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Editor of series: Ljiljana Rašković, e-mail: ral@junis.ni.ac.yu
Address: Univerzitetski trg 2, 18000 Niš,YU, Tel. +381 18 547-095, Fax: +381 18 547-950
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THE EXTRACTION OF APIGENIN AND LUTEOLIN FROM THE SAGE *SALVIA OFFICINALIS L.* FROM JORDAN

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Siniša Đorđević[1], Milorad Cakić[1], Salameh Amr[2]

[1]Faculty of Technology, Bulevar oslobodjenja 124, Leskovac, Yugoslavia
[2]Royal Science Institute, Amman- Alqwesme, Box 38365, Jordan

Abstract. *In the Kingdom of Jordan first class sage (maramia) (*Salvia officinalis *L.) grows both in wilderness and cultivated on plantations, and it is the most important plant species in the country.*
Our aim is to investigate the chemical composition of the sage originating from Jordan, and in this paper the original procedure is described for the extraction of apigenin and luteolin.
The preparative chromatography on paper was used for the isolation of these compounds from ethanol extract (1:10) of the plant (flower and top leaves) in the n-butanol-water-acetic acid system 12:2:1 v/v/v.
Considering the R_f values of IR and UV spectra (with and without adding the specific agents NaOMe, NaOAc, NaOAc+H_3BO_3, $AlCl_3$ and $AlCl_3$+Hcl) and compared with the literature data it was determined that the isolated compounds were apigenin and luteolin.

Key words: *sage, extraction, apigenin, luteolin*

1. INTRODUCTION

The sage growing in Jordan both in wilderness and cultivated on plantations is of first class quality. It is the compulsory ingredient in cooking recipes and for tea making and is known as traditional medicine for numerous diseases. The popular names for this plant are: žalfija (in towns in Serbia), plem (in Montenegro and Herzegovina), kadulja (in middle Dalmatia), kuš (in the north coast), kalaver or džiger (in eastern Serbia), meramia or marjamia (in Jordan). Beside the above, a great number of other names are also used.

The sage is one of the oldest medicinal herbs. It is mentioned by all the medical writers in the ancient Rome. The Latin name *Salvia* comes from the Latin verb "salvare" meaning to save, to heal. *Officinalis* in Latin means medicinal. The mere fact that both

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Latin names are referring to the medicinal properties, which cannot be said for any other plant, shows how much the ancient Romans appreciated the sage two thousand years ago, and used it for healing in various ways.

The sage is also important from the ecological point of view because it improves the environment, the air around its habitat, thanks to the antiseptic effect of its ether oil.

The scope of this work is the isolation, by an original technological method, of the pure bioactive components of the sage, apigenin and luteolin, and the determination of their structure by modern instrumental methods.

2. EXPERIMENTAL CONDITIONS

The plant material (flowers and top leaves), gathered at the locality of Hfashijet Al Dbajbe (The Kingdom of Jordan) in the phase of blooming, dried in dark, well ventilated place, ground by electric grinder and screened through the 1.4 mm mesh sieve, was analyzed according to the Jordanian Agricultural Products Quality Control Regulations, 1982. The extraction by the maceration method in 98% ethanol was carried out with the drug to solvent ratio 1:10, for a period of 20 hours with intermittent stirring.

The extract obtained, evaporated under vacuum at the temperature of 50^{o}C to approximately one tenth of its original volume, was purified by liquid-liquid extraction method with n-butanol and water. In a 2000 ml separation funnel 50 ml of ethanol extract was mixed with 125 ml of n-butanol, and then 40 ml portions of water were added in 15 minutes' intervals (with manual stirring), while at the same time the lower water layer was separated. The procedure was repeated 12 times. The residual upper layer (n-butanol) was applied to Whatman 1 chromatographic band, 2 cm^3 to band size 30×60 cm, and chromatographed for 48 hours in n-butanol-water-acetic acid system 12:2:1 v/v/v. In the chromatograms obtained two zones were clearly visible: R_f - 0.9 and R_f - 0.81, and these were separated by cutting the paper. The procedure was repeated several times for preparatory isolation. The substances were eluted from the chromatographic paper by ethanol. The solutions were evaporated to small volume from which, when cooled, crystallized the separated substances (substance I at $R_f = 0.9$ and substance II at 0,81).

