UV-Visible Spectroscopic Technique for Prediction of Total Polyphenol Contents for a Bunch of Medicinal Plant Extracts

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Abstract

Medicinal plants extracts are a rich natural source of active phytochemicals (Polyphenols) that are known to have important health-enhancing properties. This study determines the total content of polyphenols of nine medicinal plants extracted using the accurate UV-vis spectroscopic method, along with the Orange Data Mining Tool (ODMT). The total content of polyphenols for the selected medicinal plant extracts (Daucus Carota L., Ruta Chalepensis L., Anisosciadium DC., Thymus Vulgaris, Senna Alexandrina, Myrtus Communis, Silybum Marianum Flower, Silybum marianum Leaves, and Rosa moschata) was measured using gallic acid as a standard. The intended method requires a maximum of 1 mg of Gallic acid and only 1 mg of the plant extract. The wavelength range of the maximum absorption in the UV-vis spectrum was about 270 nm. For polyphenols, the purposed method linear dynamic concertation range (44.67 to 334.7 mg GAE/g DW) with a recovery percentage range of 95.3–104.3%. This method is easy, fast, accurate, and less expensive than the Folin Ciocalteu method.

1. Introduction

Phytochemicals originate from the Greek word (Phyto) meaning plant. They are biologically active chemical compounds that are naturally present found in plant fruits, vegetables, whole grains, nuts, seeds, legumes, and other parts. They protect plant cells from pollution, dehydration, exposure to ultraviolet rays, and toxic substances as well as diseases, insects, and exposure to the ultraviolet rays [1, 2]. They are responsible for the color, smell, and flavor of every plant. They also play a great role in protecting human health from many diseases; they provide the human body with more energy than micronutrients in industrial supplements [3]. Phenolic compounds are the largest category of phytochemicals found in plants. They can be divided into several classes, including flavonoids, phenolic acids, tannins, stilbenes, and lignins. They can have various simple and complex structures such as simple phenolic acids (e.g. vanillin, gallic acid, caffeic acid), polyphenols such as stilbenes, and flavonoids, and polymers derived from these different groups [4]. Phenolic phytochemicals are an essential component of our food as they are responsible for the color and taste of fruits and vegetables. In particular, as polyphenols precipitate salivary proteins, this property could somewhat participate in the defense against their anti-nutritional effects [5].

UV-vis spectrophotometric methods assessing total phenolic contents in plant extract are cheaper, faster, and thus more accessible methods than analytical chromatography techniques, such as high-performance liquid chromatography (HPLC) [6]. Moreover, spectrophotometric assays identify compound categories rather than individual compounds. Among different spectrophotometric techniques, UV-visible spectroscopy appears to be suitable for the quantification of phenolic contents in the plant extract [7]. Phenolic compounds contain π-conjugated systems with hydroxyl-phenolic groups. They can strongly absorb UV light where π type molecular orbitals electronic transitions of phenolic groups provide the UV-visible spectrum. UV-visible spectroscopy for phenolic analysis is reported to quantify anthocyanins [8], phenolic acids [9], stilbenes [10], flavanols [11], and tannins [12]. However, the main limitations of UV-

visible spectroscopy are overestimating the specificity of the assays and assigning results to specific phenolic compounds instead of compound categories.

Total phenolic content is widely accepted as a key measure of quality for plants were predicted for total phenolic contents using two different spectrophotometric methods. Among these methods, the Folin-Ciocalteu method [13] and prediction by Orange Data Mining Tool method are commonly used [14–16]. The Folin-Ciocalteu method is broadly used to decide complete polyphenols. This reaction happens by the phosphotungstic corrosive reduction, shaping a blue chromophore comprised of a phosphotungstic-phosphomolybdenum complex [17],[18]. The maximum absorption of chromophores mainly depends on the alkaline solution and the concentration of phenolic derivatives [18]. However, this reagent decomposes quickly and easily in alkaline solutions, so a large surplus of reagents must be used to avoid the reverse reaction and to obtain a complete reaction and this excess can result in precipitates and high turbidity, making spectrophotometric analysis impossible [19]. Many modifications of this method are found in many pharmacopeias and laboratory procedures including the amount of the Folin-Ciocalteu reagents, concentration, wavelength, the standard used, reaction time, and temperature [20].

