



# CHEMO-TAXONOMICAL STUDY FOR CULTIVATED SPECIES OF THE GENUS *MORUS* L. (MORACEAE) IN NORTHERN IRAQ

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## Abstract

The present study deals with a chemical study of the cultivars of *Morus* L. genus' species, cultivated in northern Iraq, namely Beautiful Day, Big White, Rease, Greece, Pearl, Border Sweet and Pendula for the species. *Morus alba* L., Kokuso Korean for *Morus latifolia* Poir, Amarah for *Morus rubra* L., Shami for *Morus nigra* L., King White and Dwarf for *Morus macroura* Miq, Tice and Wellington for *Morus hybrid*. Six medically important phenolic compounds were identified in the extract of leaves of the studied cultivars by using high performance liquid Chromatography (HPLC) technique. The cultivars showed variations in terms of containing the compounds and their concentrations; the phenolic acid (Gallic acid) was diagnosed in only two of the cultivars, namely (Greece) and (Big White) for the species *Morus alba* and 5 flavonoids; Apigenin, Kaemferol, Rutin, Luteolin and Quercetin in all the cultivars of the species under study. These differences between the cultivars regarding the chemical content can be relied upon as a guideline and a classification indicator in the identification and isolation of the studied cultivars. The spectral study included the use of UV ray technology and for the first time the identification of the absorption values of chlorophyll for cultivars of the genus' species adopted in the study and their wavelengths, as it showed a clear variation among the cultivars in wavelengths at the highest absorption ( $\lambda_{max}$ ), the thing that made it possible to use in the diagnosis and separation of cultivars into groups.

**Key words:** Chemotaxonomy, genus, *Morus*, Moraceae

## Introduction

The genus *Morus* L., which belongs to the mulberry family Moraceae, Rosales, grows in the form of trees and Perennial shrubs, mono or dioecious, grows in different types of soil and blooms well in acidic soils of pH (6.2-7) and grows at a temperature (18-30) C°. It is genetically complex and has great potential for variation, adaptation and spread on a wide range in different environments (Kafkas *et al.*, 2008; Wani, 2012).

Plants abound in huge numbers of different chemical compounds, which are increasingly being discovered as a result of the great progress in the field of multiple chemical analysis methods, the possibility of diagnosing and detecting these compounds and considering the great benefits and importance of these chemical compounds in the field of plants taxonomy.

Radford *et al.*, (1974) indicated that adding Chemotaxonomy information to other results' information such as phenotypic, anatomical and cellular information

can provide us with a solid basis for botanical taxonomy decisions; they also indicated that phenolic compounds are well-known chemicals that are widely used in taxonomy because they are widespread and exist usually in leaves, flowers, fruits, wood and cotyledons.

Al-Mousawi (1987) reported that chemicals in plants make an effective contribution to nature in determining the flavor and taste of the plants in which they are found and in many cases the species and the cultivar can be distinguished by the taste of these plants regardless of any other characteristics.

Samuel & Luchsinger (1987) mentioned that Flavonoids have important uses in the chemical classification due to their absolute presence in almost all higher plants and their ease of separation and diagnosis no matter how little plant matter there is, in addition to their presence in varying concentrations, the presence of fixed genetic bases for these changes and their chemical stability that contributed to the possibility of adopting these compounds as classification indicators in most Lower and

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higher plant groups.

Flavonoids are the largest known group among the more than 1,000 phenolic compounds in addition to lignin and tannins (Mullen *et al.*, 2002).

The values of flavonoids and phenolic compounds differ among species due to their genetic characteristics, environmental conditions and stages of maturity (Ercisli & Orhan, 2007).

Al Aroussi & Weassifi (2007) showed that the basis of plant division have advanced in the use of biochemical properties due to the development of methods for accurate separation of compounds, the basis of which was the detection of certain compounds to distinguish between taxa. Compounds of Low-presence in plants, such as flavonoids, have been used for this distinction.

Memon *et al.*, (2010) diagnosed many phenolic compounds in the leaves and fruits of species of mulberry, namely *M. alba* L., *M. nigra* L. and *M. laevigata* W. which grow in Pakistan.

Ju *et al.*, (2018) diagnosed 7 flavonoids such as (Rutin, Isoquercitrin, Kaemferol and Quercetin) when studying 12 cultivars of white mulberry (*Morus alba* L.) grown in Korea.

Balik and others (2019) were able to diagnose several phenolic compounds including Rutin, Quercetin, Gallic acid and Catechin when studying 13 genotypes of black and white berries.

CISER (2010) indicated that plants contain Primary pigments and secondary pigments necessary to absorb the energy used in photosynthesis. She used the most efficient wavelengths to estimate the absorption of chlorophyll in leaves of different colours of *Coleus* plant.

Abdel-Fattah *et al.*, (2010) indicated that the chemical composition may be beneficial in the spectroscopic studies of the plant in their dependence on the qualitative detection of certain substances and comparing them to another plant and to recognize the difference by measuring the highest wavelength which corresponds to its highest absorption, when using the spectral method to diagnose the absorbance of the organic effective groups to distinguish between two species of the genus *Euphorbia* spp., which wildly grows in Anbar Governorate.

The spectroscopic studies based on ultraviolet or infrared devices with the science of taxonomy will open a new horizon in which important new differences are found in isolating species and cultivars within the same genus (Al-Rajab *et al.*, 2014).

It has been noticed in recent times that great interest

in planting mulberry trees and the consumption of their fruits increased rapidly, due to their good taste, high nutritional value and biological effectiveness and given the medical and economic importance of the cultivars of the mulberry species and the lack of studies regarding the cultivars of species of this genus in Iraq, so the study aimed to diagnose Some phenolic compounds of quantitative and qualitative medicinal importance, as well as identifying the absorption values of chlorophyll in the extract of the leaves of the genus *Morus* L. in northern Iraq.

## Materials and Methods

The study relied on samples collected from several regions of northern Iraq throughout field trips during the growth period of 2018-2019. Samples diagnosed by the Ministry of Agriculture and cultivated in nurseries and fields affiliated with the ministry were collected for the studied cultivars from several regions in Nineveh and Duhok Governorates and the Nineveh and Duhok horticulture stations.

### Extraction, acid analysis and diagnosis of phenolic compounds

The method of Grand *et al.*, (1988) was followed in the preparation of ethanol extract modified from the primary method of Verporte *et al.*, (1982). The layer formed of the raw extract was taken after evaporation, as 2 g was obtained and the samples were preserved by freezing in sterile glass bottles with a tight lid until used. The acidic decomposition of the leaves extract for the cultivars under study was conducted according to the Harborne method (1973). The raw extract was dissolved in 3 ml methanol and preserved until used. Phenolic compounds were diagnosed according to the method of Mradu *et al.*, (2012) using HPLC technology via a German-origin device Sykam brand. The separation process resulted in drawing curves or peaks for each of the standard compounds and the samples to be measured in conjunction with the special retention time of each compound as demonstrated in the tables of curves regarding the separation process. Values of retention time for standard compounds were approved by matching them with the retention values for the compounds that were separated from the extract under study and that were injected into the apparatus under the same conditions as shown in table 1.