The pure compounds isolated by this method were tested for their UV spectra, as well as their respective shifts (bathochromic and hypsochromic), that occur due to addition of specific agents: NaOMe, NaOAc, NaOAc + H_3BO_3, $AlCl_3$, and $AlCl_3$ + HCl. IR spectra were also made. For the determination of R_f value in the TBA system (tertiary butanol-acetic acid-water 6:2:2 v/v/v) Whatman 1 chromatographic band was used.

3. RESULTS AND DISCUSSION

In Table 1 the investigation results are shown of common quality parameters of Jordanian sage.

Table 1. Quality parameters of sage (*Salvia officinalis* L.) originating from Jordan

Moisture %	Ashes %	Ashes not soluble in HCl %	Ether oil %	Plant drug scent
9.41	12.57	2.45	2.09	specific and pleasant

All the quality parameters tested comply with the requirements of Jordanian Regulations. Table 2 shows the R_f values of the isolated substances in various systems.

Table 2. Basic properties of chromatographically isolated compounds 1 and 2

No	Property	Compound 1	Compound 2
1.	Rf value in TBA system	0.87	0.77
2.	Rf value in n-butanol-water-acetic acid system 12:2:1 v/v/v	0.9	0.81

Some R_f values in the TBA system were identical to those of apigenin and luteolin mentioned in literature, and even more in n-butanol-water-acetic acid system 12:2:1 v/v/v due to polarity difference. Bearing in mind the limitations of the identification of substances by the chromatographic method, the two isolated substances were further tested by UV and IR spectroscopy methods for identification.

Compound 1 structure determination

The UV spectrum of the methanol solution of compound 1 has characteristic bands: I at $\lambda = 336$ nm, and band II at $\lambda = 267$ nm (Figures 1 and 2) which leads to the conclusion that the compound belongs to the group of flavonoides.

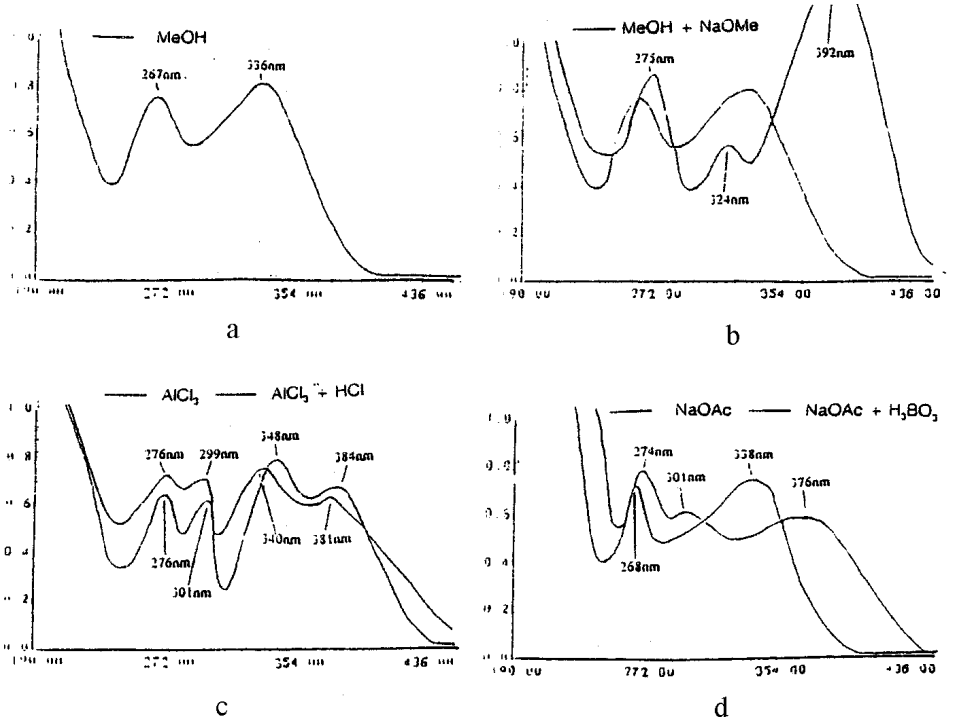

Fig. 1. UV spectra of compound 1 (a) with, and (b, c, d) without the addition of specific agents

From the bathochromic shift with $AlCl_3$ (Figure 1c), i.e. the band I shift from $\lambda = 336$ nm,

splitting into two bands with peaks at $\lambda = 384$ nm and $\lambda = 348$ nm, the presence of an OH-group was detected in position 5. After the addition of agent NaOAc (Figure 1d), the band shift II by 7 nm (274 nm - 267 nm), indicated the presence of and OH-group in position 7.