Recently, an attempt has been made based on the application of data prediction techniques for quality modeling of various products [21],[22],[23]. Due to the small amount of the natural extract of the plants and depletion of reagents used in the analysis of the phenolic compounds, it is of great importance to developing new method use consumes fewer amounts of samples and reagents. In this study, the total polyphenol content of plant extracts using new UV-Vis spectroscopy methods combined with the Orange Data Mining Tool (ODMT). The purposed method consumes a small amount of reagent and avoids possible side reactions when following the Folin Ciocalteu method. It helps to avoid potential environmental factors such as heat or cold that affect the reaction. The method developed proved to be reliable for the total polyphenols of the crude extract plants. Furthermore, the Orange Data Mining Tool (ODMT) is suitable for different kinds of users, from data mining beginners to programmers who prefer a scripting interface. Therefore, the purposed method ends up being direct, precise, reproducible, and simple to perform [24, 25]. The purposed method for the determination of polyphenol content showed excellent results, compared to those obtained directly from the Folin Ciocalteu method (Gallic acid equivalent (GAE) in mg/g of the extract) is to detect the total polyphenol content using chemical reactants.

2. Materials And Methods

2.1. Materials and Instrumentation

All chemicals used were of analytical quality; ethanol (C_2H_6O, 99.9%, Sigma-Aldrich), hexane (C_6H_{14}, 97%, Sigma-Aldrich), reagent Folin Ciocalteu ($3H_2O$. P_2O_5. $13WO_3$. $5MoO_3$. $10H_2O$) produced by PROLABO, sodium carbonate (Na_2CO_3, ≥ 99.5%, Sigma-Aldrich), and Gallic acid ($C_7H_6O_5$, 99%, PROLABO). Analytical balance (Shanghai Suisse Instrument precision 0.0001g). Rota evaporator branded (B.U.C.H.I) model R-210. equipped with a top cooler. A Shimadzu UV-Vis 1800 spectrophotometer. characterized by high resolution and an error of less than 0.01 nm. This device is linked with a microcomputer to facilitate

the processing of results. Whatman® cellulose chromatography papers 1 Chr sheets. (20 × 20) cm (GE Healthcare Life Sciences, UK) were used for paper chromatography. The absorption spectrum was measured on a UV–Vis. Spectrophotometer (UV–Vis. Cary 4000. Agilent, UK) controlled by Agilent Scan software.

2.2. Preparation of the plant extract (Maceration)

The plant samples (Daucus carota L., Ruta Chalepensis L., Anisosciadium DC., Thymus vulgaris, Senna alexandrina, Myrtus communis, Silybum marianum Flower, Silybum marianum Leaves, and Rosa moschata). Randomly selected samples were taken to the laboratory for analysis. In the laboratory, only samples with no visible malformation or bacterial damage were carefully selected. Then the samples were washed with distilled water to remove the dirt deposited on the surface of the samples. These plant samples were then naturally dried after washing with distilled water. The oven-dried samples were ground to a powder using a mortar and pestle and then sieved using a mesh sieve of 2 mm diameter. The process was left for 24 hours and the solids were filtered out using a Whatman No. 1 filter. To extract the phenolic compounds, 10 gm of each sample were taken in a conical flask and extracted with organic 100 mL solvents ethanol in a mechanical shaker with temperature control (70°C) at a constant stirring rate at 200 rpm and repeat the process three times. In the end, the solvents were separated from the extract [27]. To date, different solvents have been reported for polyphenol extreaction i.e. water, methanol, ethanol, propanol, chloroform, n-hexane, ethyl acetate, and acetone. These solvents differ in their polarity; and thus, they have different influences on the effeicny of the extraction process. However, ethanol has been selected as general solvent, as its is a safe option for plant extraction due to the fact that it leaves behind a safe to use, non-toxic oil product.

2.3. Determination of Total Polyphenol Content

Measurements of total polyphenols contents in various extracts are performed by the Singleton and Rossi method using the Folin Ciocalteu method (Gallic acid equivalent (GAE) in mg/g of the extract) [13, 26]. Volumes of 1000 µl at different concentrations of in the various extracts are carried were added to 200 µl Na_2CO_3 (10%). Approximately. 1000 µl Folin-Ciocalteu diluted ten times reagent was added to the reaction medium. After incubating in the dark at room temperature for 40 min. Folin & Ciocalteu's phenol reagent does not contain phenol. Rather, the reagent will react with phenols and nonphenolic reducing substances to form chromogens that can be detected spectrophotometrically. The absorbance was measured a λ_{max} nm. The calibration curve of Gallic acid at various concentrations (Fig. 2a) as standard [28] was given in (Fig. 2b). Therefore. The result is the milligram equivalent of Gallic acid per gram of dry weight (mg GAE / g DW). All data were presented as the mean of three separate experiments and error bars are displayed with standard error. Results were expressed as mean ± standard deviation.

