective means for protecting genetic resources.

***Key words*:** Lamiaceae, *Scutellaria*, conservation, Turkey

1. INTRODUCTION

Scutellaria L. (skullcap) is a cosmopolitan genus of Lamiaceae with approximately 350 species [1-2]. This species is found mainly in temperate regions and tropical mountains. *Scutellaria* is found on every continent except for Antarctica. Especially it is found in Eurasia which has rich areas with the most species. *Scutellaria* is represented by 34 taxa of which 15 species are endemic to Turkey in the flora of Turkey [3].

"Skullcap" describes the shape of the calyx at the base of the flower. This structure called scutellum is formed from a fold in the upper calyx lip that stands erect and enlarges during fruiting [1].

Scutellaria have been used in herb spices, fragrances, traditional and folk medicines in different parts of the world for centuries. They are well known among people as powerful medicinal herbs which are mild relaxants that affect the neural and muscular-skeletal systems [4-11]. Nowadays, many of *Scutelaria* species have been studied in the fields of health,chemistry and phytochemistry, and their therapeutic activities have been tested, such as

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spasmolytic, antidiarrhea, antifungal, antipyretic, antioxidant, anticancer, anti-HIV, antibacterial, antiviral, anti-inflammatory, anticonvulsant [12-14].

Our goal in this study is to emphasize the importance of the genus *Scutellaria*.

1.1. Diversity

Scutellaria spreads out almost all over the world, except for the Amazon basin, the lower parts of tropical Africa, South Africa, Pacific Islands, northern parts of Central Asia and the deserts of the North Pole (Figure 1). The genus has maximum diversity at the Irano-Turanian region, particularly Central Asia and Afghanistan. Eastern Mediterranean and the Andes are the second center of its speciation [1]. *Scutellaria* is well adapted to Eurasian climate. There are about 300 species of the genus in Asia [15-23].The comparison of genus *Scutellaria* with the number of taxa in Turkey and surrounding flora, the number of endemic taxa and the rate of endemism are given at Table 1. It is seen obviously, the rate of endemism in countries close to the gene center is higher than the other countries.

In Turkey, *Scutellaria* species diversity is very high and the members of the genus are distributed all over the country. Within Flora of Turkey the genus is represented by 24 species, 1 hybrid species and 13 subspecies, totally 34 taxa. 15 species of them are endemic [3]. But for some species, there exists a very high risk of extinction due to small or fragmented populations (Table 2).

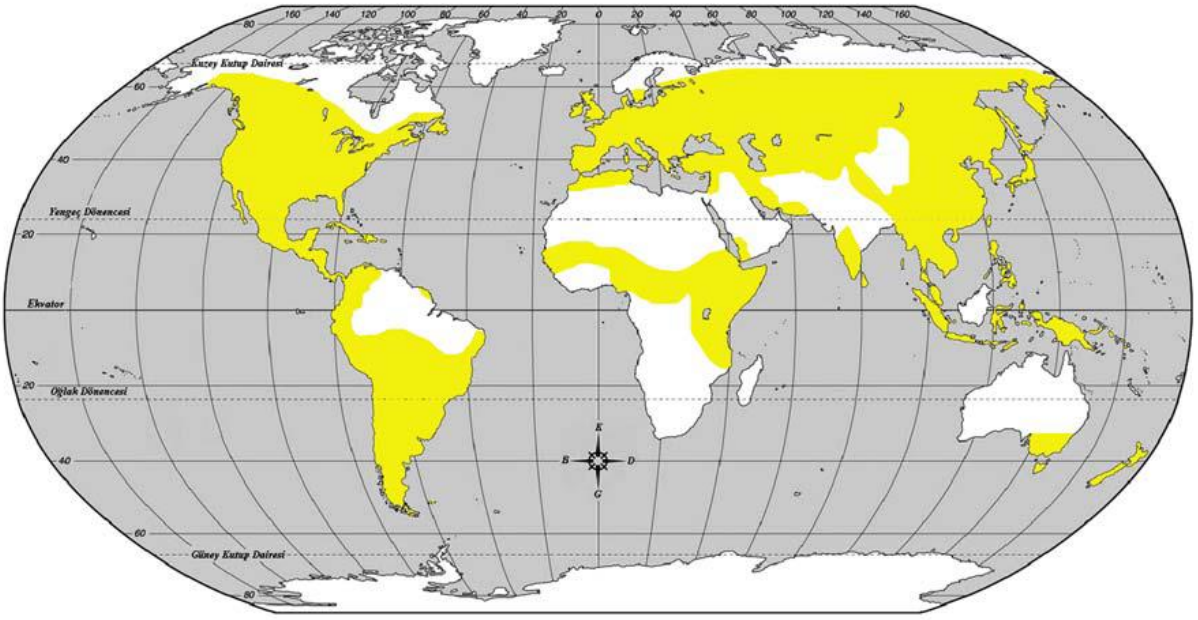

Figure1. The yellow area shows the world distribution map of *Scutellaria* species [24].

Table 1. The comparison of genus *Scutellaria* with the number of taxa in Turkey and surrounding flora, the number of endemic taxa and the rate of endemism

Countries or Regions	The number of taxa	The number of endemic taxa	The rate of endemism (%)
Flora of Turkey [3]	34	15	44
Flora of the Soviet Union [25]	148	109	74
Flora of Syria, Palestine, the Sinai Desert [26]	12	1	8
Flora of Palestine [27]	3	0	0
Flora of Iranica area [18]	54	39	72
Flora of Europe [28]	19	12	63
Flora of Cyprus [29]	3	3	100

2. ECOLOGY AND MORPHOLOGY

Scutellaria has a wide wild population and is found in very different environmental types, from xerophytic areas to flooded areas [1]. Even though *Scutellaria* can grow in very different environments on variety of continents, it is often found in disturbed areas [31-32]. Turkish species live on volcanic or calcareous rocky and stony hillside, step, hardpan and marl, alpine pasture, lake, river and water carrier bank, woodlands (from sea level to 3500 m) (Table 2). American species live in xerophytic environments [24] or low woodlands and along roadsides in moist habitats [31]. Species native to Northern China often appears in grassland, sunny grassy slopes and shrubbery or forest habitats (from 12 to 2000 m) with a cold-dry climate [33]. In New Zealand and Australia *Scutellaria* is found in frequently flooded and somewhat disturbed areas [24, 34].

Scutellaria is a perennial plant often sufirutescent at base, ± lacking aromatic odour. Its stem base prostrates on the ground or grows upward, height 30 to 120 cm. The taproot is stout and slightly conical. The stems shape is four prism and branches grow out at base. Inflorescence a raceme or a spike; flowers arising singly in axils of bracts or floral leaves, on very short pedicels, *Scutellaria* species are typically characterized by the shape of calyx with two undivided lips and the presence of a scutellum on the upper lip. Its blue-purple or white flowers bloom from June to September. Its opposite leaves, heart-shaped at the base, 1 to 6 cm long with scalloped or toothed margins. The flowers are tube shaped, hooded, with two lips, the upper lip being the hood and the lower lip having two shallow lobes. Stamens 4, anthers included under hood, ciliate, lower pair longer, monothecous, upper pair with 2 divergent thecae. Style unequally bifid. Nutlets depressed-globose to broadly ellipsoid, often tuberculate, pubescent with stellate hairs.

Table 2. Inflorescence, habitat features, threat categories, Turkey and World Distribution of Turkish *Scutellaria* species

	Taxa	Inflorescence	Habitat and Height	Threat Categorie*	Spread in Turkey	World Distribution	Phytogeographic Region
SECT. *SCUTELLARIA*	*S. galericulata* L.	June-September	lakes, rivers and waterways edges,sea-level -2500 m	LC	northern, western and eastern Anatolia	Temperate northern hemisphere;	Cosmopolitan
	S. hastifolia L.	May-June	Lake, river and stream edges 80-800 m	VU	Northwest (Ankara, Bursa, Sakarya) and W. Anatolia (İzmir)	from Europe to the Caucasus	Euro-Siberian
	S. altissima L.	June-August	*Carpinus* forest, 75-1800 m	VU	North and northeast Anatolia (İstanbul, Kars, Kastamonu Zonguldak)	from Middle East Europe to the Caucasus	Euro-Siberian
	S. porphyrantha Rech.f.	June-August	volcanic rock slopes, rocky slopes, *Quercus forest* 1800-2059 m	EN	Eastern Anatolia (Hakkari, Şırnak)	Turkey, Northern Iraq	Iran-Turan
	S. albida L. subsp. *albida*	May-July	*Quercus*, *PinusForest*, stony slopes, scrub, 50-1500 m	LC	Northwest and Western Anatolia	from southeast Europe to Transcaucasia	East Mediterranean
	S. albida subsp. *velenovskyi* (Rech.f.) Greuter & Burdet	May-July	Limestone cliffs, streams and rivers edge, the subalpine regions 200-1950 m	LC	Northwest and Western Anatolia	Bulgaria, Greece, Romania, Yugoslavia, East Aegean Islands, Turkey	East Mediterranean
	S. albida subsp. *colchica* (Rech.f.) J.R.Edm	June-August	Rocky, rocky slopes and shrubs, 445-1800 m	NT	Northeast Anatolia	From the Crimea, the northeast of Turkey to Transcaucasia	Euro-Siberian (Auxin element)
	S. albida subsp. *condensata* (Rech.f.) J.R.Edm	June-August	calcareous rocks, bushes and rocky slopes, granite cliffs, 445-1800 m	NT	Northeast Anatolia	Crimea, Turkey, Transcaucasia	Euro-Siberian (Auxin element)
	S. brevibracteata Stapf. subsp. *brevibracteata*	May-August	scrub, limestone cliffs and rocky slopes, sea level - 1830 m	LC	Southwestern Anatolia	Turkey (endemic)	Mediterranean
	S. brevibracteata subsp. *subvelutina* (Rech.f.) Greuter & Burdet	May-June	limestone cliffs and rocky slopes and shades, 400-1660 m	LC	Southern Anatolia, Central Anatolia, the North Anatolian	Turkey, Lebanon, Syria, Palestine, Saudi Arabia	East Mediterranean