The concentration of the sample was calculated according to the method of Sousse and Ali (2017) using the following formula:

$$\text{Concentration of Sample} = (\text{area of sample}) / (\text{area}$$

**Table 1:** Retention time for standard phenolic compounds prepared in a laboratory setting with applying HPLC technology to them.

	Standard sample	Retention time (Rt)
1	Epigenin	3.06
2	Kamferol	3.79
3	Rutin	4.86
4	Luteolin	5.18
5	Gallic acid	5.93
6	Quercetin	6.56

of standard) × Conc. of standard × Dilution factor

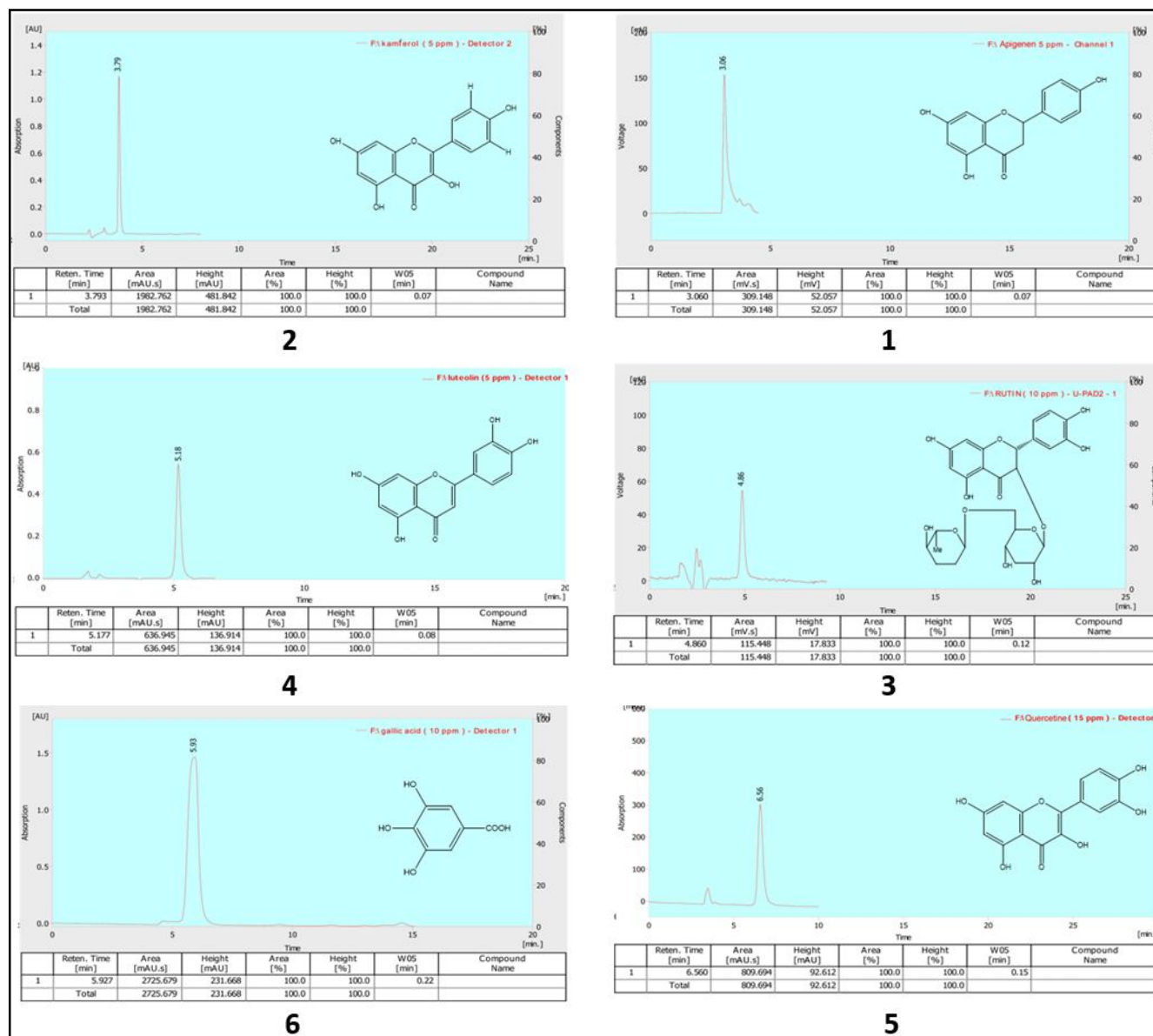
### Spectral study

The spectral study was done using the UV device, UV Spectro Photometer, of Japanese origin Shimadzu brand using (CISER, 2010) method, 0.3 g of chlorophyll

extract was prepared from the leaves of the varieties under study, then the leaves were cut into small pieces and placed in a ceramic mortar and added to it 10 ml of absolute ethanol alcohol and crushed until tiny pieces remain, then 20 ml of absolute ethanol alcohol was added to the mortar, then the solution was filtered into a baker through the separation funnel using filter paper, then the leachate was put into the device's quartz containers and the absorptions were drawn against the positive lengths of 400- 800 nanometres.

### Results and Discussion

Results of HPLC analysis showed the diagnosis of six Phenolic compounds, which are Apigenin, Kaemferol, Rutin, Luteolin, Quercetin and Gallic acid, which showed variation in the chemical content of the leaves' extract of

**Fig. 1:** Standard curves and scientific formulas of compounds.

the studied *Morus* L. genus table 2 and 3 and Fig. 2.

**Apigenin:** the highest concentration appeared in the Pendula cultivar of *M. alba*; it reached (29.30)  $\mu\text{g}\cdot\text{g}^{-1}$  and the lowest concentration was (6.87)  $\mu\text{g}\cdot\text{g}^{-1}$  in the Kokuso Korean cultivar of the *M. latifolia* species.

**Kaemferol:** the highest concentration was 4.4  $\mu\text{g}\cdot\text{g}^{-1}$  in the Pendula cultivar of type *M. alba* with the lowest concentration (0.73)  $\mu\text{g}\cdot\text{g}^{-1}$  in the pearl cultivar of the *M. alba* species, the rest of cultivars of the species ranged between these two values.

**Rutin:** its concentration varied greatly among species cultivars, as it reached the highest value of its concentration (1451.02)  $\mu\text{g}\cdot\text{g}^{-1}$  in the Pendula cultivar for the species *M. alba* and at its lowest concentration (48.63)  $\mu\text{g}\cdot\text{g}^{-1}$  in the Amarah cultivar of the species *M. rubra* and the rest of cultivars were between (110.99-493.91)  $\mu\text{g}\cdot\text{g}^{-1}$ .