2.4. Software and Tools

The Orange Data Mining Tool (ODMT) is a data-mining tool that is useful for visual programming and exploratory data analysis that can be written in Python. Orange has many components known as widgets. Each widget includes some tasks of data retrieval, preprocessing, visualization, modeling, or

evaluation. The combination of different user interface elements in a workflow allows users to create comprehensive data analysis charts on the go. With a large library of tools [29–32]. This study proposes a comparison of the Folin Ciocalteu method (Gallic acid equivalent (GAE) in mg/g of the extract) [13], and prediction methods in the Orange Data Mining Tool (ODMT) are used in this study for analysis and prediction of total polyphenols contents in the plant extracts. The methodology flow used in this study is illustrated in the following Fig. 1. In this Fig. 1, the program was designed using prophecy models that are present in Orange software to extract the desired results. which are the polyphenols contents for a bunch of plant extracts.

The purposed method consumes a small amount of reagent, estimated at 1 mg, for one time. It also helps to avoid possible side reactions when following the Folin Ciocalteu method. The method also avoids potential environmental factors such as heat or cold that affect the reaction. As shown in Fig. 1, method steps start with using a new file function. The data table function is used for adding the spreadsheet of a gallic acid solution of known is entered in this file, as predicted before in Fig. 1a. Linear regression function is used for finding the relationship between the gallic acid concentration and studied samples of a bunch of plants. Test and score function used to extract the correction factor and calculate the success rate of the prediction model and the result associated with the prediction models previously used. The second new file function is used to enter the plant extract information (sample of unknown concentration), where the second select column function is used for adding the UV-vis data (spreadsheet) of plant extracts. Finally, the matrix function is used to link the concentrations of Gallic acid (standard) and the plant extracts (unknown samples).

3. Results And Discussion

Total polyphenol contents in the Plants of weed were estimated in two ways, where the closeness ratio between the two results was observed. The first result was obtained by the method (Gallic acid equivalent (GAE) in mg/g of the extract) of Singleton and Rossi [26]. The second result obtained by a new method by ODMT predictor is also presented in this paper. Therefore, from this research it can be said that the value of polyphenols can be predicted in any plant in the future, as mentioned in the introduction, the total polyphenol content can be predicted and apply it to all medicinal plants using the new method.

3.1. Prediction of Various Concentrations of Gallic acid

Various concentrations of Gallic acid in ethanol at 70°C (standard solutions) were prepared and recorded using a UV-vis spectrophotometer (Fig. 2). Figure 2 shows the calibration curve of the absorbance at xx nm versus the concertation of the Gallic acid, with a correction factor (R^2 = 0.9998). Finally, the data of the UV-vis were entered into the Orange Data Mining Tool (ODMT), to be considered as the database detection of unknown total polyphenols concentration in plant or mixture. The prepared solutions were recorded in the UV-vis spectrophotometer as given in Fig. 2, and then the data matrices were stored in the Orange Data Mining Tool (ODMT).

2.2. Determination of total content of polyphenols in plant extract

The total content of polyphenols for a group of plant extracts (Daucus carota L., Ruta Chalepensis L., Anisosciadium DC., Thymus vulgaris, Senna alexandrina, Myrtus communis, Flower of Silybum marianum, leaves of Silybum marianum, and Rosa moschata) was measured using gallic acid as a standard. The percentage of the contents of polyphenols in the different plant extract reveal that the polyphenols concentration in the samples ranges from 44.67 to 334.7 mg GAE/g DW).

Table 1
The percentage of the contents of polyphenols in the different plant extract measured using the Folin Ciocalteu method as a reference method

Plant extract	Polyphenol mg GAE/g DW
Daucus carota L.	51.10 ± 17.56
Ruta chalepensis L.	54.94 ± 20.86
Anisosciadium DC.	44.67 ± 1.10
Thymus vulgaris	141.8 ± 2.86
Senna alexandrina	45.14 ± 2.84
Myrtus communis	334.7 ± 15.17
Silybum marianum Flower	44.23 ± 2.89
Silybum marianum Leaves	64.73 ± 17.59
Rosa moschata	258.59 ± 8.29

3.3. Mathematical Calculations

Equation 2 was used to estimate the polyphenols contents in different plant extracts using data (Fig. 2a) stored in the Orange Data Mining Tool (ODMT)

$$C_{Sample} = \left(\frac{C_{ODMT}}{Absor \times C_{Manuelle}}\right) \times 100 \qquad (2)$$

Where

C_{Sample}: *Concentration of polyphenol of plant extract (mg GA / g DW)*

C_{ODMT}: *Concentration calculated by Orange Data Mining Tool in* $\frac{mg}{ml}$.

$C_{Manuelle}$: Concentration of plant extract (mg /ml) .

Absor: Sample absorbance in λ max in (nm), it varies from one plant to another plant.

λ max : Larger absorption of the sample in (nm).

