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IDENTIFICATION OF FLAVONE AGLYCONES AND FLAVONOL GLUCOSIDES IN ETHYL ACETATE EXTRACT OF *MENTHA ARVENSIS*

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ABSTRACT

Four flavonoids (two flavonol glycosides and two flavones) were isolated from ethyl acetate extract of *Mentha arvensis leaves*. The structures were determined by usual spectroscopic methods (UV and ^{1}H NMR). This study confirmed that *Mentha arvnsis* can be a good source of antioxidant polyphenols. In recent study kaempferol and apigenin and their derivatives, have been found to possess preventive and therapeutic potential against several kinds of cancer.

KEYWORDS: ***Mentha arvensis*, flavone aglycones, flavonols glucosides**

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INTRODUCTION

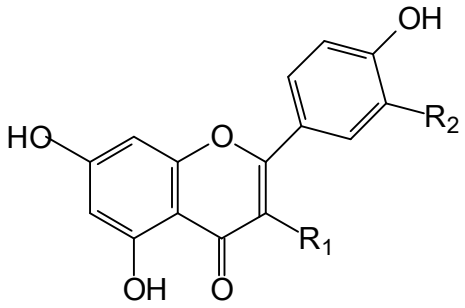
Flavonoids are polyphenolic compounds commonly found in many plants, vegetables, and flowers. The flavonoid family comprises 15 classes of compounds, including the flavones, flavonols, chalcones, and isoflavones [1, 2.] In this study we are interested in the genus of *mentha* (*Lamiaceae*) which is widely used in many region of Algeria as traditional treatment in several diseases. There are about 70 species of mint, probably even more, because the mint hybridizes easily. The species *Mentha arvensis* (field mint), is an herbaceous perennial plant growing to 10–40 cm tall. The leaves are in opposite pairs, simple, 2–6.5 cm long and 1–2 cm broad, hairy, and with a coarsely serrated margin. The flowers are pale purple (occasionally white or pink), in clusters on the stem, each flower 3–4 mm long [3, 4]. The genus *mentha arvensis* has many therapeutic properties as: Anesthetic, Anti-microbial, Antispasmodic, anti-inflammatory, Carminative, Digestive, Expectorant, Stimulant, and Stomachic [5], Field mint's essential oil is considered to be a sedative and a regulator to the nervous system. It can alleviate digestive ailments such as flatulence, gastrointestinal and gallbladder disorders [6]. Essential oil composition of *Mentha arvensis* is represented by Monoterpenol 65-75%, Monoterpens 10 – 20%, and 25-30% Cetone[7]. Our preliminary results have shown that some flavonoids (two glucosides flavonols and two aglycones flavonones) can be isolated from ethyl acetate extract of leaves of *Mentha arvensis*. We will continue our investigation to the rest of extracts (chloroformic and n-butanolic) in order to identify the actives principles of this plant, responsible of the therapeutic effects of this plant.

EXPERMENTAL

Harvesting equipment plant (*Mint arvensis*) was harvested in El-milia region (east of Algeria) in July 2010. The parts collected were dried away from direct sunlight. Drying time varies between four and six weeks. Aerial parts were powdered and weighed (M = 437 g). Plant extraction: Dried aerial parts (437 g) of plants were powdered and soaked in methanol/water (7/3, 4L) for 24 hours at room temperature, and then filtered, this operation was repeated three times .The recovered extract was concentrated under reduced pressure at a temperature between 35 ° C and 40 ° C. At the hydro-alcoholic extract was added with stirring, distilled water and the resulting solution was allowed to stand at cold overnight to decantation, and then filtered to obtain an aqueous solution and a residue. The aqueous phase was extracted (liquid – liquid) successively with Petroleum ether, chloroform, ethyl acetate and n-Butanol. The extracts were evaporated to dryness to get three extracts: petroleum ether extract (not used), chloroform extract (2.1 g), ethyl acetate extract (4.82 g) and n-butanol extract (12.67 g) respectively. About 2 g of the ethyl acetate extract was tested on PC (Whatman® 3MM) eluted with SI (15 % AcOH) and SII (BAW; n-butanol-AcOH-H_2O (4:1:5),top layer) then chromatographed on a column of silica gel, elution is carried out using a mixture (ethyl acetate / water / acetic acid, (9 / 0.5 / 0.5), (8/1/1) and (6/1/1) with split each 25 ml. monitoring the composition of the fractions was performed by thin layer chromatography TLC on silica gel on aluminum support. Fractions with similar chromatographic behavior were gathered giving nine fractions. The fractions 1-4 were separated and identified using preparative TLC of silica gel 60G-F254 using $CHCl_3$/MeOH (15 to 9/1) as systhems.The pure compounds were purified for spectral analysis by using the methanol over a sephadex LH20 column.