**Luteolin:** Its highest concentration value was found to be (6.64)  $\mu\text{g}\cdot\text{g}^{-1}$  in the King White cultivar of *M. macrourea* species and the lowest concentration value was (2.66)  $\mu\text{g}\cdot\text{g}^{-1}$  in the Big White cultivar of the *M. alba* species.

**Quercetin:** The highest concentration of it was found in the cultivar Pendula of the *M. alba* species and was (41.94)  $\mu\text{g}\cdot\text{g}^{-1}$  and the lowest concentration was (4.78)  $\mu\text{g}\cdot\text{g}^{-1}$  in the cultivar pearl of the *M. alba* species and the remaining cultivars of species were between (6.16 - 36.01)  $\mu\text{g}\cdot\text{g}^{-1}$ . It is clear from the results that all cultivars of the genus' species contained the compounds Apigenin, Kaemferol, Rutin, Luteolin and Quercetin. The gallic acid, on the other hand, was diagnosed only in the Big White and Greece cultivars of the *M. alba* species, which can be considered of Taxonomic importance in isolating them from other species under study.

**Table 2:** Phenolic compounds separated from the leaves of cultivars of the *Morus* L genus' species using HPLC technique.

Species	Cultivars	Apigenin	Kaemferol	Rutin	Luteolin	Gallic acid	Quercetin
<i>M. alba</i>	Beautiful Day	+	+	+	+	-	+
	Big White	+	+	+	+	+	+
	Rease	+	+	+	+	-	+
	Greece	+	+	+	+	+	+
	pearl	+	+	+	+	-	+
	Border Sweet	+	+	+	+	-	+
	Pendula	+	+	+	+	-	+
<i>M. latifolia</i>	Kokuso Korean	+	+	+	+	-	+
<i>M. rubra</i>	Amarah	+	+	+	+	-	+
<i>M. nigra</i>	Shami	+	+	+	+	-	+
<i>M. macrourea</i>	King White	+	+	+	+	-	+
	Dwarf	+	+	+	+	-	+
<i>M. hybrid</i>	Tice	+	+	+	+	-	+
	Wellington	+	+	+	+	-	+

**Table 3:** Concentrations of phenolic compounds in leaves of cultivars of the *Morus* L genus' species, measured in  $\mu\text{g}\cdot\text{g}^{-1}$ .

Species	Cultivars	Apigenin	Kaemferol	Rutin	Luteolin	Gallic acid	Quercetin	Total
<i>M. alba</i>	Beautiful Day	10.76	2.13	263.13	4.24	-	14.98	297.24
	Big White	7.62	0.77	149.71	2.66	1.48	6.61	168.85
	Rease	10.52	1.67	216.73	5.38	-	12.59	246.89
	Greece	12.14	1.54	172.48	5.91	1.42	12.97	206.46
	pearl	8.47	0.73	110.99	4.32	-	4.78	129.29
	Border Sweet	8.51	1.56	214.67	4.52	-	12.92	242.18
	Pendula	29.30	4.40	1451.02	7.03	-	41.94	1533.69
<i>M. latifolia</i>	Kokuso Korean	6.87	1.37	472.46	6.12	-	36.02	522.84
<i>M. rubra</i>	Amarah	10.01	1.74	48.63	4.37	-	27.14	91.89
<i>M. nigra</i>	Shami	8.58	1.21	316.65	4.89	-	24.63	400.96
<i>M. macrourea</i>	King White	29.21	1.75	400.58	16.64	-	16.00	464.18
	Dwarf	12.65	1.26	312.99	4.90	-	26.13	357.93
<i>M. hybrid</i>	Tice	10.05	1.09	250.19	4.96	-	21.07	287.36
	Wellington	8.07	1.45	493.91	4.89	-	25.10	533.42

The results of the HPLC technique showed the presence of differences in the content of phenolic compounds in the alcoholic extract of leaves, that were diagnosed and detected among the cultivars of the studied genus, these variations were adopted as important taxonomical evidence. The flavonoids; Apigenin, Kaemferol, Rutin, Luteolin and Quercetin were diagnosed in all cultivars of the species of the genus *Morus* L. under study and this constitutes an evolutionary phenomenon of significance, since such a presence of flavonoids in all cultivars of species supports the existence of a common bond between the cultivars of species of this genus in terms of their chemical properties.

As for the phenolic acid, Gallic acid, it was only diagnosed in the Big White and Greece cultivars of *M. alba* species.

This finding is consistent with what Pieprzyk-kokcha *et al.*, (2013) and Rodrigues *et al.*, (2019) found in mulberry leaves.

It also agrees with what Divis, Heywood (1963) and Al-Moadadi (2003) stated that the study of phenolic compounds has a high taxonomic value in isolating species and cultivars, especially phenotypically similar ones and in creating evolutionary links between them. The current study also stated the presence of variations in the concentrations of diagnosed phenolic compounds cultivars of the species significantly, which consolidated the taxonomic importance of this study and are taken as taxonomic evidence to isolate and separate them from each other, as the cultivar Pendula of the species *M. alba* recorded the highest concentration of four flavonoids, namely, Apigenin, Kaemferol, Rutin and Quercetin, which further strengthened its clear isolation status from the

**Table 4:**  $\mu$  max spectrum and its absorption values in cultivars of the *Morus* L. genus' species under study.

Species	Cultivars	Absorption	lmax
<i>M. alba</i>	Beautiful Day	3.055	664
	Big White	4.000	650
	Rease	3.424	664
	Greece	2.994	664
	pearl	2.813	664
	Border Sweet	3.151	662
	Pendula	2.987	664
<i>M. latifolia</i>	Kokuso Korean	4.000	458
<i>M. rubra</i>	Amarah	3.534	414
<i>M. nigra</i>	Shami	4.000	468
<i>M. macroura</i>	King White	3.593	438
	Dwarf	3.745	428
<i>M. hybrid</i>	Tice	3.342	664
	Wellington	2.951	664