2.4. Spectroscopic Prediction of Total Polyphenols Content

A series of dilutions were prepared for each plant extract, as shown in Table 2. Then, the UV-vis spectrum of each sample and their series of dilutions were recorded separately, given in Fig. 3 and Fig. 4. The data's a matrix that connects the concentrations and absorption of each plant is then entered for each of the total polyphenol concentrations for the plant extracts (dilution series) in the second part of the program and then the following concentrations from the Orange Data Mining Tool (ODMT) were obtained. Table 3 indicates that the results obtained in the reaction method are very close to the results obtained from the Orange Data Mining Tool, with a recovery percentage range of 95.3–104.3%.

Table 2
Comparison of the results of the two methods.

Daucus carota L.							
Concentration [1] (mg / ml)	0.3000	0.2700	0.2500	0.2300	0.2140	0.2000	0.1875
Concentration [2] (mg / ml)	0.0242	0.0238	0.0231	0.0219	0.0202	0.0190	0.0174
Absorbance at λ_{max} = 255nm	1.9740	1.8000	1.6530	1.5070	1.3900	1.3010	1.2100
Ruta chalepensis L.							
Concentration [1] (mg / ml)	0.0100	0.1000	0.0500	0.2500	0.2083	0.1785	—
Concentration [2] (mg / ml)	0.0011	0.0110	0.0564	0.0248	0.0219	0.0201	—
Absorbance at λ max = 261 nm	0.1290	0.9210	0.4860	2.1950	1.8410	1.5970	—
Anisosciadium DC.							
Concentration [1] (mg / ml)	0.5000	0.4545	0.4166	0.3846	0.3571	0.3333	0.3125
Concentration [2] (mg / ml)	0.0270	0.0268	0.0265	0.0255	0.0240	0.0234	0.0230
Absorbance at λ max = 266 nm	2.1580	2.0060	1.8280	1.6720	1.5670	1.4900	1.4220
Thymus Vulgaris							
Concentration (1) (mg / ml)	0.2300	0.2142	0.2000	0.1875	0.1764	0.1666	0.1166
Concentration (2) (mg / ml)	0.0262	0.0260	0.0259	0.0246	0.0241	0.0233	0.0201
Absorbance at λ max = 282 nm	1.6700	1.5430	1.4480	1.3140	1.2840	1.2180	0.9920
Senna Alexandrina							
Concentration (1) (mg / ml)	0.3000	0.2727	0.2500	0.2307	0.2142	0.2000	0.1875
Concentration (2) (mg / ml)	0.0271	0.0266	0.0258	0.0252	0.0238	0.0234	0.0224
Absorbance at λ_{max} = 265 nm	2.3560	2.1160	1.9060	1.7520	1.6050	1.5180	1.4190
Myrtus communis							
Concentration (1) (mg / ml)	0.0500	0.0454	0.0416	0.0384	—	—	—
Concentration (2) (mg / ml)	0.0146	0.0131	0.0122	0.0109	—	—	—
Absorbance at λ_{max} = 265 nm	1.0310	0.9300	0.8700	0.7840	—	—	—

Daucus carota L.							
Silybum marianum Flower							
Concentration (1) (mg / ml)	0.3000	0.2727	0.2500	0.2300	0.2140	0.1875	—
Concentration (2) (mg / ml)	0.0265	0.0257	0.0245	0.0236	0.0235	0.0210	—
Absorbance at λ_{max} = 265 nm	2.2150	1.9720	1.8450	1.7150	1.6030	1.4150	—
Silybum marianum Leaves							
Concentration (1) (mg / ml)	0.3000	0.2727	0.2500	0.2307	0.2142	0.2000	0.1875
Concentration (2) (mg / ml)	0.0263	0.0246	0.0224	0.0216	0.0212	0.0197	0.0183
Absorbance at λ_{max} = 267nm	1.6310	1.4370	1.3290	1.2370	1.1380	1.0620	0.9890
Rosa moschata							
Concentration (1) (mg / ml)	0.1250	0.1040	0.0892	0.0781	0.0694	0.0625	
Concentration (2) (mg / ml)	0.0260	0.0230	0.0189	0.0167	0.0154	0.0134	
Absorbance at λ_{max} = 266 nm	1.6560	1.4380	1.1960	1.0430	0.9720	0.8440	

Concentration [(1)]: Concentration of the extract of samples of the for the sample of the plant which in manual preparation (mg/ml).

Concentration [(2)]: Concentration of the extract of samples from the plant sample of the plant can be calculated in software ODMT (mg/ml).

Absorbance (nm): Absorbance of the plant extract in λ_{max} of the plant extract in (nm).