When agent NaOAc+H_3BO_3 was added (Figure 1d) a slight shift of band I by 2 nm (338 nm - 336 nm) indicated the absence of OH-group in position 3.

From the UV spectrum the presence of OH-group in positions 5, 7, and 4' was recorded. However, the UV spectra do not furnish information whether the compound tested belongs to the group of flavon glycosides, i.e. whether it contains more OH-groups involved in the forming of glycoside bond with some sugar. That is why the IR spectrum was made, as shown in Figure 2.

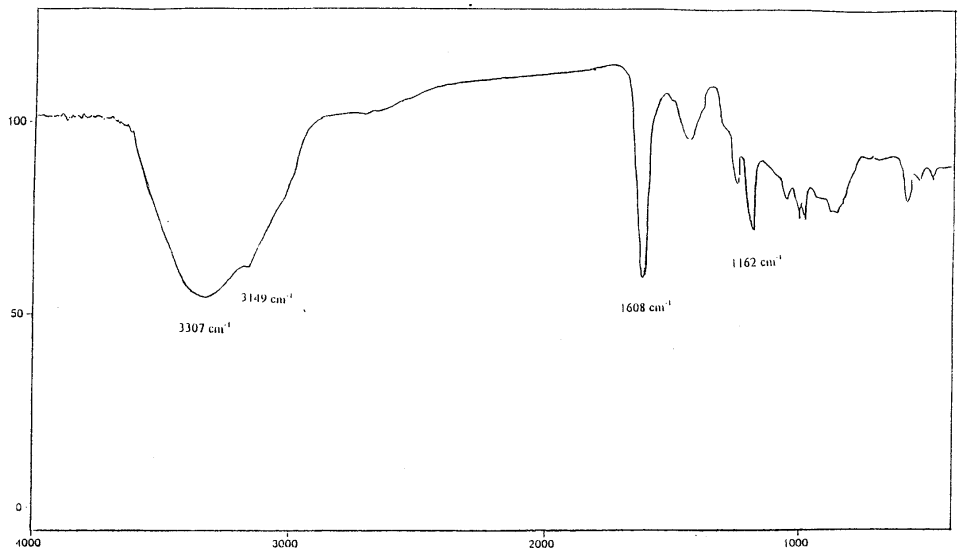

Fig. 2. IR spectrum of compound 1

It can be seen that there are no intensive bands in the 1100-1000 cm^{-1} span that are characteristic of glycoside bond, nor band of any sugar type. For example, there are no bands of ν_{CH} vibrations of CH_2 group at approx. 2950 cm^{-1} and 2850 cm^{-1}, or any δ_{CH} vibrations at approx. 1470 cm^{-1} or sugar vibration band ν_{OH} (expected frequency about 3400 cm^{-1}). In the spectrum of ν_{OH} vibrations two bands are found, one at approx. 3307 cm^{-1}, and the other at approx. 3149 cm^{-1}, that are most probably the result of ν_{OH} vibrations of phenol OH groups. The intensive band at 1608 cm^{-1} is most probably the result of $\nu_{C=O}$ vibrations of C=O group from the central heterocyclic ring, while the ν_{C-O} vibration should occur at approx. 1162 cm^{-1}. The above spectral characteristics indicate with high probability that the compound is apigenin. These data were compared with data from the book by T.J. Mabry and the identical UV spectra made with typical agents are proof that the isolated compound 1, the structure of which is shown in Figure 3, is apigenin.

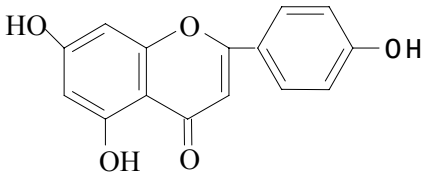

Fig. 3. The structure of apigenin Compound 2 structure determination -

The UV spectrum of the methanol solution of the compound 2 has two characteristic bands, I at $\lambda = 349$ nm, and band II at $\lambda = 253$ nm, which indicates that the compound belongs to the group of flavonoides (Figures 4a). From the bathochromic shift with $AlCl_3$ (Figure 4c), i.e. the band I shift from $\lambda = 349$ nm, splitting into two bands with peaks at $\lambda = 426$ nm and $\lambda = 328$ nm, indicated the presence of an OH-group in position 5.