RESULTS AND DISCUSION

Fractionation by column chromatography on silica gel of the ethyl acetate extract resulted in the isolation of four flavonoids. They were identified by spectroscopic methods (UV and ^1H NMR).



Compound	*R_1*	*R_2*
1	*-O-Glu*	*H*
2	*-O-Glu*	*OH*
3	*H*	*H*
4	*H*	*OH*

Identification of compounds isolated

Kaempferol O-B-D- glucoside 1 : The UV-Vis spectra of flavonoids exhibit two major absorption peaks: band I (usually 300 – 380 nm) and band II (usually 240 – 280 nm) [8] The black violet color under UV, with the presence of two maxima of absorption at 354 nm (band I) and 260 nm (band II) recorded in methanol, oriented to a structure of flavonol with a hydroxyl substituted in position 3. Bathochrome displacements of band II after addition of the reagents NaOH, $AlCl_3$, $AlCl_3$+ HCl, NaOAc and NaOAc + H_3BO_3 in the presence of methanol indicate the presence of 3 free hydroxyls in positions 5, 7 and 4 table.1 [9,10.]

Table 1

Reagents	Band I (nm)	Band II (nm)	Other band(nm)	Observation
MeOH	354	260		Flavonol 3-OR
NaOH	400	254		4'-OH
NaOH after 5 min	400	254		Absence of OH in C-3
NaOAc	390	276	310	7-OH
NaOAc+ H_3BO_3	370	260		Abs of system orto di OH
$AlCl_3$	414	266		5- OH
$AlCl_3$ + HCl	414	264	360	Abs of systhem orto di OH

^{1}H NMR ($CDCl_3$, δ): Multiple signals in the aromatic zone δ (6 - 8) ppm indicating the presence of aromatic rings. A signal with δ = 8.1 ppm, integrations 2 H in the form of a doublet of doublet attributed to H-2' and H- 6'. A signal with δ = 6.9 ppm, integrations 2 H in the form of a doublet of doublet characteristic of the H-3' and H-5' protons. A signal with δ = 6.3 ppm, integrations 2H in the form of a broad singlet, cha racteristic of the H-6 and H-8 of ring A. A doublet with δ= 5.2 ppm, attributed to the proton anomeric of a sugar connected to the aglycone by oxygenated bridge with the proton anomeric in C-3 of the ring C. A multiply between intervals 3.1 - 3.75 ppm attributed to the protons of the sugar substituent. The combinations of these spectral data (UV and ^{1}H-NMR) directs towards the following partial structure: 5, 7, 4' - 3-O-Glucose flavonol. To determine the nature and the position of sugar we carried out the acid hydrolysis of compound. After concentration of the organic and aqueous phases, we proceeded to the analytical Co chromatography on siliceous plate analytical eluted by the system Acetone - Water (9: 1) of the aqueous phase in the presence of authentic sugar samples available in the laboratory. The preliminary results show that sugar is glucose. The whole of these data makes it possible to allot to the flavonole considered structure 5,7, 4'- trihydroxy-3-O-β-D-glucosideflavonol.This identification is confirmed by comparison between the spectral data and that published in literature [11,12]

5,7,4'-trihydroxyflavone (Apigenin) 2: Black violet fluorescence under wood light (365 nm) allows us to propose a structure of the flavonoid. The ultraviolet absorption spectrum recorded in methanol shows two absorption bands I

and II, both are intense. It can be a flavone or flavonol. The position of the band I (350 nm) moving towards a flavone or flavonol substituted with hydroxyl in 3. The band II shows a peak around 255 nm, this absorption band allows in certain way to know the number of hydroxyl of the ring B. This value reflects a monosubstitution. On the other hand the two spectra with $AlCl_3$ and $AlCl_3$ + HCl are superposable implying a single substitution of ring B. Orthodihydroxyl system on ring A is reflected by a bathochromic shift of the band II(+10 nm) in the presence of $AlCl_3$ compared to spectrum $AlCl_3$ + HCl . The addition of NaOH causes a bathochromic displacement of the band (ΔλI = + 50 nm) with an increase in intensity compared to that recorded in methanol, reveals the presence of a free OH in C - 4 '. The bathochromic shift of the band II (ΔλII = + 5 nm) after addition of NaOAc reagent compared to that recorded in methanol, reveals the presence of a free OH in C -7. This hypothesis is confirmed by the appearance of a new band at 330 nm in the spectrum recorded in the presence of NaOH. The addition of the reagent $AlCl_3$ + HCl causes a bathochromic shift of the bond I (ΔλI = + 50 nm) compared to that recorded in methanol reveals the presence of a free OH in C-5. The bathochromic shift I (ΔλI = + 55 nm) after addition of $AlCl_3$ compared to that recorded in methanol confirmed the presence of a free OH in C-5. The low hypsochromic shift of the band I in the presence of $AlCl_3$ + HCl compared to that of $AlCl_3$. (ΔλI = - 5 nm) means the absence of free orthodihydroxyle group on ring B of the skeleton flavonoid. The results of the UV spectral range are shown in Table 2.