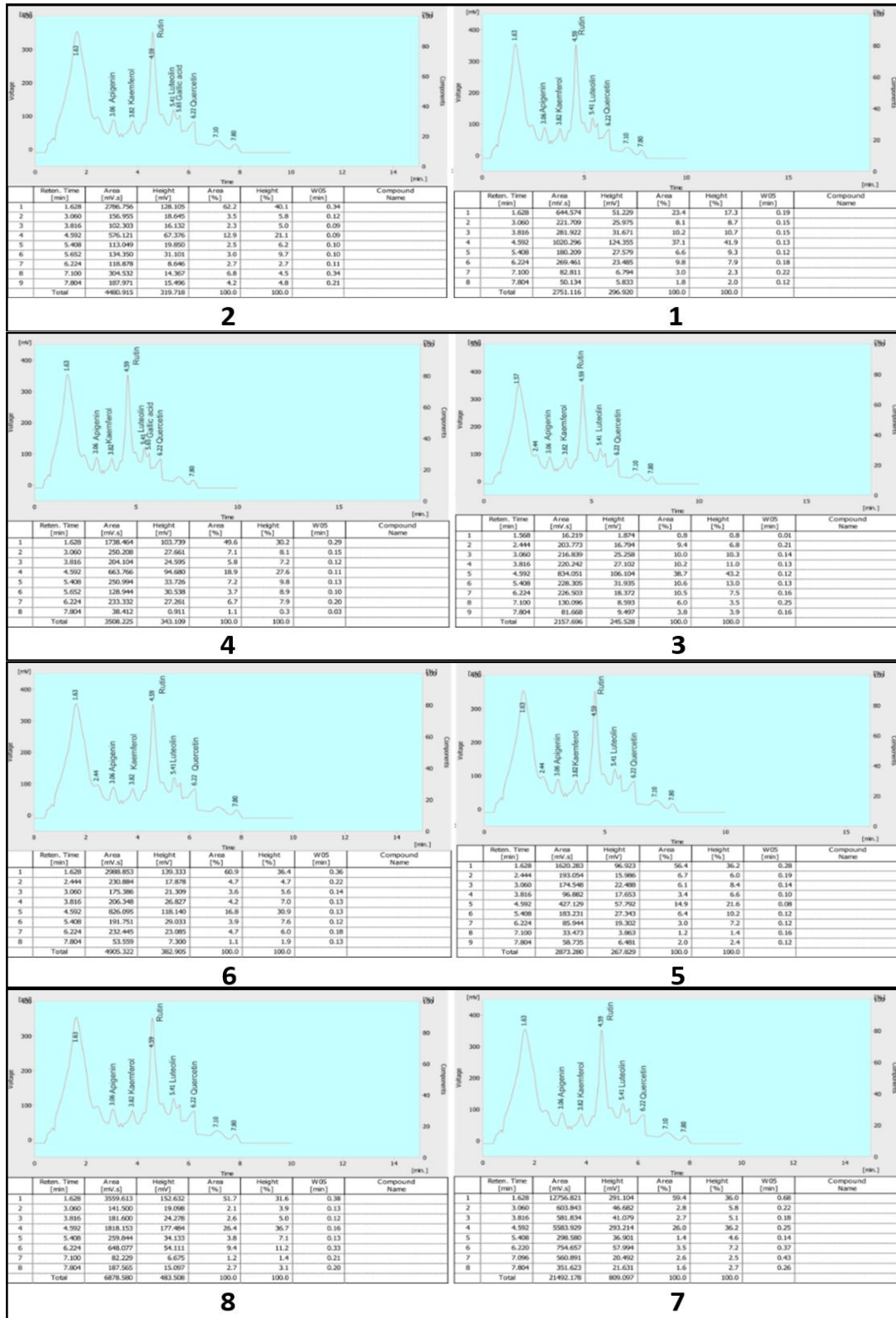
other varieties adopted in the study.

The results of the study also showed that the compound Luteolin recorded the highest concentration (16.64)  $\mu\text{g. g}^{-1}$  in the King White cultivar of the species *M. macroura* and the lowest concentration (1.42)  $\mu\text{g. g}^{-1}$  in the cultivar Greece of *M. alba* species. These results are consistent with what Ercisli & Orhan (2007) confirmed that the values of phenolic compounds differ between the species and cultivars of mulberries due to their genetic characteristics, environmental conditions and stages of maturity.

The spectral study showed clear variations of taxonomic importance in the alcoholic extract of chlorophyll, between the cultivars of species studied and based on the  $\lambda_{\text{max}}$  values which represent the highest absorption the cultivars of species were distinguished into four groups: The first included all types of the two species, *M. alba* and *M. Hybrid*, which recorded the highest absorption at wavelength between (650-664) nanometers and the second of which the cultivars of the species *M. nigra* and *M. latifolia* had the highest absorption at wavelength that ranged between (468-458) nanometers and the third represented by the cultivars of the species *M. macroura*, in which the highest absorption was at wavelength between (428-438) nanometers and the fourth was unique to the cultivar of species *M. rubra* at a wave length (414) nanometers for the highest absorption, as shown in table 4 and Figs. 3 and 4.

The spectroscopic study proved an important and prominent role in assisting and supporting other taxonomic indices as it showed clear differences among the cultivars of *Morus* L. genus' species, as it was possible to use them for classification in separating and isolating the cultivars of species, based on the  $\mu$  max values of the leaf chlorophyll extract, into four groups: the first: its  $\mu$  max values ranged between (650 - 664) nanometers and included the cultivars of the species *M. alba* and *M. Hybrid*, the second: its  $\mu$  max values ranged between (458-468) nm and included the cultivars of species *M. latifolia* and *M. Nigra*, the third: the values of which were between (428-438) nanometers and included the cultivars of *M. macroura* species and the fourth of which the cultivar Amarah of the species *M. rubra* was its sole member and recorded the minimum  $\mu$  max value (414) compared to the other species.