Table 3
Comparison of total polyphenols content in leaves, twigs end flowers by Orange Data Mining Tool (this work) and Folin Ciocalteu method (reference method) in terms of the Gallic acid equivalent (GAE) in mg/g of the extract.

Samples	Polyphenol (mg GAE/g DW)		Recovery %
	Method (ODMT)	Method (GAE)	
Daucus Carota L.	48.71 ± 7.60	51.10 ± 17.56	95.32
Ruta Chalepensis L.	57.66 ± 12.56	54.94 ± 20.86	104.95
Anisosciadium DC.	46.59 ± 3.69	44.67 ± 1.10	104.29
Thymus vulgaris	144.73 ± 29.82	141.8 ± 2.86	102.06
Senna Alexandrina	46.26 ± 7.93	45.14 ± 2.84	102.48
Myrtus Communis	337.61 ± 26.99	334.7 ± 15.17	100.86
Silybum Marianum Flower	45.41 ± 4.51	44.23 ± 2.89	102.66
Silybum Marianum Leaves	64.19 ± 11.10	64.73 ± 17.59	99.16
Rosa Moschata	250.38 ± 4.45	258.59 ± 8.29	96.82
ODMT = Orange Data Mining Tool.			

GAE = the gallic acid equivalence method

Recovery % = Conc given by Method (ODMT) x 100 / Conc given by Method (ODMT)

4. Conclusions

UV-vis spectroscopic methods for the prediction of the total polyphenol contents of medicinal plants extracts can be improved by adjusting the reagent concentration, pH, reaction time, temperature, and absorption wavelength. In this study, we developed an accurate UV spectrophotometric method for the determination of polyphenol contents in nine medicinal plant extracts by the mean of with the Orange Data Mining Tool (ODMT). The method used very small amounts of reagents; requires a maximum of 1 mg of Gallic acid reagent and only 1 mg of the studied plant extract. The determination of total polyphenol contents is based on creating a database (standard calibration curve) associated with the Gallic acid reagent and the Orange Data Mining Tool (ODMT). The total polyphenolic contents obtained by the standard calibration curve of the Folin Ciocalteu method (Gallic acid equivalent (GAE) in mg/g of the extract) are in agreement with the results predicted by the ODMT. The purposed method linear dynamic concertation range (44.67 to 334.7 mg GAE/g DW) with a recovery percentage range of 95.3–104.3%. The purposed method opens up for researchers to other studies such as determining the proportion of flavonoids, flavanols, dragon and each substance is related to plants and so the oxidation value of plants can be determined.

Declarations

Author Contributions: Conceptualization. S.E.L. F.G., and A.B. (Abderrhmane Bouafia) and A.B. (Ahmed Barhoum); methodology. A.B. (Abderrhmane Bouafia) and A.B. (Ahmed Barhoum); software. F.G. and A.B. (Abderrhmane Bouafia); validation. A.B. (Abderrhmane Bouafia). F.G. and A.B. (Ahmed Barhoum); formal analysis. S.E.L.. A.R., A.T., and F.G.; investigation. A.B. (Abderrhmane Bouafia); resources. A.R.; data curation. S.E.L.; writing—original draft preparation. F.G., A.B. (Abderrhmane Bouafia), and A.B. (Ahmed Barhoum); writing—review, and editing. F.G.. A.B. (Abderrhmane Bouafia). and A.B. (Ahmed Barhoum); supervision. S.E.L. A.R.. authors have read and agreed to the published version of the manuscript.