	S. brevibracteata subsp. *pannosula* (Rech.f.) Greuter & Burdet	July-August	rocky limestone slopes 1113- 1123 m	CR	Southern Anatolia (Mersin)	Turkey (endemic)	Mediterranean
	S. glaphyrostachys Rech.f.	June-August	limestone cliffs and rocky slope, scrub, 305-2135 m	NT	South and southwest Anatolia	Turkey (endemic)	East Mediterranean
SECT. *SALVIIFOLIAE*	*S. salviifolia* Benth.	April-August	stony and rocky slopes, steppe, scrub, 400-2200 m	LC	North, South, Central and Western Anatolia	Turkey (endemic)	not clear
	S. diffusa Benth.	July-August	Subalpine regions 700-2200 m	NT	Southern Anatolia	Turkey (endemic)	East Mediterranean
	S. pontica K.Koch.	July-August	alpine and sub-alpine meadows, steppe and shrubs, granite and stony-rocky volcanic slopes, 1600-3100 m	LC	Northeast Anatolia; Artvin, Bayburt, Rize, Trabzon	Turkey, Transcaucasia	Euro-Siberian (Auxin element)
	S. heterophylla Montbret et Aucher ex Benth.	May-June	stony slopes, scrub, clay hills 150-800 m	NT	Southern Anatolia (Adıyaman, Kilis, Gaziantep, Hatay, Mersin, Osmaniye)	Turkey, Syria, Lebanon	East Mediterranean
SECT. *LUPULINARIA*	*S. virens* Boiss. & Kotschy	June-September	Limestone rocky slopes 1690-3000 m	LC	Eastern Anatolia (Van, Bitlis, Muş)	Turkey, the Caucasus	Iran-Turan
	S. orientalis L.	May-June	volcanic rock slopes, clay slopes, granite and metamorphic hills 300-1750 m	LC	Northeast Anatolia	Crimea, Central Russia, Turkey, Transcaucasia	not clear
	S. sosnowskyi Takht. subsp. *sosnowskyi*	May-July	rocky, stony and clay slopes, dry steppes, volcanic rock slopes 1520-2700 m	LC	Eastern Anatolia	From Turkey's eastern to Transcaucasia	Iran-Turan
	S. bicolor Hochst., J.A.Lorent	May-July	limestone cliffs and rocky slopes 530-2000 m	LC	Eastern Anatolia	Turkey (endemic)	Iran-Turan
	S. macrostegia Hausskn. ex Bornm.	May-June	rocky slopes, rocky cliffs and eroded slopes 930-1650 m	LC	Eastern Anatolia, western Anatolia	Turkey (endemic)	Iran-Turan
	S. pectinata subsp.	May-September	arid steep slopes, calcareous soils,	LC	Central, Northeast and East Anatolia (Ankara,	Turkey, Iraq, Syria (Sinai Desert)	Iran-Turan

	pectinata Montbret & Aucher ex Benth.		limestone slopes 870-2160 m		Erzincan Sivas and Trabzon)		
	S. sosnowskyi subsp. *pinnatifida* M.Cicek et O.Ketenoglu	April-August	limestone cliffs and rocky slopes, gypsum, clay and lime lands 300-2700 m	LC	West, South, Southwest, North, Northwest and Central Anatolia	from Southeast Europe to Turkey	not clear
	S. pichleri (Stapf) Rech.f.	May-August	dry rocky hills and slopes, steppe, metamorphic areas 1690-2590 m	VU	Eastern Anatolia (Erzurum/Bayburt, Hakkari, Van)	Turkey, Iraq and Iran	Iran-Turan
	S. sintenisii Hausskn. ex Bornm.	May-July	rocky limestone slopes, calcareous soils 880-1700 m	LC	West Central Anatolia and Eastern Anatolia (Ankara, Elazığ, Erzincan)	Turkey (endemic)	Iran-Turan
	S. haussknechtii Boiss.	May-July	rocky limestone slopes 680-1150 m	VU	Southeast Anatolia (Batman, Mardin)	Turkey (endemic)	Iran-Turan
	S. bornmuelleri Hausskn. ex Bornm. subsp. *bornmuelleri*	June- July	dry stony and rocky limestone slopes,	LC	Southeast Anatolia (Hakkari, Van)	Turkey, Iraq	Iran-Turan
	S. tomentosa Bertol.	May-July	lime-stony soils and slopes, stony dry areas 380-1200 m	LC	Southeast Anatolia	Turkey, the Sinai desert of Syria, Palestine, Iraq and Iran	Iran-Turan
	S. tortumensis (Kit Tan & Sorger) A.P.Khokhr.	June-July	eroded cliffs and rocky slopes 1200-1330 m	NT	Northeast Anatolia (Erzurum)	Turkey (endemic)	Iran-Turan
	S. anatolica M.Cicek & O.Ketenoglu	June	volcanic rocky-stony slopes 1540-1610 m	CR	South Anatolia (Adana, Niğde)	Turkey (endemic)	Iran-Turan

*Critically endangered (CR), Endangered (EN), Vulnerable (VU), Nearly Threatened (NT), Least Concern (LC) [30].

3. USES IN ALTERNATIVE MEDICINE

Some species of *Scutellaria* used in alternative medicine for at least a few hundred years [10]. The dry root of *Scutellaria* is one of the most popular and multi-purpose herb used in China and in several oriental countries. Some *Scutellaria* species used in the traditional Chinese medicines are *Scutellaria baicalensis* Georgi, *Scutellaria viscidula* Bge, *Scutellaria amoena* C.H., *Scutellaria rehderiana* Diels, *Scutellaria ikonnikovi* Juz., *Scutellaria likiangensis* Diels, and *Scutellaria hypericifolia* Levl [35]. It has been widely used in the treatment of hepatitis, jaundice, tumour, leukaemia, arteriosclerosis, diarrhea, and inflammatory diseases [36].

Scutellaria is not widely used in Turkey; however it has been used as styptic, wound for healing and strengthening in Anatolian folk medicine [37].

4. BIOACTIVE COMPOUNDS

Phytochemicals of *Scutellaria* are flavones, flavonoids, chrysin, iridoids, neo-clerodanes, scutapins, and isoscutellarein. Flavonoids work as antitumor. *Scutellaria baicalensis* has high flavonoids includings: Wogonin (5,7-dihydroxy-8-methoxyflavone), Wogonoside (Wogonin-7-glucuronic acid), Baicalin (7-glucuronic acid, 5,6-dihydroxy-flavone), and Baicalein (5,6,7-trihydroxyfavone) [38-40].

Shin and Lee (1995) produced successfully baicalin in callus cultures of *S. baicalensis* using the hairy root culture system [41]. Thus it is suggested that hairy root cultures could possibly be used in herbal medicine as a substitute for *Scutellaria* radix [42, 43].

Hirotani (1999) isolated 16 flavones and five phenylethanoids from *S. baicalensis* [43]. Saraçoğlu et al. (1995) isolated 10 glycoside compounds from *Scutellaria salviifolia* Benth. and determined that some phenylpropanoid glycoside exhibit antitumor activities [44]. Gousiadou et al. (2013) isolated irioid glycoside from *Scutellaria albida* L. subsp. *albida*. It shows antioksidant properties [45].

Iridoidglucosides were isolated from *S. albida* subsp. *colchica*, phenylethanoidglycosides were isolated from *Scutellaria albida* subsp. *colchica* (Rech. f.) J.R.Edm, *S. orientalis* L. subsp. *pinnatifida* and *S. salviifolia* Benth. several neo-clerodane diterpenoids with insect antifeedant activity have been reported from *S. galericulata* L. [46-48].

5. MEDICAL STUDIES

The components of *Scutellaria* have a wide range of pharmacological actions, such as antitumor, anti-angiogenesis, hepatoprotective, antioxidant, anticonvulsant, antibacterial and antiviral activities [14].

The flavones isolated from the roots of *Scutellaria* were showed to exert antioxidant [49], anti-viral [50-54], anti-thrombotic [55-56], anti-inflammatory [57] and anti-cardiovascular illness [58]. The bioactive phytochemical flavones of *Scutellaria* have anti-inflammatory effect. This activity of the *Scutellaria* flavones are at least in part due to their ability to suppress expression of monocyte chemotactic protein-1, a crucial factor for early inflammatory responses, and to down-regulate several inflammation-associated genes such as inducible nitric oxide synthase, cyclooxygenases, and lipoxygenases and, consequently, inhibit production of nitric oxide (NO) and prostaglandin [59-65]. It has also been recognized as a muscle relaxant.