Table 2
UV spectral data of compound 2.

Reagents	Band I (nm)	Band II (nm)	New Band (nm)	Interpretation
MeOH	350	265		Flavone
NaOH	400	270	330	4 – OH
NaOH + 5min	400	270		Stable spectra
NaOAc	375	275		7- OH
NaOAc+H_3BO_3	360	270		Abs of group O-dihydroxyle
$AlCl_3$	405	270		5 – OH
$AlCl_3$ + HCl	400	270	350	5- OH

^{1} H NMR (δ, CDCl $_3$): Multiple signals in the aromatic region δ = 6.8 ppm indicating the presence of aromatic rings. A signal at δ = 7.8 ppm 2H integration as a doublet of doublets due to H'-2 and H'-6. A signal at δ = 6.8 ppm 2H integration as a characteristic doublet of doublet protons H'-2 and H'-5 d (J = 8.5 Hz). A signal at δ = 6.2 ppm 2H integration as a broad singlet characteristic of the protons H -6 and H-8 of the A ring of a flavonoid. These preliminary results allow us to assign the following partial structure: 5, 7, 4'-trihydroxyflavone.

Quercetin 3-O-β-D- glucoside 3: Rf on TLC cellulose 0.19 (SI); 0.31 (SII)
Δλ (NaOH/ MeOH) = + 50 nm presence of OH in 4' and absence of 3'-OH. UVλ ($AlCl_3$) = 267, 360, 350-406. AlCl3+HCl: 264, 369, 305 – 426, Δλ ($AlCl_3$+HCl)/OH) = 51nm, presence of 5-OH. NaOAc: 264, 360, 301, (NaOAc/MeOH) = +43 nm presence of 7-OH. NaOAc + H_3BO_3 : 261, 378, 301, Δλ (NaOAc/MeOH) = 43 nm, presence of ort-di-OH (3'-OH, 4'-OH). ^{1} H - NMR (300MHz, CDCl $_3$) δ: 6.20 (1H, d, J= 1.6, H6), 6.40 (1H, sb, H-8), 6.92(1H, d, J= 8.2, H'-5). 7.63(1H, dd, J=2.8.2, H'-6, 7.68 (1H, d, J=2.1, H'-2), 5.15(1H, d, J= 7.3, H'-1), 3.40 – 5.15 (m, Sugar).

5-7-3'-4'- tetrahydroxyflavone (Luteolin) 4: RF on TLC 0.9 (SI), (SII), UVλmax (MeOH) = 265, 345 (flavone) UVλmax (AlC_3+HCl) 257, 360, 395 (presence of 5-OH, UVλ (NaOH) = 275, 400 presence of 4-OH, UVλ (NaOAc) = 270, 380 (presence of 7-OH), (NaOAc+H_3BO_3) = 270, 355 (presence of did-OH, UV ($AlCl_3$) λ = 405 , 275, 350 (presence of 5-OH. UV ($AlCl_3$+HCl) λ = 275, 360, 395 (presence of 5-OH).^{1}H-NMR(300 MHz,$CDCl_3$)δ: 7.40(dd, J=2.1Hz; 8.2Hz, H-6'). 7.38 (d, J=2.1Hz, H-2'). 6.87 (d, J=8.2Hz, H-5'). 6.65 (s,H-3).6.43(d,J=2Hz, H-8; 6.15(d, J=2.1Hz, H-6

CONCLUSION

This study showed that the *mentha arvensis* is rich in flavonoids (Kaempferol, quercetine and apigenin) as natural substances that are at the origin of several therapeutic properties. kaempferol and their derivatives, have been found to possess preventive and therapeutic potential against several kinds of cancer, especially on the reduction of breast cancer in postmenopausal women after hormone therapy breast, according to a study published in the journal Cancer Prevention Research [13,14] . The flavonoids kaempferol and quercetin and their derivatives seems to act synergistically in reducing cell proliferation of cancer cells, meaning that the combined treatments with quercetin and kaempferol are more effective than the additive effects of each flavonoid. This was a conclusion from a study by ML Ackland et al (In Vivo, Feb 2005) titled "Synergistic ant proliferative action of the flavonols quercitin and kaempferol in cultured human cancer cell lines". The two other extracts (ethanolic and chloroformic) will be identified later and probably will contain other anti-oxidant polyphenols.

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