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Figures

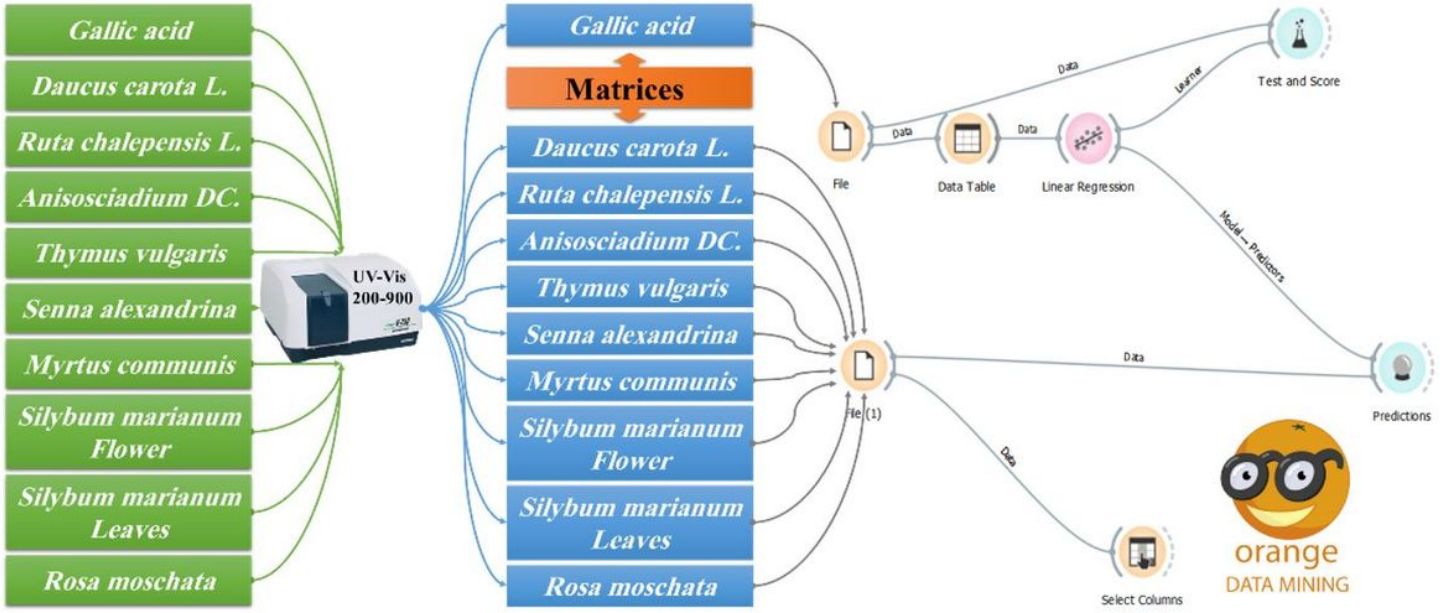

Figure 1

Flow chart showing the method steps and the workflow in Orange Software to extract the desired results.