Besides the anti-inflammatory activities, the flavones obtained from *Scutellaria* have been also shown cytostatic and cytotoxic activities against many human cancer cells. Importantly, they have no (or very little) toxicity against normal epithelial and normal peripheral blood and myeloid cells. Xiao-Chai-Hu-Tang showed that *S. baicalensis* alone could inhibit proliferation of several human myeloma cell lines in vitro [66] and stopped tumour

growth in vivo in bladder, head/neck squamous and sarcoma mouse tumour models [67-69]. In contrast, *S. baicalensis* has not growth inhibitory effects on non-tumorigenic oral squamous cells [68]. Because of this point *S. baicalensis* is a very attractive as a new anticancer drug.

6. CULTIVATION

Not only *Scutellaria* species grow in the wild, but also some *Scutellaria* species like *S. baicalensis*, *S. agrestis* A. St.-Hil. ex Benth, *S. incana*, *S. lateriflora*, and *S. floridana* were cultivated in China, US and Central Europe [70-71]. In Turkey any systematic cultivation methods for crop production have not been worked out yet.

7. CONCLUSION

Because of having a wide range of pharmacological actions, numerous members of *Scutellaria* genus are very important as medicinal plants. Especially having cytostatic and cytotoxic activities against many human cancer cells are very noteworthy.

In recent years, *Scutellaria* based herbal formulations have been employed to establish its medical/scientific value using in vitro cell culture systems. *Scutellaria baicalensis* and *S. lateriflora* are the two species which have been used in most of the herbal formulations. They are currently available in the market in some countries like US, China, etc. Our current research here focuses on *Scutellaria* species found in and around Turkey. Few studies have been carried out on Turkish *Scutellaria* species and further work is needed on these species. Despite the fact that there are the numbers of *Scutellaria* taxa in Turkey, very few studies on their phytochemicals were conducted. If the work to be done on this matter is increased, maybe there will be a chance to find new bioactive compounds and more *Scutellaria* species can be used as drugs containing.

Some of these species are becoming rare or threatened because of population pressure, environmental pollution, and destruction of their natural habitat. But, there is not any regulation for the protection of the endangered *Scutellaria* species. It is very important that it must be taken preventive measures to conserve this species. It is also necessary that we should initiate preservation of endangered seeds of these taxa in gene banks.

Conservation by cultivation is an effective means for protecting genetic resources. We suggest that these species should be cultivated thus gaining economic and ecological benefits. Actually some countries such as the US and China achieved cultivation of this genus and they grow some species of *Scutellaria* as a crop production.

Scutellaria is not only medicinal plant but also it has brilliant beautiful blooms. For this reason there is a great potential for these species as ornamentals.

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Review Article

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Studies on *lilium* species

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Abstract: In this review, some studies on *Lilium* genus that have about 100 species and belong to Liliaceae were investigated and compiled. *Lilium* is used in different fields as medicine, food, landscape and perfumery industry. There are a lot of molecular and genetic studies on *Lilium* species because of its large kromosoms. The antioxidant, cytotoxic, genotoxic activities of some species were determined and some phyochemical compounds were isolated from *Lilium* species. According to researches, *Lilium* species are potential antioxidant sources and include some compounds as steroidal saponins, glycoalkaloid and phenolic glyceride was found. Consequently, this review strongly suggest that this genus may serve for pharmaceutical applications and can continue to be used as an ornamental plant.

Keywords: Lilium, Liliaceae, genetic, cytotoxic, antioxidant

Lilium türleri üzerine yapılan çalışmalar

Özet: Bu derlemede Liliaceae familyasına üye olan ve yaklaşık 100 tür içeren *Lilium* cinsi üzerine bazı çalışmalar araştırılmış ve derlenmiştir. Zambaklar tıp, gıda, peyzaj ve parfüm endüstrisi gibi birçok alanda kullanılmaktadır. Büyük kromozomlarının sağladığı kolaylıkdan dolayı *Lilium* türleri üzerine birçok moleküler ve genetik çalışmalar bulunmaktadır. Bazı türlerinin antioksidan, sitotoksik, genotoksik aktiviteleri tespit edilmiştir ve bazılarından fitokimyasal bileşenler izole edilmiştir. Araştırmalara göre, *Lilium* türlerinin potansiyel bir antioksidan kaynağı oldukları ve steroidal saponin, glikoalkaloid ve fenolik gliserit gibi bazı bileşenleri içerdikleri bulunmuştur. Sonuç olarak bu çalışma, *Lilium* cinsinin farmasötik uygulamalara hizmet edebileceği ve süs bitkisi olarak kullanılmaya devam edilebilceğini vurgulamaktadır.

Anahtar kelimeler: Lilium, Liliaceae, genetik, sitotoksik, antioksidan

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1. Giriş

Dünyada Liliacea familyası 250 cins ve 3500 tür içermekte ve bu türlerin yaklaşık %12.8'i ülkemizde bulunmaktadır. Liliaceae (Zambakgiller) familyasından olan *Lilium sp.* insanların tanıdığı en eski bitkilerdendir. Ülkemizde *Lilium* sp. Zambak, Beyaz Zambak, Ak Zambak, Kokulu Zambak, Misk Zambağı, Orak Zambağı ve Türk zambağı olarak isimlendirilir. Dünya üzerinde başlıca 10-60^{o} kuzey enlemleri arasında olmak üzere Avrupa, Asya ve Kuzey Amerika'da, ülkemizde ise Doğu ve Batı Anadolu'da doğal olarak yayılış gösterir. Liliaceae familyasına üye olan *Lilium* cinsi, ağırlıklı olarak kuzey yarıkürenin ılıman bölgelerinde yayılış gösteren yaklaşık 100 tür içermektedir [1-4]. *Lilium* L. Cinsi, farklı yazarlar tarafından 5 ile 10 arasında bölüme ya da altcinse ayrılmıştır [5-11]. Bu cinsin, biri endemik 6 türü, doğal olarak yetişir. Bu türlerden sarı veya pembe çiçekli olup Kuzeydoğu Anadolu'da yetişen *L. martagon* L.(Türk Zambağı) ve Güneybatı Anadolu'da *L. candidum* L. (Ak Zambak) bulunur [12-14].

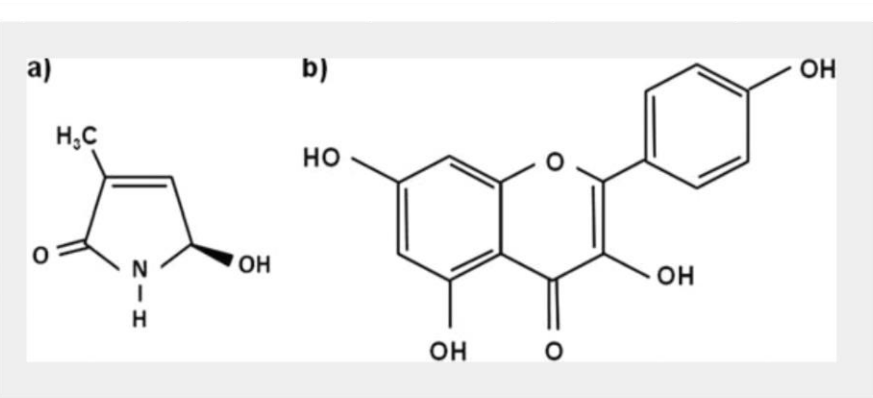
Zambaklarla ilgili tarihsel kayıtlar, *Lilium* yetiştirmenin antik Grit uygarlığında M.Ö. binlerce yıl önceye dayandığını [15], Mısırlıların cenazelerde çelenk yapımında *Lilium* kullandıklarını ve Romalıların saray bahçelerinin yapımında nergis ve sümbüllerle beraber zambakları ekdiklerini göstermektedir. 5000 yıl öncesine dayanan Sümer tabletlerinden İran'daki Susa şehrinin zambak bahçeleri ile çevrili olduğu ve şehrin bu bitkiden sonra isimlendirildiği tespit edilmiştir [16, 17]. Son yıllarda *Lilium*'un gerek kesme çiçek, gerekse saksılı çiçek olarak yetiştiriciliğinde; birim alandan oldukça yüksek kar getirmesi, yıl boyu yetiştirilebilmesi, yeni çeşitlerin bulunması, çiçeğe talebin artması gibi nedenlerle önemli bir artış dikkati çekmektedir [18].

Lilium L. cinsi hem bahçe bitkileri hem de kültüre alınan çiçekleriyle en önemli süs bitkileri grubu olarak konumlarını korumaktadırlar [19, 20]. *Lilium* türleri soğanlı ve çok yıllıktır. Gösterişli çiçeklerinden dolayı park ve bahçelerde süs bitkisi olarak yetiştirilen *Lilium* türleri ayrıca birçok alanda kullanılmaktadır. Bazı *Lilium* türleri, aromatiktir ve parfüm endüstrisinde kullanılmaktadır [21]. Etken maddelere sahip olan bazı *Lilium* türleri cilt hastalıkları, apse, sivilce tedavisinde ilaç hazırlamada kullanılmaktadır [22]. Bazı *Lilium* türleri ise yapıştırıcı ve boya olarak kullanılmaktadır [23]. Dünya çiçek pazarında bu cinsin hibritlerinin ve kültürlerinin genişliği ve çeşitliliği ticari olarak çok önemlidir [24]. Ayrıca bazı türler, tıbbi ve gıda değeriyle bilinmektedir [25-28].