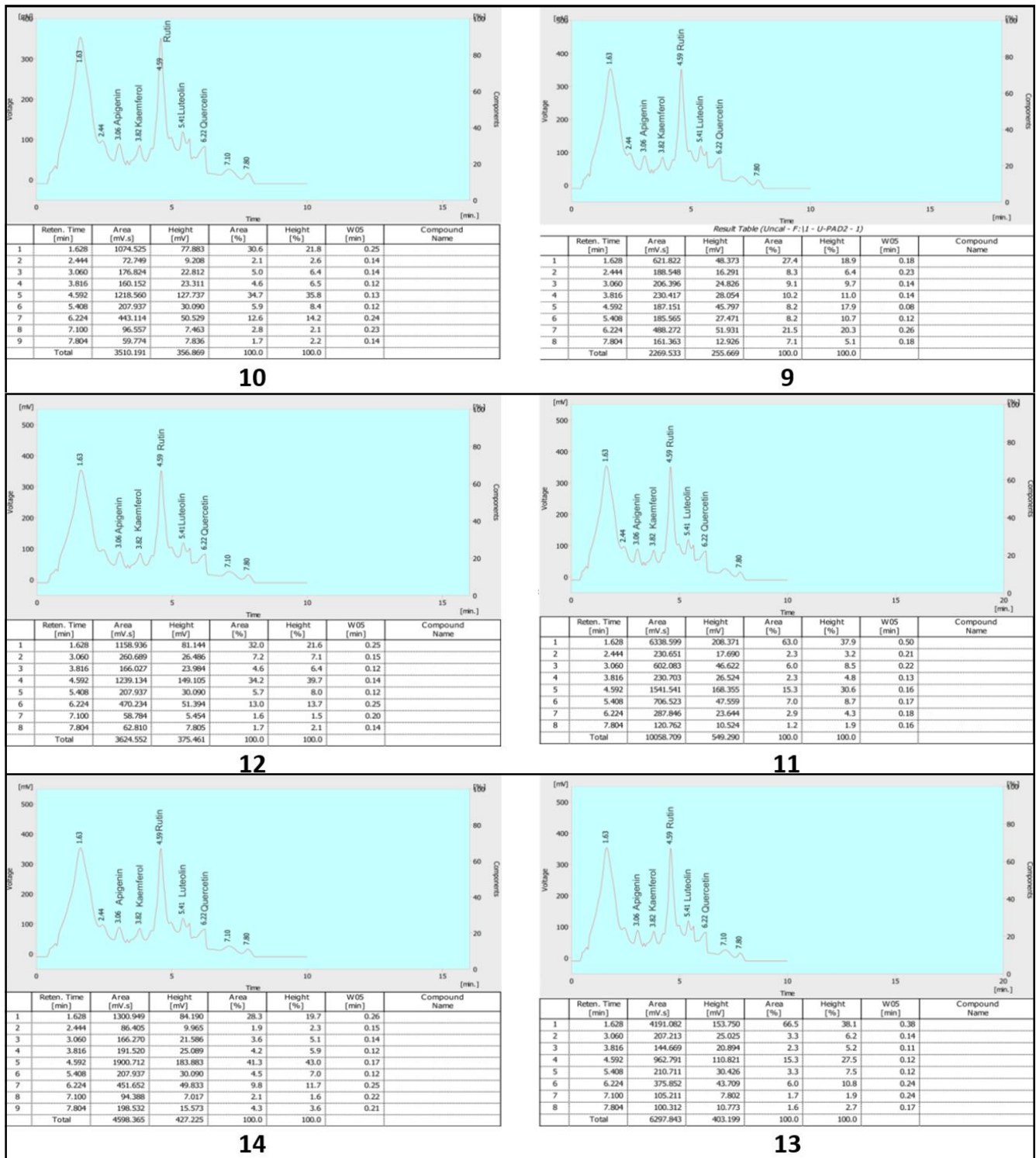
Based on the  $\mu$  max values, the validity is clear for the affiliation of the cultivars with their types, as the values of the cultivars belonging to the same species have converged significantly from each other and have been isolated from the rest of the cultivars of other species.



**Fig. 2:** Curves of the phenolic compounds separated from the leaves of cultivars of the *Morus L.* species under study.

1. *M. alba* (Beautiful Day).
2. *M. alba* (Big White).
3. *M. alba* (Rease).
4. *M. alba* (Greece).
5. *M. alba* (pearl).
6. *M. alba* (Border Sweet).
7. *M. alba* (Pendula).
8. *M. latifolia* (Kokuso Korean).
9. *M. rubra* (Amarah).
10. *M. nigra* (Shami).
11. *M. macroura* (King White).
12. *M. macroura* (Dwarf).
13. *M. hybrid* (Tice).
14. *M. hybrid* (Wellington).

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**Fig. 2:** Curves of the phenolic compounds separated from the leaves of cultivars of the *Morus* L. genus' species under study. 1. *M. alba* Beautiful Day. 2. *M. alba* Big White. 3. *M. alba* Rease. 4. *M. alba* Greece. 5. *M. alba* pearl. 6. *M. alba* Border Sweet. 7. *M. alba* Pendula. 8. *M. latifolia* Kokuso Korean. 9. *M. rubra* Amarah. 10. *M. nigra* Shami. 11. *M. macroura* King White. 12. *M. macroura* Dwarf. 13. *M. hybrid* Tice. 14. *M. hybrid* Wellington.

This result is consistent with Rajab *et al.*, (2014) in that  $\mu$  max values are among the physical constants that can consolidate and support taxonomic indices in plant taxonomy and make each value a specific identity with

which we differentiate the different species and cultivars of plants and that linking spectroscopic studies with taxonomy will open a new door in which important new differences are found for isolating species and cultivars