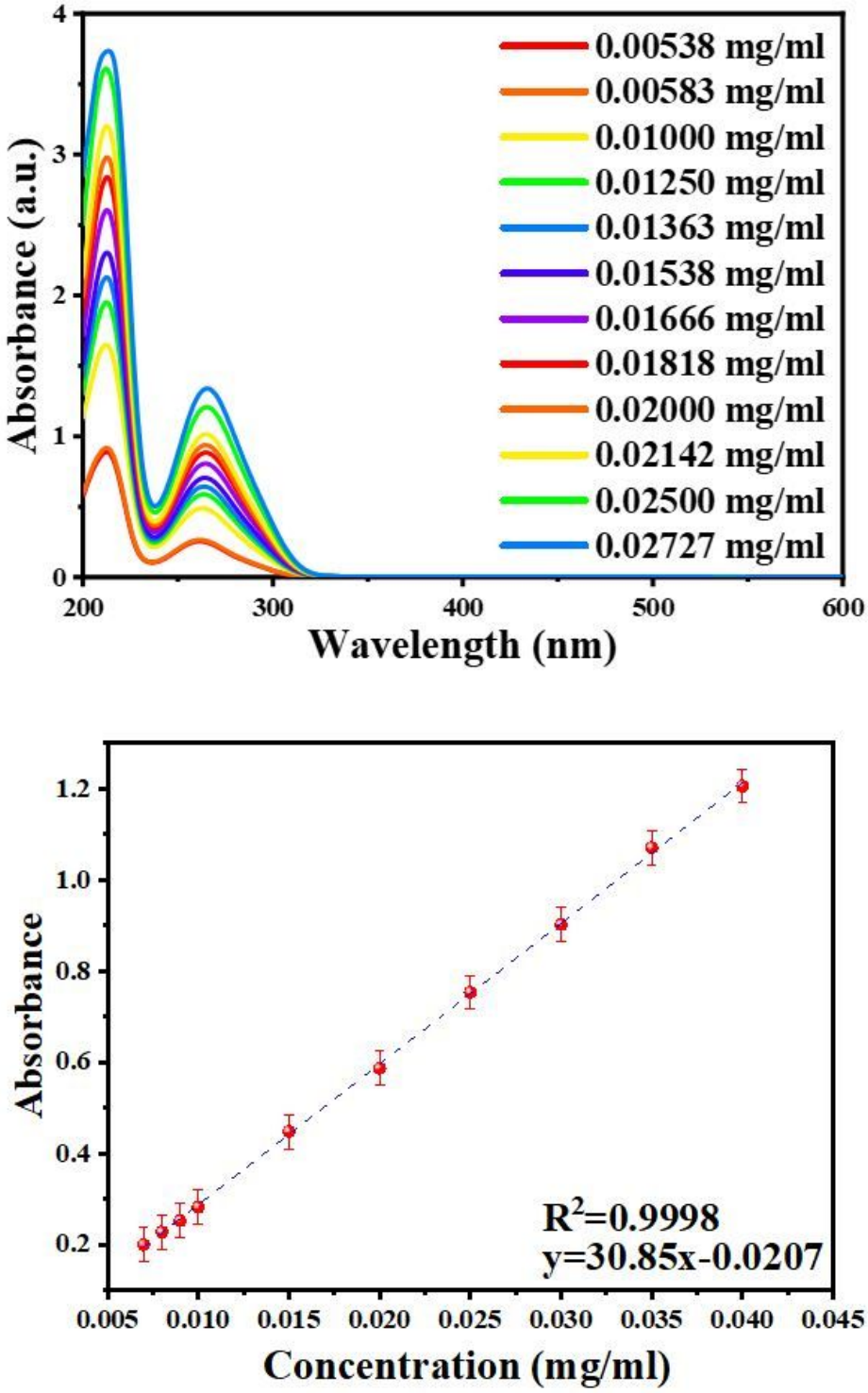

Figure 2

UV-vis spectrophotometric analysis of gallic acid: (a) UV-absorbance curve at different concentrations from 0.00538 to 0.02727 mg/ml; (b) Standard solutions calibration curve.

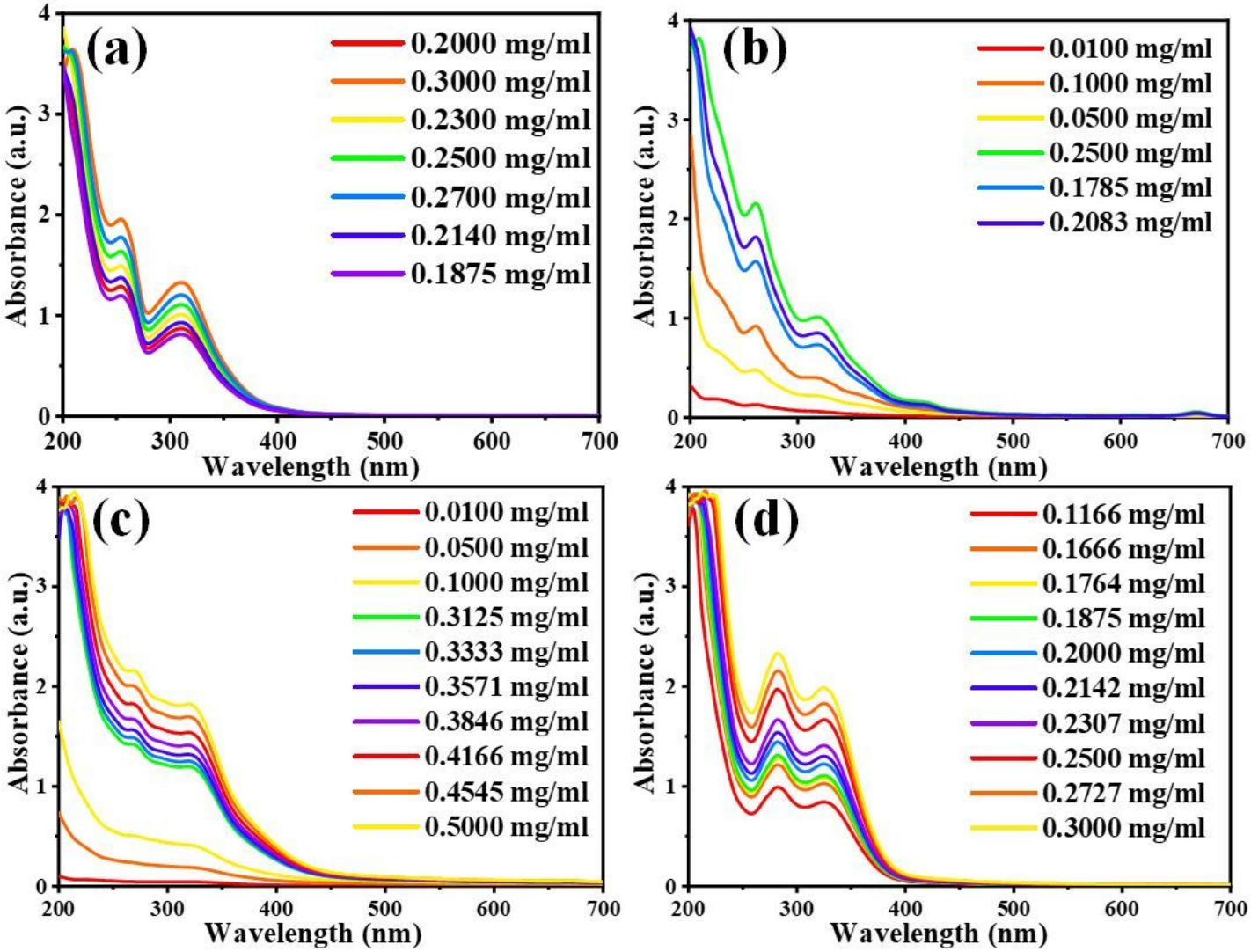

Figure 3

UV-absorbance curve of the plants: (a) Daucus carota L. (b) Ruta Chalepensis L. (c) Anisosciadium DC. and (d) Thymus vulgaris at deferent concentration.