Lilium türleri üzerinde farklı amaçlarla bilim adamları tarafından birçok çalışmalar yapılmıştır:

2. Sitotoksik ve Genotoksik Çalışmalar

Organizmalar, çevresel DNA-hasar faktörlerinin zararlı etkilerine sürekli maruz kalmaktadırlar. Çevresel genotoksinlerin zararlı etkileri, antimutajenler kullanılarak uygun bir şekilde azaltılabilir. Mutajenik yükü azaltmakla alakalı modern yaklaşımlardan biri; genotoksinlere karşı koruyucu ve antimutajenik potansiyele sahip olan doğal ve sentetik bileşenlere dayanmaktadır. *L. candidum*'dan izole edilen kampferol ve jatrofam (Şekil 1) doğal bileşenlerinin; *in vitro* koşullardaki *Hordeum vulgare* ve insan lenfosit hücreleri ile kurulan ökaryotik test sisteminde sitotoksik ve genotoksik aktivitelerinin yanı sıra radyomimetik zeocin'e karşı hücreleri koruma potansiyelleri de test edilmiştir. Kampferol ve jatrofamın her ikisinin de, deney düzeneğine ve test sistemine bağlı olarak, sitotoksisiteyi ve zeocin'in genotoksik etkisini modüle etme ve azaltma potansiyeli olduğu gösterilmiştir. Bu veriler, sağlık araştırmaları için özellikle doğal bitki bileşenlerinin aktivitesi ve farmakolojik potansiyellerinin açıklığa kavuşturulmasında faydalı olabilir [29].



Şekil 1. *Lilium candidum*'dan izole edilen (a) jatrofam-5-O-bglucopyranosideve (b) kamferol-3,40,5,7-tetrahydroxyflavone kimyasal yapıları (Jovtchev ve ark. 2013' den uyarlanmıştır).

Dizel egzoz gazı partikülleri en önemli hava kirleticileri olarak kabul edilmektedir. Dizel egzoz partiküllerinin, hem yaygın alerjenlere karşı duyarlılığı artırıcı aktivitesi hem de duyarlı kişilerdeki alerjik semptomlar üzerine arttırıcı etkileri gösterilmiştir. Bir çalışmada, antialerjik madde olarak bilinen *L. martagon'un* polen taneleri toplanmış ve 5-10 gün egzoz gazına maruz bırakılmıştır. Farklı polen ekstraktlarının alerji potansiyelleri, tedavi edilen hayvanlarda kandaki eozinofil sayısı ve immünoglobulin E seviyesi analizlerinin yanında deri testi yoluyla karşılaştırılmıştır. Alerjik bantlar immünoblot yöntemiyle incelenip kontrol edilmiştir. İncelenen alerji testleri sonuçları, egzoz gazına maruz kalan polen tanelerinin alerjik semptomları uyarmada etkili olduğunu göstermektedir. Uygun koşullarda, egzoz gazı partiküllerinin suda çözünen parçaları polen proteinlerinin salınımını etkileyen değişikliklere neden olmaktadır. Dizel egzoz partikülleri polen alerjen molekülleri taşıyabilir, yeni proteinleri (alerjen) indükleyebilir ve alerjenler için adjuvant olarak hareket edebilirler [30].

Herpes Simplex Virüsü-1 ve 2 (HSV-1 ve HSV-2) ve Varicella-Zoster Virüsüne (VZV) karşı bitki etanol ekstraktlarının antiviral aktivitesi *in vitro* olarak incelenmiş ve bitki virüslerine dirençli olan *Ficus binjamina* ve bitki virüslerine karşı yüksek duyarlılığa sahip olan *L. candidum* kullanılmıştır. *F. binjamina*'nın meyveleri sadece VZV'yi inhibe ederken; yaprak ekstraktları çalışılan bütün virüsleri inhibe etmiştir. *L. candidum* yaprak ekstraktları VZV üzerinde bir etkiye sahip değildir ancak HSV-1'i güçlü bir şekilde ve HSV-2'yi az bir şekilde inhibe etmiştir [31].

3. Moleküler ve Genetik Çalışmalar

Liliaceae bitkilerinin sitolojisi başından beri, büyük kromozomlara sahip olmalarından ve sitolojik tedavilerde kolaylık sağlamalarından dolayı uygun materyal olarak görüldüğü için defalarca çalışılmıştır. Ancak, Levan (1939), *Allium ammophilum* ve *A. cernuum*'daki karşılıklı translokasyon durumunu kaydetmiş [32], bir başka çalışmada, *Lilium hansonii* Leicht. de benzer bir duruma rastlamıştır [33,34].

Lilium'da uzaktan akraba türler arasındaki çoğu hibrit, özellikle embriyo kültürüyle üretilen hibritler, bazı istisnalar dışında polen kısırlığı göstermektedir [35,36]. Bu kısırlıklar öncelikle metafaz 1'deki düzensiz kromozom birleşmeleriyle oluşmuştur [37,40].

Çin'de *Lilium*'un 32 taksonunu temsil eden 52 populasyon karyolojik olarak analiz edilmiştir. Sonuçlar bütün populasyonların aynı temel kromozomlara (x=12) sahip olduğunu ve triploid olan (2n=36) *L. tigrinum* dışındaki bütün türlerin diploid olduğunu göstermektedir. Ek olarak, *L. sulphureum*'un bir populasyonu anöploiddir (2n=23). *Lilium* cinsindeki karyotip

evrimi, ağırlıklı olarak kromozomların ince yapısının değişiklikleri bakımındandır, ploididekiveve temel kromozom sayısı varyasyonlarından değildir [41].

Zambaklar 7 alt şubeye ait 80'den fazla tür içermektedirler. Bu bölümler içinde Sinomartagon, Archelirion ve Leucolirion'den yetiştirilen kültürler ticari marketlerde en önemlileridir. Şu anda, zambak yetiştiriciliğinde en umut verici atılım, ticari ıslah için şimdiye kadar kullanılmayan yabanıl türlere üye olan ıslah materyallerinin genetik özelliklerinin transferiyle türlerarası melezleme yoluyla yeni kültürlerin arttırılmasıdır. Bu çalışmada hemen hemen tüm farklı çapraz kombinasyonları denenmiştir ve 1980'den beri 28'den fazla çaprazlama kombinasyonunda başarılı olunmuştur. Umut verici çaprazlamalar Asyalılar ve Trompet zambakları gibi birkaç tür arasında gözlemlenmiştir. Bu bağlamda, zambaklar arasındaki türlerarası melezlemelerin bazı değerli örnekleri ve olası yöntemleri gösterilmiştir [42].

Bosna Hersek'ten Balkan endemik takson *Lilium*'un dört populasyonu incelenmiştir. Geleneksel karyolojik çalışma herhangi bir önemli farklılık ortaya koymamıştır. Moleküler-sitogenetik çalışmalar göreceli en yakın *L.carniolicum* ile ilgili olarak ribozomal genlerin düzenlenmesinde önemli farlılıklar ve populasyonlar içi ve populasyonlar arası değişkenliğe dikkat çekmektedir. *L. bosniacum* için FISH deneyi ve florokrom bantlaşmasının sonuçları ilk kez burada bildirilmiştir. Tüm incelenen populasyonların, kladanj populasyonundaki bazı bireyler hariç, aynı aktif Nükleolus Organizatör Bölge (NOR) sayılarına sahip olduğu mevcut sonuçların iki endemik *L.camiolicum* ve *L.bosniacum* arasındaki türler arası farklılaşmayı net olarak ortaya çıkardığı bildirilmiştir [43].

Yapılan başka bir çalışmada olgun sporun oluşturulmasından sonra *L. regale*'nin gametofitik gelişmesi incelenmiştir. Generatif hücre kromozomlarının kendine özgü konfigürasyonları için açıklamalar sunulmuştur. Veriler, kromozomun daha önce kabul edilmiş mitoz sırasında bir otozom biriminin daha fazla olduğunu göstermektedir [44].

Rastgele çoğaltılmış polimorfik DNA (RAPD) markırları, Türk zambağı (*L. martagon*) populasyonları arasındaki ilişkiyi ve populasyonlar içindeki genetik değişimi tahmin etmek için kullanılmıştır. İsveç, Danimarka, Norveç, Litvanya'dan oniki evcil populasyon ve Litvanya'dan dört yerli populasyon, İsviçre ve İtalya altı dekamer primerleri kullanılarak analiz edilmiştir. Primerler toplam 55 polimorfik bant ürün vermiştir. İncelenen populasyonların, çoğu durumdaki sınırlı bir nüfus büyüklüğüne rağmen, şaşırtıcı derecede heterojen olduğu gözlemlenmiştir. Gen çeşitliliği değerleri 0,15 (İsviçre yerli populasyonu) ile 0,26 (İsveç'in evcil populasyonlardan biri) arasında değişmektedir. Genel olarak, evcil populasyonların yerli populasyonlardan daha az değişken olduğu gözükmemektedir [45].

Çin'e ait olan 5 varyete ve 44 türü kapsayan, 98 tür ve 5 varyeteden 214 örnek ile nükleer ribozomal DNA (nrDNA)'dan kapsamlı bir filogeni çalışması yapılmıştır. Çin'de yaygın olan cinsin (Comber) beş bölümünü kapsayan 25 tür ve beş çeşit (44 örnek) ITS (Internal Transcribed Spacer)'nin veri tabanına dahil edilmiştir. Verilere göre, Çin türleri beş bölüme ayrılmıştır: *Martagon, Archelirion, Leucolirion, Sinomartagon* ve *Lophophorum'*dur [19].