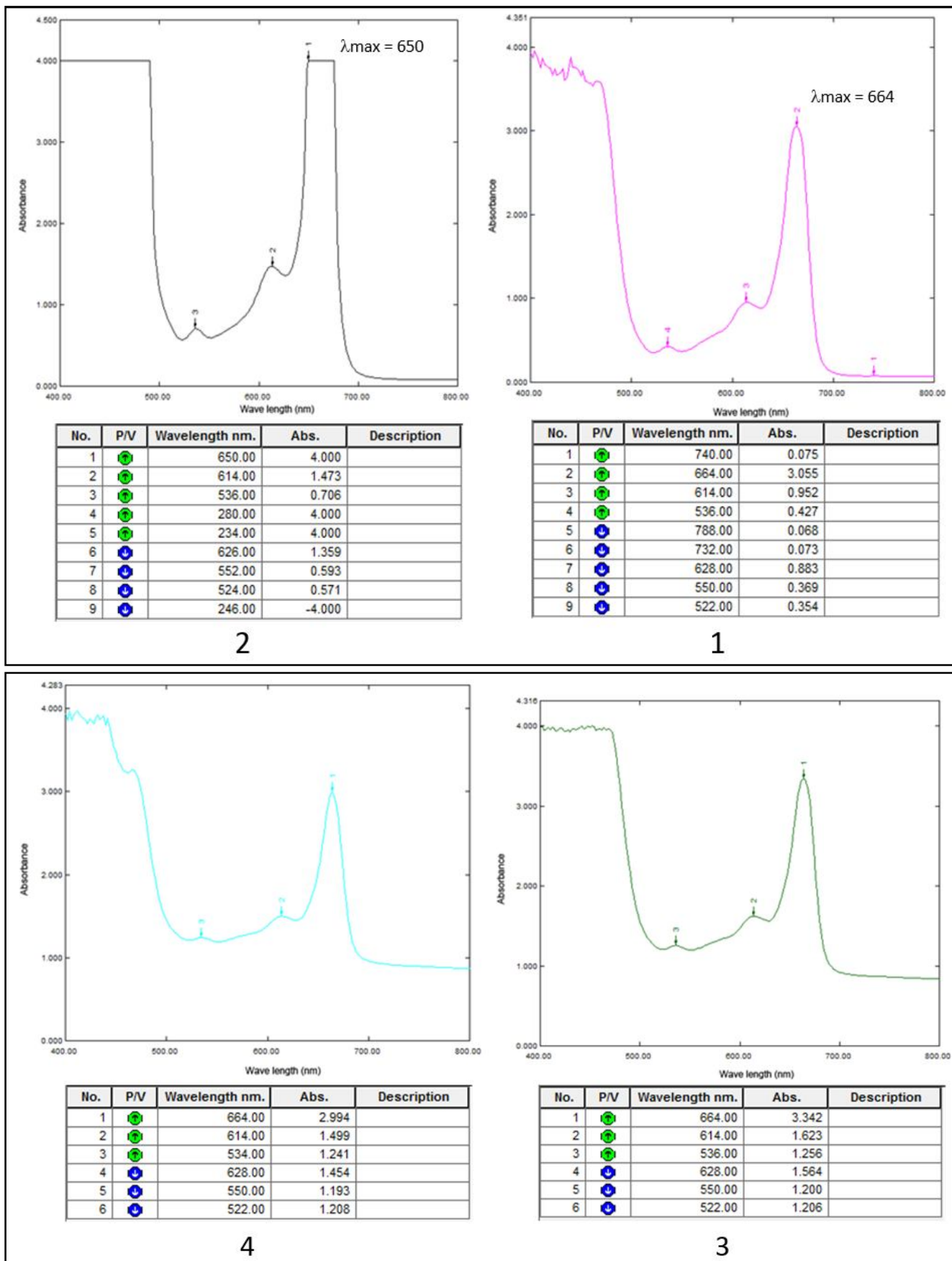


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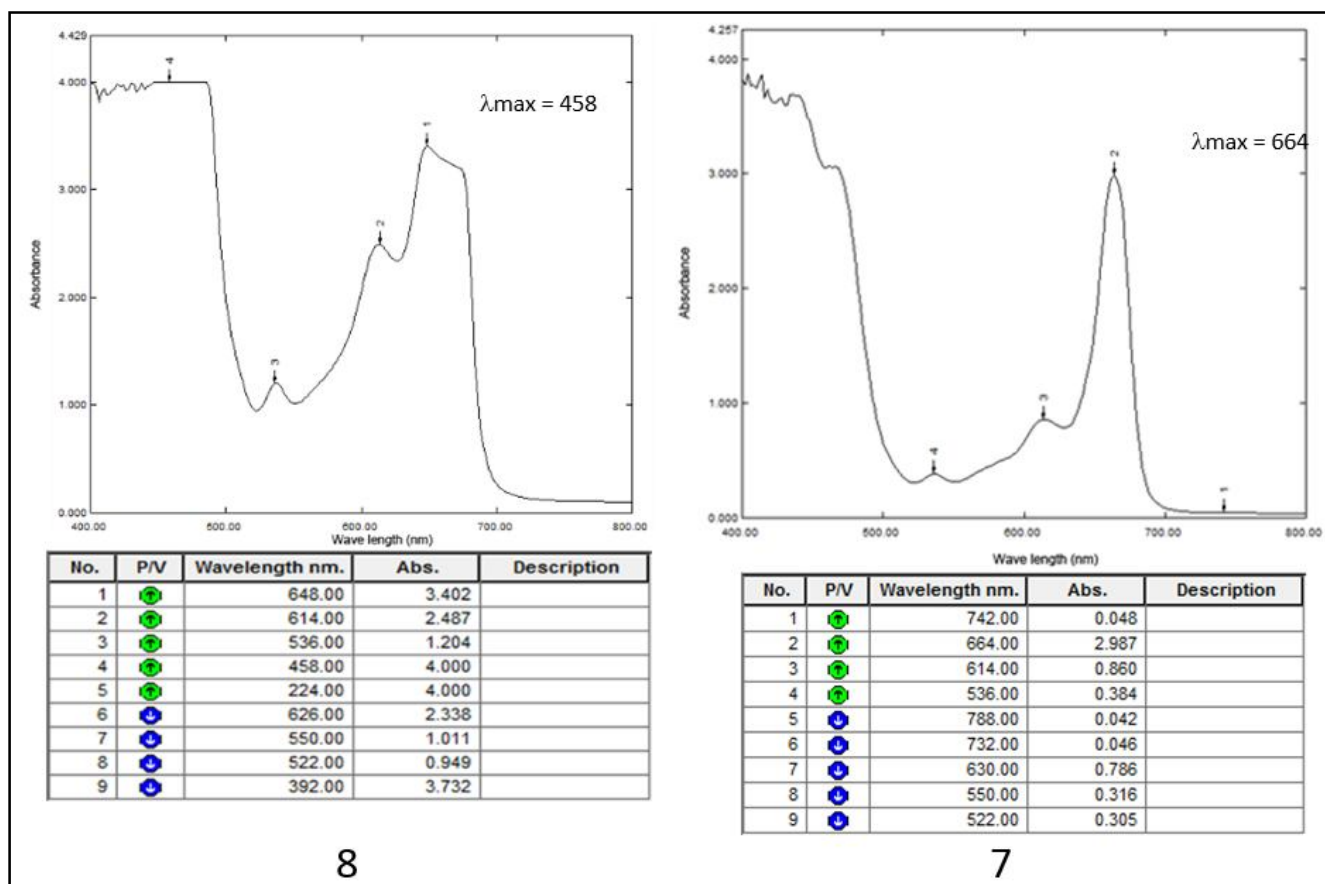
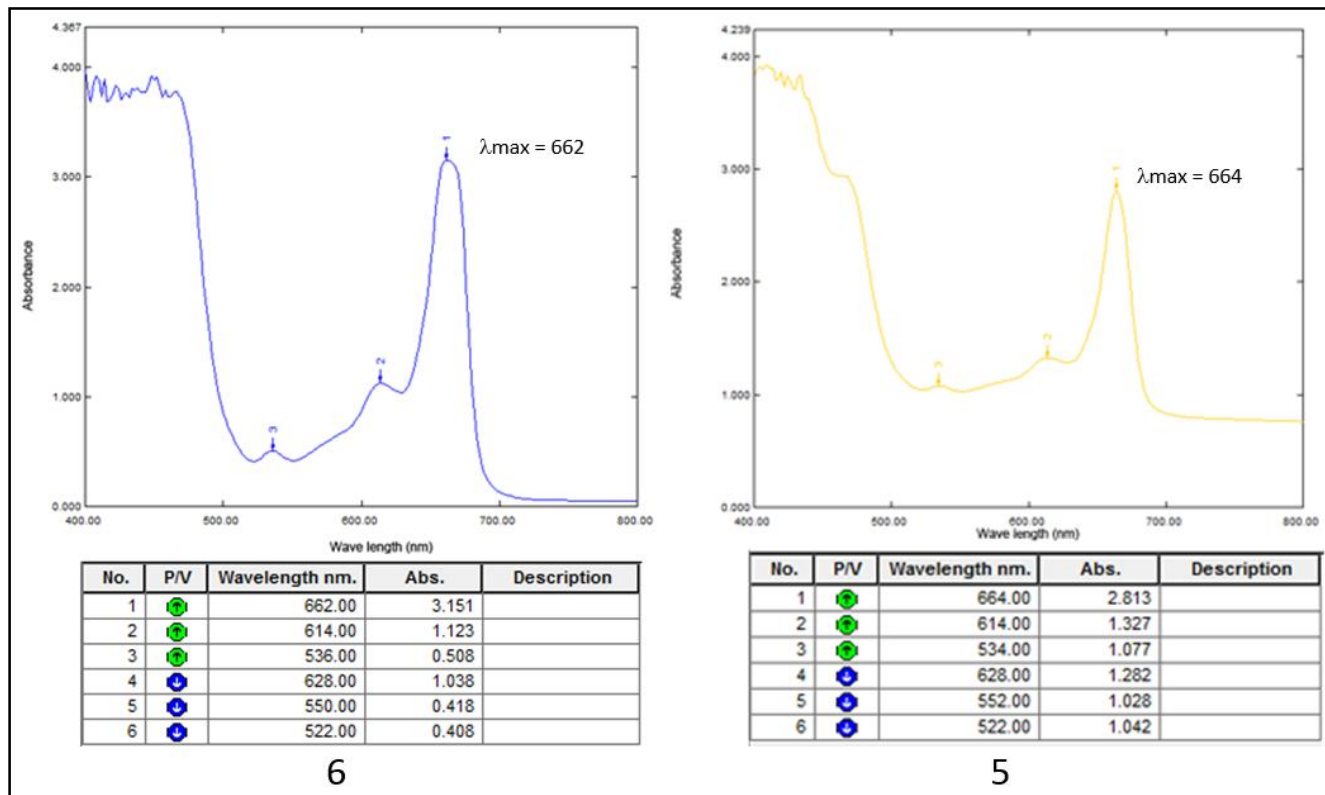