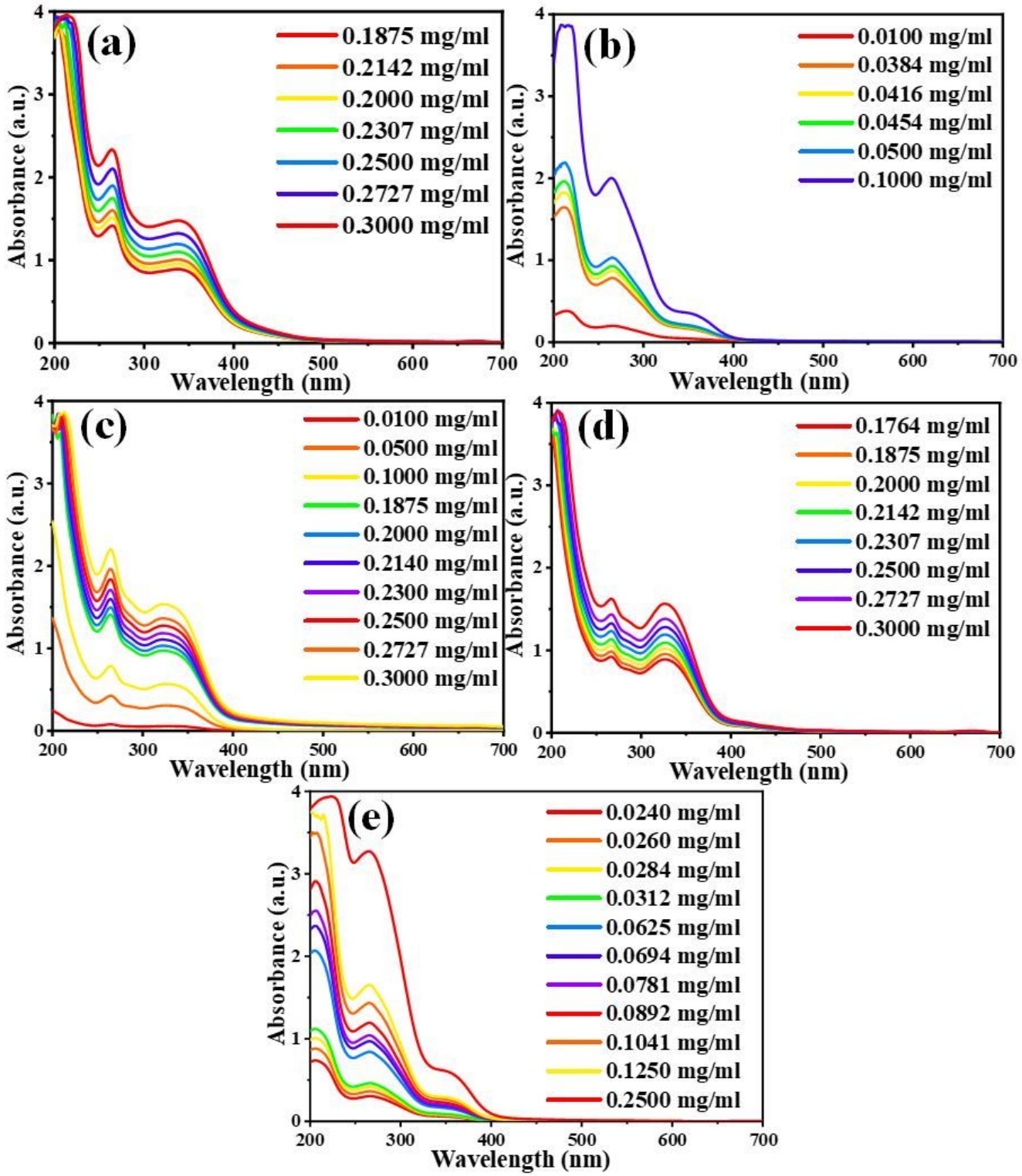

Figure 4

UV-absorbance curve of the plants: (a) Senna alexandrina. (b) Myrtus communis. (c) Silybum marianum Flower. (d) Silybum marianum Leaves. and (e) Rosa moschata at deferent concentration.