Bir çalışmada RAPD markırları, tesadüfi üreme yoluyla üretilen *Lilium sp.*'nin *in vitro* olarak çoğaltılmış soğanlarının klonal uygunluğunu görüntülemek için kullanılmıştır. Doku kültürü ile çoğaltılan yavrularda hiçbir varyasyon gözlenmemiştir. Elde edilen bu sonuçlar, Asya melezlerinin *in vitro* soğanlarından tesadüfi olarak çoğalanların klonal olarak aynı ve stabil olduğunu göstermektedir [46].

Bu veriler *Lilium*'un filogenisi, kökeni ve sınıflandırılmasını anlamamıza katkıda bulunmuştur.

4. Botanik, Morfolojik, Anatomik ve Evrimsel Gelişim Üzerine Çalışmalar

Eski uygarlıklarda saflık ve temizliği simgeleyen *Lilium* sp., Liliaceae familyasından, çok yıllık soğanlı bir bitkidir. Soğan; besin maddeleri içeren kalın etli pulların birbiri üzerine katlanmasından oluşan, içerisinde gelişim halinde büyüme konisi, yaprak ve çiçek tomurcuğu içeren toprak altı organıdır. Soğanlar *Tulipa* (Lale) ve *Narcissus* (Nergis) gibi kabuklu veya *Fritillaria* (Ağlayan Gelin veya Adıyaman Lalesi) gibi kabuksuz olabilir [14, 47].

Bir çalışmada, *Lilium* L. cinsinin filogeni ve biyocoğrafyası incelenmiştir ve *Liriotypus* için ayrışma süreleri hesaplanmıştır. Plastid DNA dizisi verisi (trnC-petN interjenik ayıracı ve petN geni) ve nükleer DNA ITS dizi verisi *Liriotypus* bölümünün filogenetik tarihini anlaşılması için kullanılmıştır. Moleküler analizler, *Liriotypus* üyelerinin yaklaşık 9 milyon yıl önce *Lilium* cinsleri ile ayrıldığını göstermektedir ve bu şubede, son 6 milyon yıl içinde türleşmenin arttığı gözlemlenmiştir [48].

Hengduan Dağları (H-D) Çin'de Qinghai- Tibet (Q-T) yaylasının doğusunu kuşatır ve ılıman kuşak bitki çeşitliliğinin önemli bir merkezidir. Bu dağların jeolojik geçmişi ve karmaşık topoğrafik özellikleri buradaki çoğu çeşidi ve endemik türleri evrimleşmeye yönlendirmiştir. Bir hipoteze göre Q-T yaylası ve H-D dağlarının 4-3 milyon yıl önce meydana gelen bir ayrılma sonucu oluştuğu ve 8-7 milyon yıl önce nihai hızla bir yükselme evresine girdiği ileri sürülmüştür. Bu hipotezi değerlendirmek amacıyla, *Nomocharis*'leri de kapsayan *Lilium* cinsinin çeşitlenme oranı, filogenisi, biyocoğrafik özellikleri, divergens zaman analizleri üzerine bir çalışma yapılmıştır. Filogenetik çalışmalar, Nomocharis'in *Lilium* içine yerleştiğini gösteren önceki çalışmaları desteklemektedir. Ancak, melezleme çalışmalarından kaynaklanan sonuçlar iki gen arasında uyuşmazlık tespit etmiştir. ITS veri kümesi kullanılarak yapılan zamanlama analizleri, *Lilium* ve *Nomocharis*'lerin içindeki büyük soy evriminin yaklaşık 4-3 milyon yıl önce H-D dağlarının yükselişi ve 8-7 milyon yıl önce oluşan Q-T platosunun yükselişiyle hemen hemen aynı zamanda olmuş olabiliceğini göstermektedir [49].

Bilim adamları bir çalışmada, *Nomocharis gongshanensis* yeni türünü sunmuştur ve ITS ve psbA-trnH markerleri kullanarak *Lilium-Nomocharis* kompleksindeki sistematik yerini belirlenmiştir. Analizler, *Lilium- Nomocharis'*in monofilisi ve bu iki cinsin ortak parafilisini desteklemektedir [50].

Lilium'un Kore türlerinin evrimini ve kökenini belirlemek için *Lilium* ile ilgili infrajenerik ilişkileri araştırılmıştır. Sonuçlar *Lilium* içindeki birçok önemli soyu tespit etmiştir ve yalnızca *Martagon* şubesinin, monofiletik olduğunu ortaya koymuştur [51].

Lilium türleri ile yapılmış çeşitli sitolojik çalışmalar da bulunmaktadır. Vesque (1879) *Uvularia grandiflora'*daki embriyo kesesinin bir megaspore ana hücresinin iki veya üç kardeş hücresinin herbirinden geliştiğini gözlemlemiştir [52]. Dixon (1895), *L. longiflorum*'un embriyo kesesinin döllenmesi hariç merkez kısmında çok sayıda çekirdeğe sahip olduğunu bulmuştur [53].

L. henryi'nin makrospor ana hücresinin çekirdeği ve onun kardeş çekirdeği, hücre bölünmesi olmaksızın heterotipik ve homotipik bölünmeler geçirir ve böylece 4 çekirdekli embriyo kesesi oluşur [54].

L. martagon'un rejenerasyonu ve kallusların başlatılmasındaki iki bitki büyüme regülatörünün etkisinin araştırıldığı bir çalışmada, modifiye MS medyumu üzerinde çalışılmıştır. Kültürler tohum kullanılarak başlatılmıştır. Eksplantlar, ek soğanların ve fidelerin (hipokotil, fide soğanı ve kök) farklı kısımlarından izole edilmiştir. Büyüme regülatörleri çeşitli kallus tiplerini uyardığı, benziladeninin (BA) yalnızca krem-beyaz, sıkı kallusları uyarırken; 4-amino-3,5,6-trikloropiridin-2-karboksilik asitin (pikloram) sarı, kırılgan, granüler kallusları uyardığı gözlenmiştir [55].

Polen tüpünün büyüyen ucunda lokal olarak artan Ca^{+2} yükselişinin polen tüpünün uzaması için gerekli olduğu bilinmektedir. Burada bu lokalize Ca^{+2}'nin zamansal olarak düzenlenildiği ve pulsatile ucu büyümesiyle yakından ilişkili olduğu gösterilmiştir [56].

Polonya'da iki ticari serada yetiştirilen oryantal zambak hibridleri üzerinde ağır yanık belirtileri oluşmuştur. Belirtiler yaprak nekroz ve malformasyonunu, çiçek tomurcuk kesilmesi ve çiçek düşmesini kapsamaktadır. 1999 yılında ciddi semptomları olan doğal infekte zambak bitkilerinde büyüme ve yaprak klorozisinin gerilediği ve ertesi yıl ise çiçeklenemediği kaydedilmiştir. Bu bitkilerden elde edilen DNA fitoplazmasının sınırlanmış profillerinin sarı yıldız çiçeği fitoplazmasının referans şuslarına karşılık geldiği bildirilmiştir [57].

1946-2002'de seçilen bahçe bitkilerinin birinci çiçeklenme tarihlerinin grafiksel analizleri, sıcaklığa ve global ısınmaya bağlı olarak görülen bir eğim gösterilmiştir. Bazı türler, ancak, ışıksal periyotlara tepkiyle bağlantılı olabilen bu eğim, kovaya benzemektedir [58].