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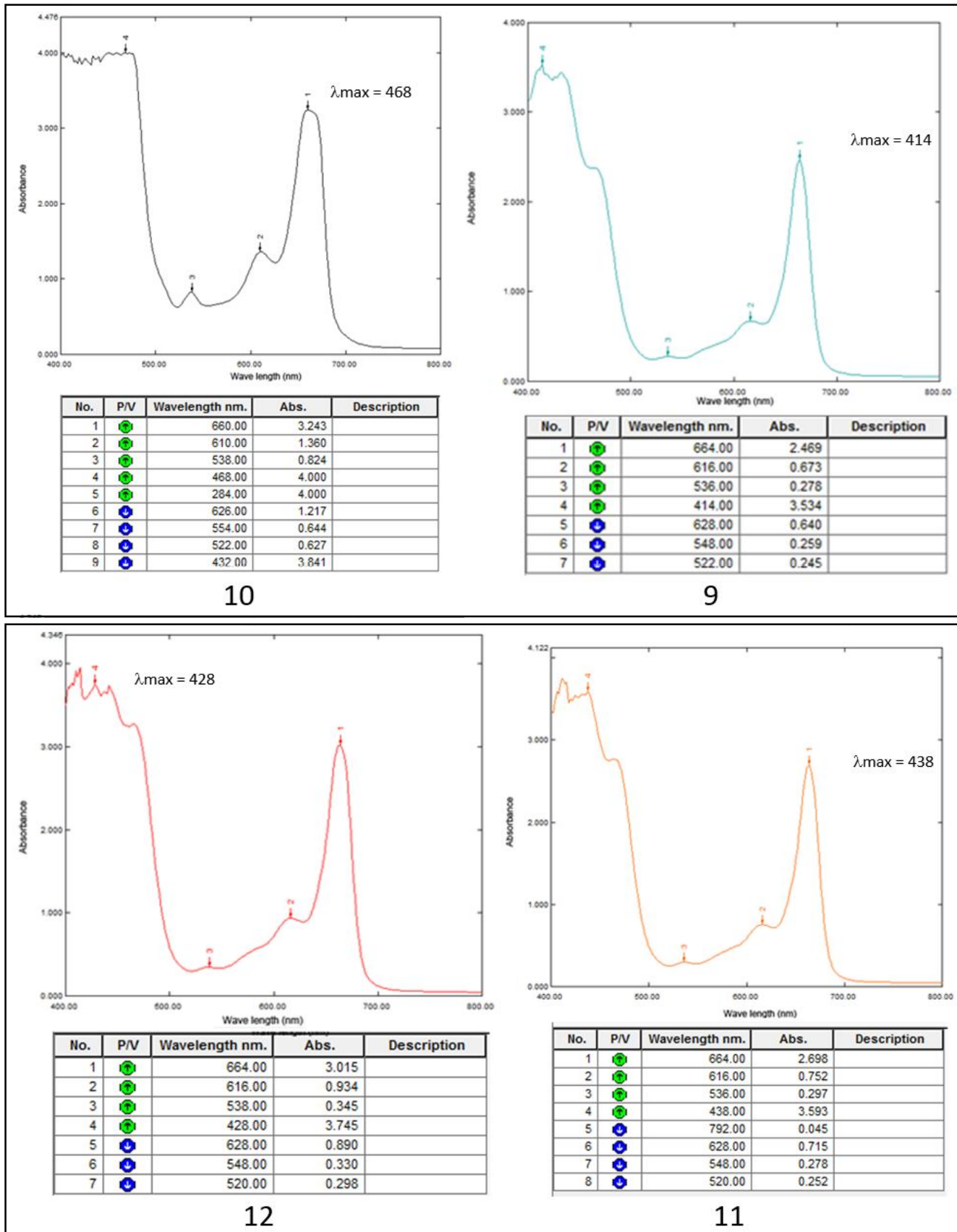