After adding the agent NaOAc (Figure 4d), the band shift II by 16 nm (269 nm - 253 nm), indicated the presence of and OH-group in position 7.

When agent NaOAc+H_3BO_3 was added (Figure 4d), the shift of band I by 21 nm (370 nm - 349 nm), indicated the presence of OH-group in positions 3 and 4.

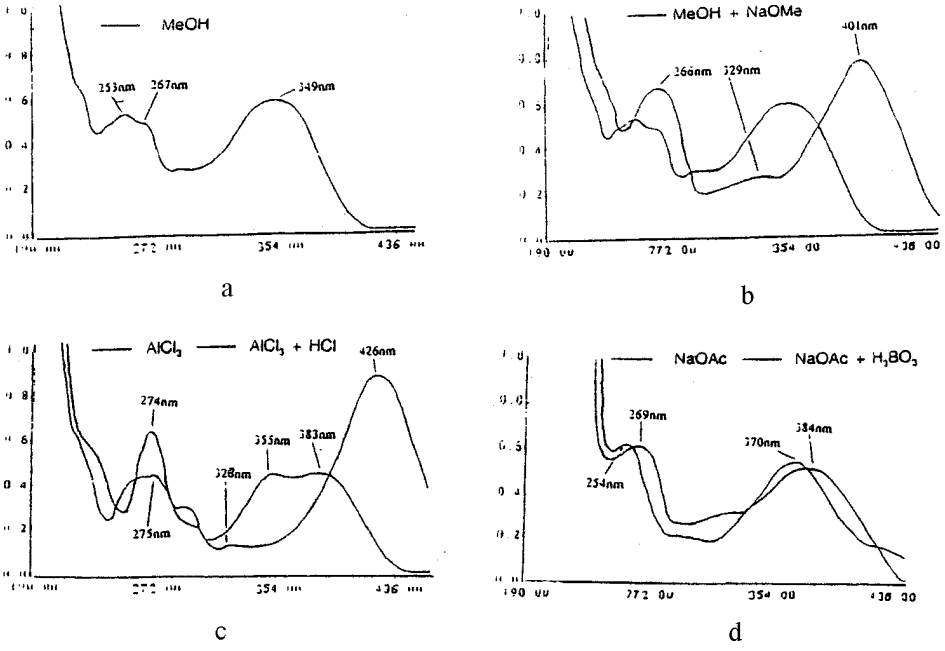

Fig. 4. UV spectra of compound 2 (a) with, and (b, c and d) without the addition of specific agents

From the UV spectrum the presence of OH-group in positions 5, 7, 3' and 4' was recorded. However, the UV spectra do not furnish information whether the compound

tested belongs to the group of flavon glycosides, i.e. whether it contains more OH-groups involved in the forming of glycoside bond with some sugar. That is why the IR spectrum was made, as shown in Figure 5.