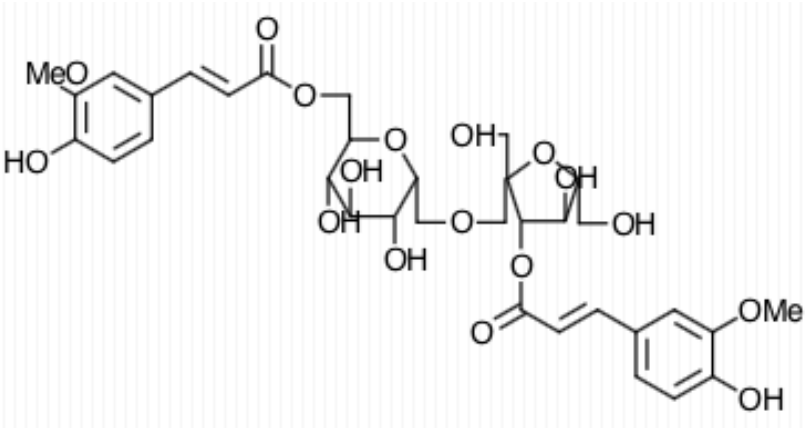
5. Fitokimyasal ve İçerik Tanımlama

Paskalya zambağı (*L. longiflorum* Thunb.) dünya çapında çekici bir süs bitkisi olarak görülmektedir, ancak çiçek tomurcukları ve soğanları dünyanın pekçok yerinde hem mutfak hem de tıbbi amaçlarla da kullanılmaktadır. *L. longiflorum*, bitki patojen savunmasına, ultraviyole ışınlardan korumaya ve zambakların tıbbi kullanımına katkısı olan fenilpropanoid gliserol glukositlerinin önemli bir miktarını içermektedirler. Bitkideki bu bileşenlerin doğal yayılımlarını belirlemek için, seçilmiş iyon izlemede kullanılan sıvı kromotografi kütle spektroskopisi (LC-MS) metodu, *L. longiflorum*'un farklı organlarından alınan 5 fenilpropanoid gliserol glukositlerinin [1:(2S)-1-O-caffeoyl-2-O-β-D–glukopiranosilgliserol, (2S)-1-O-kafeoil-2-o-β-D-glukopiranosil gliserol; 2: (2R)-1-O-β-D-glukopiranosil-2-o-p-kumarogliserol; 3: (2S)-1-O-p-kumarol-2-o-β-D-glukopiranosil gliserol; 4: (2S)-1-O-kafeoil-2-o-β-D-glukopiranosil-3-o- asetilgliserol ve 5: (2S)-1-O-p-kumarol-2-o-β-D-glukopiranosil-3-o-asetilgliserol) kantitatif analizleri için kullanılmıştır (Şekil 2). 3 temelli p-kumarol ve onun asetillenmiş 5 türevinin paskalya zambağının soğanlarında bulunan fenilpropanoid gliserol glukositleri arasında en bol miktarda olduğu belirlenmiştir (776,3±8,4; 650,7± 32,6 μg/g). Asetillenmiş p-kumarolun ve 5 ve 4 bazlı kafeoilin en yüksek konsantrasyonları kapalı çiçek tomurcuklarında birikmiştir (4925,2±512,8 ve 3216,8± 406,4 μg/g). Bileşen 5 ve 1'in ardından bileşen 4'ün olgunlaşmış çiçeklerde en bol bulunduğu kanıtlanmıştır (6006,2±625,8; 2160,3±556,5 ve 1535,8±174,1 μg/g). Fenilpropanoid gliserol glukositlerinin toplam konsantrasyonu, etli kökleri ile kıyaslandığında bitkinin yer üstü organlarında 10-100 kat daha yüksektir. Beş bileşenin ikisi, 1 ve 2, ilk kez *L. longiflorum*'da belirlenmiştir. *L. longiflorum*'un farklı bitki organlarındaki fenilpropanoid gliserol glukositlerinin kantitatif analizi, bitkinin fizyolojisinde ve kimyasal ekolojisinde bu bileşenlerin nasıl bir fonksiyon işlediklerini tanımlamayı ve ayrıca henüz araştırılmamış insan sağlığı üzerine olası etkilerini belirlemeyi amaçlayan araştırmalara yön verecektir [59].

Paskalya zambağının (*L. longiflorum* Thunb.) soğanları Asya kültüründe gıda ve ilaç olarak kullanılmaktadır ve dünya çapında süs bitkisi olarak ekilmektedir. Yeni bir steroidal glikoalkoloid ve iki yeni furostanol saponin, bilinen iki steroidal glikozitle birlikte, *L.longiflorum'*un soğanlarından izole edilmiştir (Şekil 3). Yeni steroidal glikoalkoloid, (22R, 25R)-spirosol-5-en-3beta-il O-alfa-l-ramnopiranosil-(1-->2)-[6-O-asetil-beta-D-glukopiranosil-(1-->4)]-beta-D-glukopiranosid olarak belirlenmiştir. Yeni furostanol saponinleri, (25R)-26-O-(beta-D-glukopiranosil)-furost-5-en-3beta, 22alfa,26-triol 3-O-alfa-l-ramnopiranosil-(1-->2)-alfa-L-arabinofuranozil-(1-->3)-beta-D-glukopiranozid ve (25R)-26-O-(beta-D-glukopiranosil)-furost-5-en-3beta,22alfa, 26-triol 3-O-alfa-l-ramnopiranosil-(1-->2)-R-L-xylopyranosyl-(1-->3)-beta-D-glukopiranosid olarak belirlenmiştir. Önceleri steroidal glikozit olarak bilinen (22R, 25R)-spirosol-5-en-3beta-el O-A-L-ramnopiranosil-(1-->2)-[6-O-

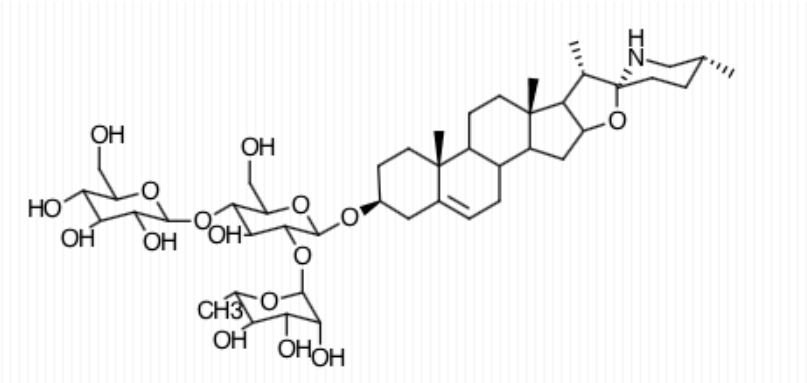
asetil-β-D-glukopiranosil-(1-->4)]-beta-D-glukopiranosid ve (25)-26-O-(beta-D-glukopiranosil)-urost-5-en-3beta,22alfa,26-triol 3-O-alfa-l-ramnopiranosil-(1-->2)-beta-D-glıkopiranosil-(1-->4)-beta-D-glukopiranosid, ilk kez *L. longiflorum*'da tespit edilmiştir. *L. longiflorum*'daki bu yeni bileşenler ve izolasyon metodu bitki gelişimi ve bitki-patojen etkileşiminde, ayrıca gıda ve insan sağlığında steroidal glikozitlerin biyolojik önemi üzerine yapılan çalışmalarda kullanılmaktadır [60].

Beş yeni spirostanol saponin ve yeni furostanol saponin *L. candidum*'un taze soğanlarından izole edilmiştir. Bunların yapıları, asit hidrolizi sonucu ve iki boyutlu NMR spektroskopik teknikler dahil olmak üzere spektroskopik analizler temelinde aydınlatılmıştır. İzole saponinler ortak yapısal özelliği olarak aglikon'un C-3'e bağlı bir *O*-glikosidik'in oluşumu ile O-α-L-ramnopiranosil-(142)-O-[β-D-glukopiranosil- (146)]-beta-D-glukopiranoz gibi bir dallı triglikozit parçası içermektedir. Na^+/K^+ ATPaz'daki saponinlerin inhibe edici aktivitesi değerlendirilmiştir (Şekil 4) [61].



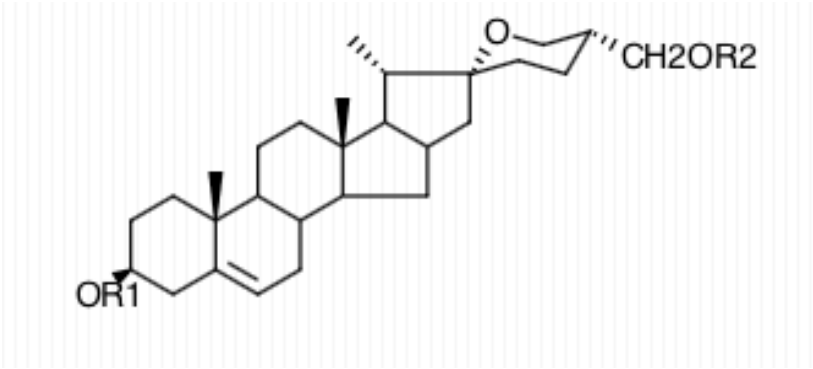
3-O-diferuloylglycerol

Şekil 2. *Lilium longiflorum'*dan izole edilen fenolik gliserol glukosit



22R, 25R)-spirosol-5-en-3beta-il O-alfa-l-ramnopiranosil-(1-->2)-[6-O-asetil-beta-D-glukopiranosil-(1-->4)]-beta-D-glukopiranosid

Şekil 3. *Lilium longiflorum'*dan izole edile steoridal glikoalkaloid



O-α-L-ramnopiranosil-(142)-O-[β-D-glukopiranosil- (146)]-beta-D-glukopiranoz

Şekil 4. *Lilium brownii var. viridulum*'dan izole edilen steoridal saponin

6. Antioksidan Çalışmalar

Yağlar proteinler ve nükleik asitler üzerine reaktif oksijen türlerinden kaynaklanan oksidatif hasar, kalp hastalıkları kanser ve yaşlanma [62] gibi kronik hastalıkları tetiklemektedir. Meyve, sebze ve yüksek antioksidan içeren diğer gıdaların alımı vücuttaki oksidatif hasarı minimize etmeye ve bu hastalıkların riskini azaltmaya yardımcı olmaktadır. Böylece, besinsel kaynaklar verimlilikleri ve toksik olmamaları sebebiyle güvenli ve yeterli antioksidanlar olarak tanımlanmaktadır [63].