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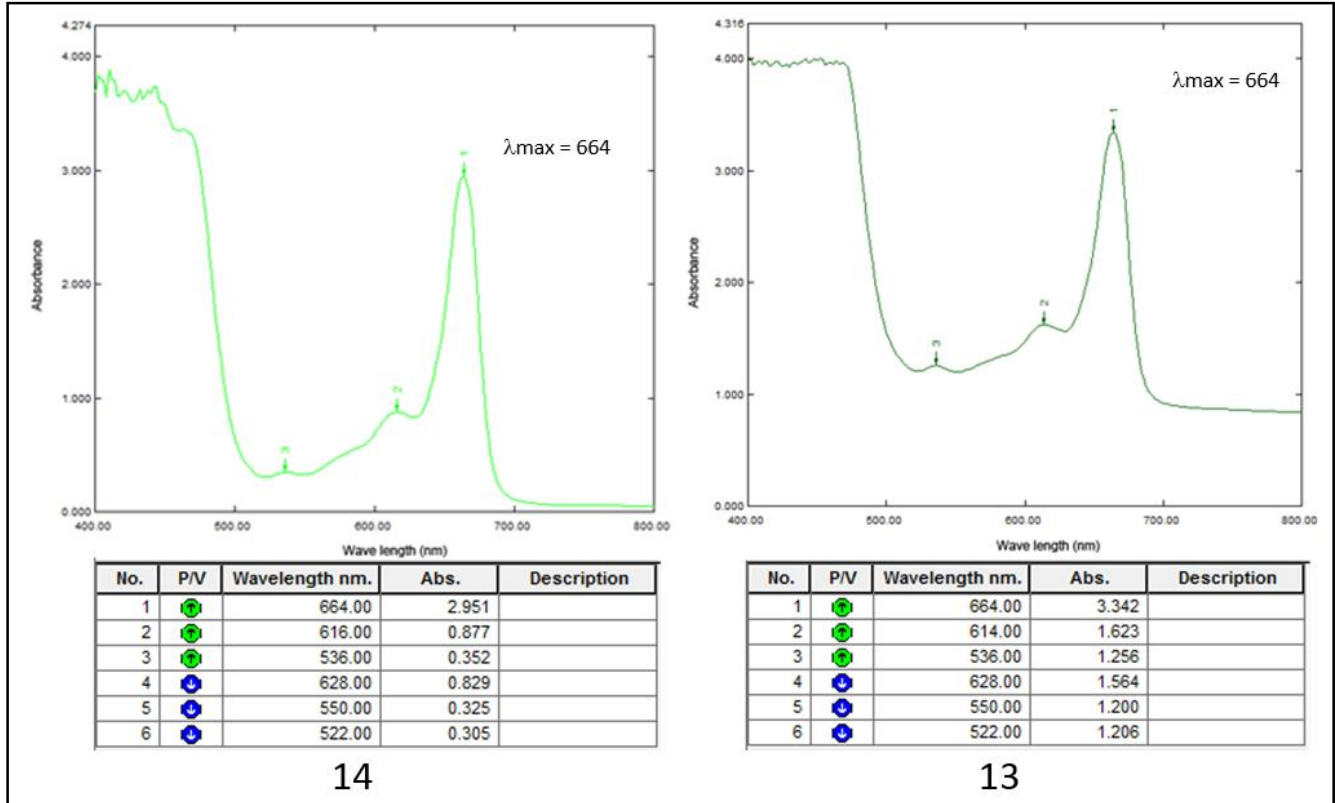


Fig. 3: Absorption spectrum and  $\mu$  max values for chlorophyll of leaves of cultivars of the *Morus* L. genus' species.

1. *M. alba* (Beautiful Day). 2. *M. alba* (Big White). 3. *M. alba* (Rease). 4. *M. alba* (Greece). 5. *M. alba* (pearl). 6. *M. alba* (Border Sweet). 7. *M. alba* (Pendula). 8. *M. latifolia* (Kokuso Korean). 9. *M. rubra* (Amarah). 10. *M. nigra* (Shami). 11. *M. macroura* (King White). 12. *M. macroura* (Dwarf). 13. *M. hybrid* (Tice). 14. *M. hybrid* (Wellington).

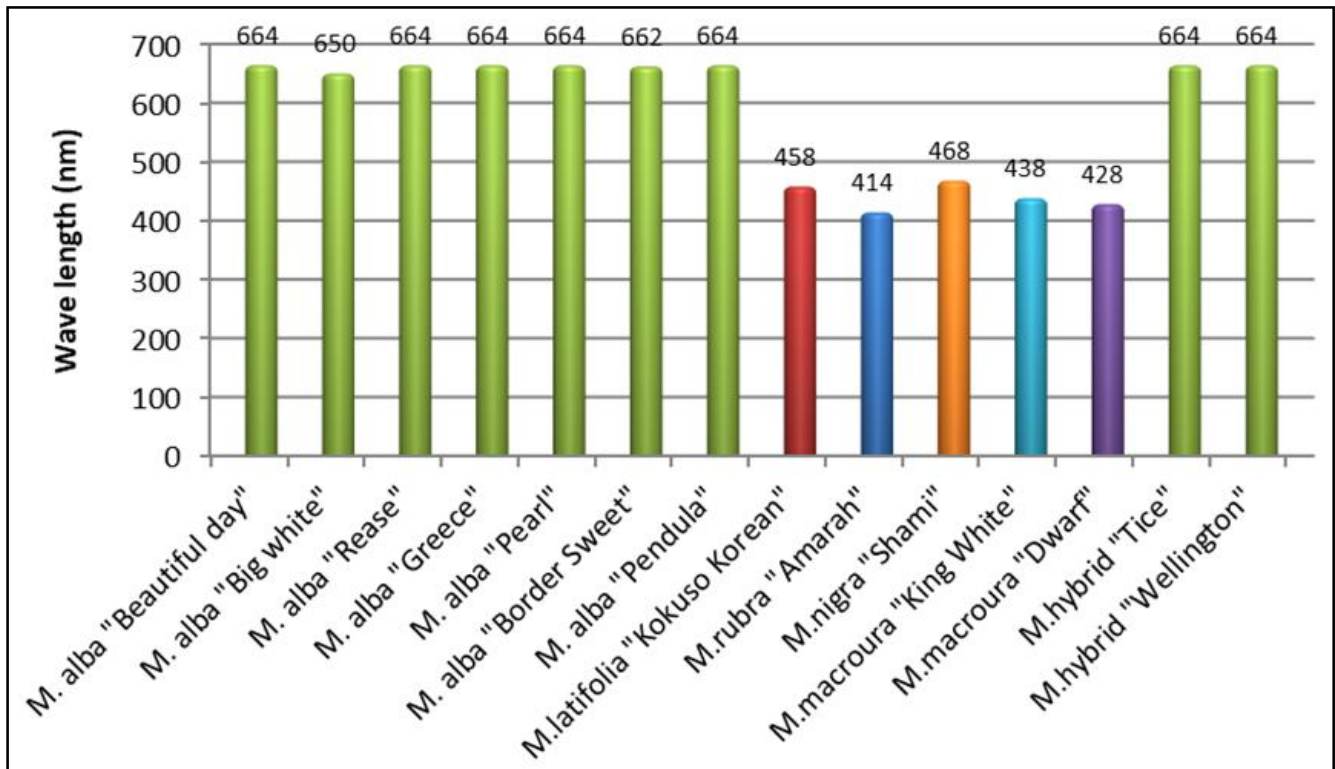


Fig. 4:  $\mu$  max values for cultivars of *Morus* L. genus' species under study.

within the same species.

It is also consistent with Abdel Fattah *et al.*, (2016) in that the chemical composition may be helpful in the spectroscopic studies of the plant in their dependence on the qualitative detection of certain substances in the plant and their comparison with another plant and knowing the difference by measuring the highest wavelength, which corresponds to its highest absorption.

### Conclusions

Phenolic compounds diagnosed using HPLC technology are among the medicinally important compounds that have shown marked variations in the qualitative and quantitative isolation of the cultivars under study by their presence or the difference in their concentrations.

The spectral study contributed to the differentiation and separation of the studied cultivars of genus' species through the spectral variation in  $\mu$  max values for the alcoholic extracts of their chlorophyll.

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