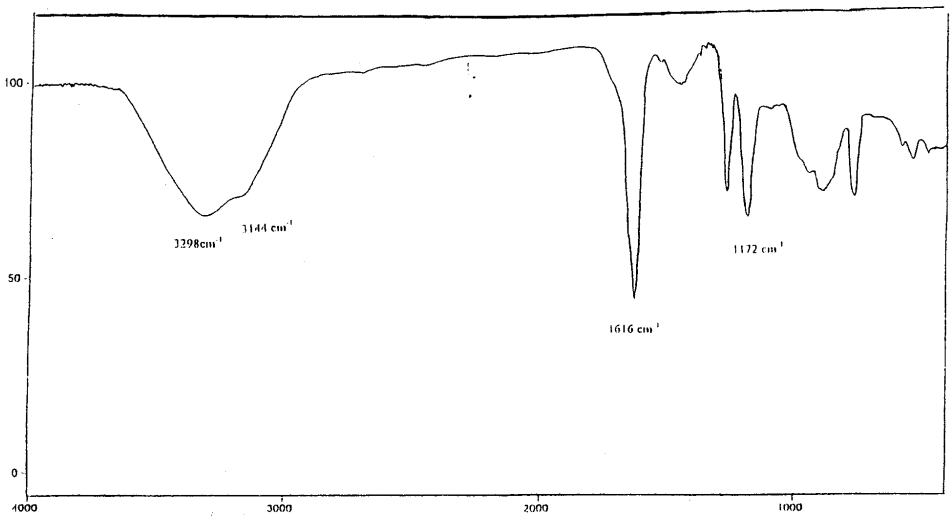

Fig. 5. IR spectrum of compound 2

It can be seen that there are no intensive bands in the 1100-1000 cm^{-1} span that are characteristic of glycoside bond, nor band of any sugar type. For example, there are no bands of ν_{CH} vibrations of CH_2 group at approx. 2950 cm^{-1} and 2850 cm^{-1}, or any δ_{CH} vibrations at approx. 1470 cm^{-1} or sugar vibration band ν_{OH} (expected frequency about 3400 cm^{-1}). In the spectrum of ν_{OH} vibrations two a band is found, at approx. 3144 cm^{-1}, that is most probably the result of ν_{OH} vibrations of phenol OH groups. The intensive band at 1616 cm^{-1} is most probably the result of $\nu_{C=O}$ vibrations of C=O group from the central heterocyclic ring, while the ν_{C-O} vibration should occur at approx. 1172 cm^{-1}. The above spectral characteristics indicate with high probability that the compound is luteolin. These data were compared with data from the book by y T.J. Mabry and the identical UV spectra made with typical agents are proof that the isolated compound 2, the structure of which is shown in Figure 6, is luteolin.

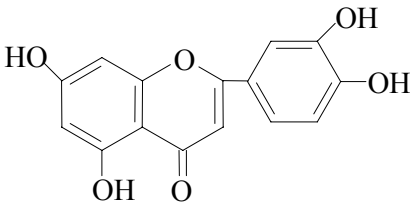

Fig. 6. The structure of luteolin

CONCLUSIONS

The sage *Salvia officinalis* L. originating from Jordan complies with the requirements of the Jordanian Agricultural Products Quality Control Regulations with respect to general quality parameters.

The original procedure with n-butanol-water-acetic acid system 12:2:1 v/v/v was used to isolate two bioactive substances, that were identified, through PC, UV-Vis and IR spectroscopy as compound 1, which is apigenin, and compound 2, which is luteolin.

REFERENCES

1. Medicinal Plants *Encyclopedia, First Edition* , Editor Al-Arabia Lidirasad, Jordan, 1993
2. *Flora SR Srbije*, Naučno delo, Beograd VI, 1974.
3. *Pharmacopoea Jugoslavica III*, Izdanje saveznog zavoda za zdravstvenu zaštitu, Beograd, 1972.
4. Harborne, J.B., *Chromatographic Identification of Anthocyanin Pigments*, Chromatogr. Rev. 1(1959).
5. Mabry, T. J., K. R. Markham and M. B. Thomas, *The Systematic Identification of Flavonoides*, Springer Verlag, New York-Heidelberg-Berlin 1970.
6. *Jordanian Regulations For The Quality Control Of Agricultural Products*, 1982.

IZOLOVANJE APIGENINA I LUTEOLINA IZ ŽALFIJE *SALVIA OFFICINALIS L.* POREKLOM IZ JORDANA

Siniša Đorđević, Milorad Cakić, Salameh Amr

U Kraljevini Jordan spontano raste, ali se i plantažno gaji žalfija (maramija) (Salvia officinalis L.) izvanrednog kvaliteta i najvažnija je biljna vrsta u ovoj zemlji.

Naš cilj je istraživanje hemijskog sastava žalfije poreklom iz Jordana, a u ovom radu izložen je originalni postupak za izolovanje apigenina i luteolina.

Za izolovanje ovih jedinjenja primenjena je preparativna hromatografija na papiru etanolnog ekstrakta (1:10) biljke (cvet + gornji lisni deo) u sistemu n-butanol-voda-sirćetna kiselina 12:2:1 v/v/v.

Na osnovu R_f vrednosti, IR i UV spektra (sa i bez dodataka specifičnih reagensa NaOMe, NaOAc, NaOAc+H_3BO_3, $AlCl_3$ i $AlCl_3$+HCl) kao i poredjenjem sa literaturnim podacima utvrdjeno je da su izolovana jedinjenja apigenin i luteolin.

Ključne reči: *žalfija, ekstrakcija, apigenin, luteolin*