Lilium, karmaşık taksonomik sınıflandırması ve Çin'deki uzun tarihiyle, önemli yenilebilir ve tıbbi bitki türleri olarak kullanılmaktadır. Çin'e özgü 6 *Lilium* türünün soğanları(*L. regale, L. concolor, L. pumilum, L. leucanthum, L. davidii var. unicolor* ve *L. lancifolium*) doğal antioksidanlarının potansiyel kaynakları fenolik bileşenlerine ve besinsel antioksidan potansiyellerine göre araştırılmıştır. Bu türler arasında en yüksek toplam fenolik madde miktarını içerenler sırasıyla *L. regale* (10381,49±49,12), *L. pumilum* (4177,39±57,19), *L. concolor* (3897,60±42,54), *L. lancifolium* (2827,25±55,50), *L.leucanthum* (2336,00±29,28) ve *L. davidii* var. *unicolor* (2017,17±140,20) şeklindedir. Sonuçlar soğan ekstraktlarının, total fenolik, total flavonoid ve total flavanol içerikleriyle pozitif korelasyon gösteren güçlü antioksidan aktivite sergilediklerini göstermiştir. Yüksek Basınçlı Sıvı Kromotografisi (YPSK) analizi, ekstraktlarda büyük fenolik bileşenler olan rutin ve kamferolü ortaya çıkarmıştır. Hiyerarşik takım analizi *L.regale*'nin yüksek fenolik içerik ve güçlü antioksidan aktiviteye sahip bir grupta olduğunu göstermiştir. *L. leucanthum, L. davidii var. unicolor ve L. lancifolium* düşük fenolik içerikleri ve zayıf antioksidan kapasiteleriyle üçüncü grupta toplanırken, *L. concolor* ve *L. pumilum* uygun fenolik içerik ve andioksidan kapasiteyle karakterize olan bir grupta toplanmıştır. *Lilium* soğanlarının, potansiyel bir doğal antioksidan kaynağı olarak gıda ve farmasötik uygulamalara hizmet edebileceği önerilmiştir [64, 65].

Son çalışmalarda, birçok doğal polisakkaritin radikal temizleyici olarak yaşayan organizmalardaki oksidatif stres ile indüklenmiş hasarı engellemedeki önemli rolleri gösterilmiştir [66-68]. Yeni bir polisakkarit kısmı (LP2-1), DEAE selüloz kromotografisi ve Sefakril S-400 boyut dışlama kromotografisi ile *L. lancifolium'*un yenilebilir soğanlarından izole edilmiştir ve saflaştırılmıştır. LP2-1'in yapısal karakterizasyonu, fizikokimyasal özellikleri ve antioksidan aktivitesi araştırılmıştır. Sonuçlar LP2-1'in 8,52 kez; 10(3) kDa ortalama moleküler ağırlığa sahip olduğunu ve genel olarak 1,88:2,13:1,00:2,50 mol oranında L-ramnopiranozu, D-arabinofuranoz, D-glukopiranoz ve D-galaktopiranoz'u içermektedir ve LP2'nin basilica fonksiyonel grupları olan COO ve OH'tır. Viskoelastik özellikli LP2-1 sistemleri, kayıp modülünden daha yüksek olan depolama modülüyle ve her iki modülün Ca^{+2} konsantrasyonunu arttırmasıyla jel benzeri bir davranış sergilemektedir. Ek olarak, LP2-1

DPPH ve hidroksil radikallerini giderim aktivitesine ve ayrıca demir iyonunu güçlü bir indirgeme gücüne ve şelatlama aktivitesine sahiptir. Bu sonuçlar LP2-1'in iyi bir antioksidan aktiviteye sahip olduğunu ve gıda endüstrisinde kullanılabilceğini desteklemektedir [69].

Lilium, çeşitli renk, kalıtım ve şekilleriyle birçok tüketiciyi çeken, peyzaj ve saksı bitkileri endüstrisinde dünya çapında önemli bir türdür [70]. *Lilium*'lar, soğuk-nemli iklim koşullarında ve yaklaşık 18-22°C optimum sıcaklıkta yetişmektedirler. Yaz aylarındaki yüksek sıcaklık *Lilium* üretimine önemli bir engel oluşturmaktadır. Bazı çalışmalarda yüksek sıcaklığın zambaklardaki antioksidan enzim aktivitesinin yükseltilmesine sebeb olabileceğinin rapor edilmesine ragmen [71, 72], ROS metabolizması ve kısa süreli ısı stresinin antioksidan sistemi yükseltmesi arasında bir ilişkiye dair çalışma bulunmamaktadır. Ek olarak, Pinhero ve ark. (1997), antioksidan enzimlerin yeni izoformlarının ROS metabolizması için daha faydalı olduğunu önermektedir. Başka bir çalışmanın sonuçları, zambak bitkisinin artan sıcaklığa (37°C-42°C) yüksek tolerans gösterdiğini ve antioksidan enzimlerin aktivitesinin arttığını göstermektedir [73].

7. Sonuç

Dünyada Liliacea familyası 250 cins ve 3500 tür içermekte ve bu türlerin yaklaşık %12,8'i ülkemizde bulunmaktadır. Liliaceae familyasına üye olan *Lilium* cinsi, ağırlıklı olarak ılıman bölgelerde kuzey yarıkürede yayılış gösteren yaklaşık 100 tür içermektedir. Zambaklarla ilgili tarihsel kayıtlar çelenk ve saray bahçelerinin yapımında kullanıldıklarını göstermektedir. En önemli süs bitkileri olarak konumlarını koruyan *Lilium* türleri aromatik bitkilerdir ve parfümeri, tıp ve gıda alanları gibi birçok alanda kullanılmaktadırlar.

Lilium türlerinin antioksidant, sitotoksik (antialerjik, antiviral) ve genotoksik etkilerinin olduğu gösterilirken, gliserol, saponin gibi birçok kimyasal bileşen içerikleri saptanmıştır. Büyük kromozomlara sahip olmalarından ve sitolojik tedavilerde kolaylık sağlamalarından dolayı moleküler ve genetik çalışmalarda defalarca uygun materyal olarak kullanılmıştır ve melezleme çalışmalarından sonra hibritlerdeki kısırlığın kaynaklandığı sebebler bulunmuştur.

Lilium türlerinin patojen savunmasına, ultraviyole ışınlardan korumaya ve tıbbi kullanıma katkısı olan etken madderi tespit edilmiştir. *Lilium* türleri ile yapılan antioksidan çalışmalar ise bazı *Lilium* türlerinin total fenolik, total flavonoid ve total flavanol içerikleriyle pozitif korelasyon gösteren güçlü antioksidan aktivite sergiledikleri gösterilmiştir ve *Lilium* soğanlarının potansiyel bir doğal antioksidan kaynağı olarak gıda ve ilaç sanayine hizmet edebileceği önerilmektedir.

8. Kaynaklar

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SUMMARY

Lilies are one of the most one of important ornamental plant group as garden plants, pot cultured and cut flowers, and has been used as food and medicine fort the 2000 y. Lily is a bulbous perennial herbaceous plant belonging to Liliaceae family. *Lilium* genus consists of about 100 species distributed mainly in temperature regions of the northern hemisphere. The importance of the genus in the world flower market is due to diversity and large number of hybrids and cultivars commercially available. However, some species are also known for medicinal and food value, which increased its economic importance many folds. The lily bulb not contains nutritive material but also accumulates various phytochemical compounds, including saponin, colchicine and polysaccharides**.** Several saponins have been extracts of the bulbs a flowers of some species. Some exracts of *Lilium* species is well known in folk medicine fort the treatment of burns, ulcers, inflammations and for healing wounds. In addition, some species of the *Lilium* are used as adhesive, perfume and paint. Also, Liliaceous plants have been repeatedly studied from various pointsof view as favorable material on account of their large-sized chromosomes and easiness in cytological treatments. According to molecular research, most hybrids in distantly related *Lilium* species, especially hybrids produced by embryos culture, indicate polen sterility with some exceptions. 32 taxon were analysed karyological and was indicated that all of species except *L. tigrinum* is diploid. Also, new cultures are created by transfer useful genetic characteristics of the species by cross-species hybridization. However, antioxidant activity and total phenolic contents of some *Lilium* species were determinated and lily bulbs are considered to be a source of antioxidant. In a recent work that antioxidant activity of 6 *Lilium* species were examined, the highest amount of total phenolic content is repectively *L. regale* (10381.49±49.12), *L. pumilum* (4177.39±57.19), *L. concolor* (3897.60±42.54), *L. lancifolium* (2827.25±55.50), *L. leucanthum* (2336.00±29.28) ve *L. davidii* var. *unicolor* (2017.17±140.20) This work showed that all bulb extracts exhibited strong antioxidantactivities, which generally correlated positively with the total phenolic contents (r = 0.68 to 0.94), total flavonoid contents (r = 0.51 to 0.89) and total flavanol contents. The results of phytochemical studies showed that extracts of *Lilium* species have some compunds as saponin, glycerol glyceride and glycoalkaloids. So, these review suggest that lily bulbs may serve as potential source of natural antioxidant for food and pharmaceutical applications and lilies can serve commercially as an ornamental plant worldwide.

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The Development and the Growth Features of Sprouts of *Malus Orientalis* Uglitzk.Species Introduced in Absheron

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Abstract: Apple is cultivated in most of the temperate regions due to the fruit's quality, its easiness to propagate, and its natural aptitude to bear. *Malus* classifications differ primarily in the taxonomic level at which infrageneric groupings of species are recognized. Object of the study was oriental apple (*Malus orientalis* Uglitzk) from Azerbaijan flora which was introduced to Absheron. The conducted experiments showed that the first sprouts of the seeds of the *Malus orientalis* sown in autumn were observed in the third decade of March. The first embryo roots which gives start to the main roots begin growth in germinal period before the ontogenesis. Thus, experiments have shown that Malus orientalis specie have normal growth in the ontogenesis initial development - germinal stage and it can be used as a perspective species for greening of Absheron.

Key Words: *Malus orientalis***,** Absheron, Taxon, Embryo

1. Introduction

The genus *Malus* Mill. comprises 25–47 species, depending upon the rank given to several taxa and the acceptance of putative hybrids. Robinson et al. (2001) explained that the number of species in genus *Malus* depends upon the rank given to several taxa, species being subspecies and putative hybrids, and the nomenclature of the taxa is complex [1]. The genus *Malus* consists of about thirty wild species and thousands of domesticated cultivars. *Malus* classifications differ primarily in the taxonomic level at which infrageneric groupings of species are recognized. Rehder (1920, 1927, and 1949) proposed a classification system which is nowadays well accepted [2-4]. Newer reports divided the genus *Malus* in six [5] or even in seven sections [6]. *Malus orientalis* is distributed in the Caucasus, the south of Russia, the north of Anatolia, Armenia, the east of Georgia, in Turkey, the mountainous belt in the northern part of Iran [7-11] as well as in the west, east and centre of Iran [7, 12]. Apple is cultivated in most of the temperate regions due to the fruit's quality, its easiness to propagate, and its natural aptitude to bear. Apples are considered a healthy fruit, as the saying goes 'an apple a day keeps the doctor away'. An apple tree can reach up to 10 m height above its own roots, having a

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globose canopy and the longevity between 60 and 100 years. *Malus orientalis* was described by a lower diversity of fruit quality, but due to the high variability in populations *M. orientalis* could have contributed to the domestication of apple by introgression of some traits [13]. *Malus orientalis* from the East, Asia and Central Asia may be an early ancestor of the do-mesticated apples. The domesticated apple is one of the most important fruit crops of the colder and temperate parts of the world [14] (Stephen, 2002).

Investigation of the biomorphological features of the introduced species sprouts are main factors which show their adaptation to the new soil-climate condition. Oriental apple is the widespread wild apple species in Caucasus. Bioecological features of the oriental apple which distributed in Azerbaijan forests were investigated by many scientists.

2. Material and Methods

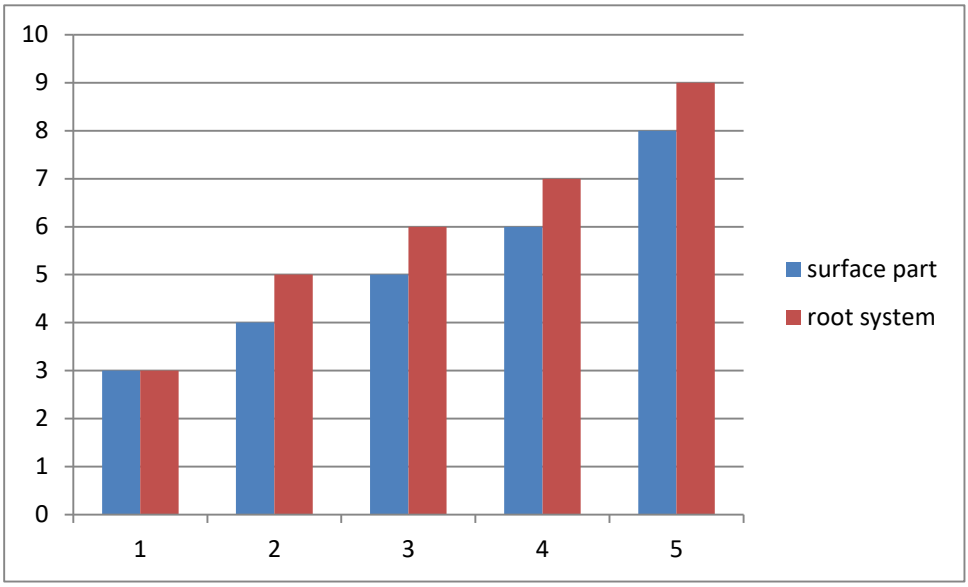
Object of the study was oriental apple (*Malus orientalis* Uglitzk.) from Azerbaijan flora which was introduced to Absheron. Experiments were carried out in Central Botanical Garden of the Azerbaijan National Academy of Science. Biomorphological features of the specie sprouts were carried out according N.T. Vasilchenkoy [15], growth and development features according to Najafova J.N. and Jacson J. E. methods and Central Botanical Garden general concepts [16, 17].

3. Results and Discussion

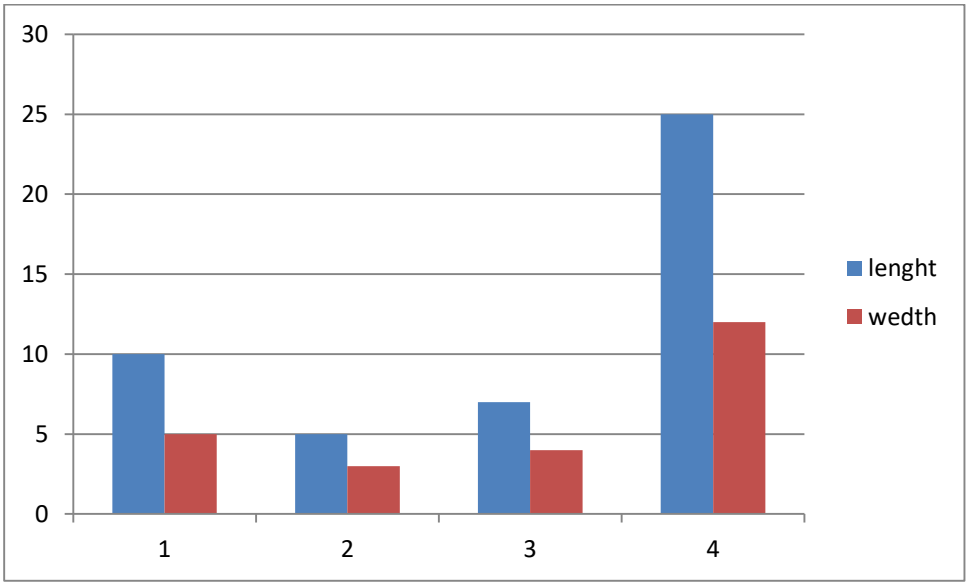
The conducted experiments showed that the first sprouts of the seeds of the Malus orientalis sown in autumn were observed in the third decade of March. The first embryo roots which gives start to the main roots begin growth in germinal period before the ontogenesis. The lateral roots and the lobe leaves begin to growth. The cotyledon elbow length was 35-45 mm, width 1 mm, the top surface was green, the bottom surface was reddish. Cotyledon was in 10-12 mm length and 5-8 mm width. On the contrary, oval or egg-shape, naked, green, fleshy, bright, stalk is short. The first leaves are egg-shaped, Uneven and double toothy, basis is wedge shaped, the length of the stalk equal to half length of the leaf. The upper surface is dark green, the bottom surface is pale green, the vascularization is net-shaped, the internode distance is 5-6 mm (Pic 1).

Picture 1. The initial development stages of sprouts of Malus orientalis.
1) seed, 2) begining of the third decade of March, 3) end of the third decade of March,
4) the first decade of April, 5) the second decade of April, 6) the third decade of April,
7) the first decade of May

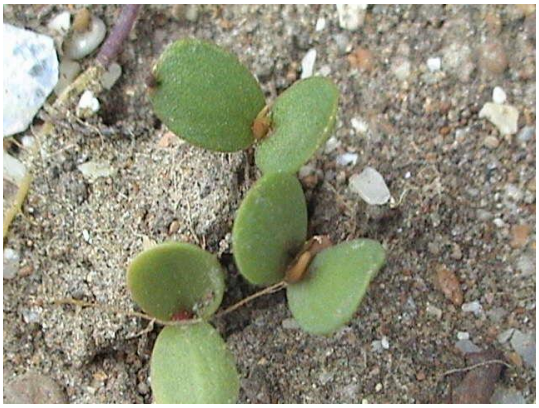
During juvenile period at the end of the third decade of March the diameter of the spronts root was 2 mm, the length of main root 3cm, the surface part was 3cm. In the first decade of April the diameter of root was 2 mm, the length of main root 5 cm and the surface part was 4 cm. In the virginal period of the growth in second decade were formed true leaves. The roots diameter was 3 mm, the length of main root 6 cm, surface part 5 cm, the length of first leaves 5 mm, the width 3 mm. The third decade of April, formed the second true leaves, root length was 7 cm, above-ground part 6 cm, cotyledon length 10 mm, width 5 mm, diameter of the root 3 mm, the first leaf length 7 mm, a width 4 mm, and the second true leaf width 3 mm and a length 4 mm. in the first decade of May cotyledon started to fall gradually. Root diameter was 4 mm, the main root length 9 cm, above-ground part 8 cm, the first leaf length 25 mm, width 12 mm, the second leaf length 15 mm, a width of 8 mm (Pic. 2, 3, 4).



Picture 2. The biometric indicators of the Height and the root system of *Malus orientalis* sprout. 1) and of the third decade of March, 2) the first decade of April, 3) the second decade of April, 4) the third decade of April, 5) the first decade of May



Picture 3. The size and growth indicators of the *Malus orientalis* species leafs. 1) The first decade of April 2) the second decade of April 3) the third decade of April 4) the first decade of May



(1)

(2)

Picture 4. The formation of the cotyledon; (1) and the true leafs, (2) of *Malus orientalis*

Thus, experiments have shown that *Malus orientalis* specie have normal growth in the ontogenesis initial development - germinal stage and it can be used as a perspective species for greening of Absheron.

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