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Research Article

PHYTOCHEMICAL INVESTIGATION OF THE MEDICINAL PLANT *MORICANDIA ARVENSIS* L. FROM ALGERIAN SAHARA

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ABSTRACT

Objective: The aim of this research was to isolate and identify flavonoids extracted from the leaves of *Moricandia arvensis*.

Methods: The phytochemical screening reaction and thin-layer chromatography have been used to characterize the chemical groups, before they were identified by nuclear magnetic resonance.

Results: The leaves contain essentially flavonoids, tannins, cardenolides, saponins, and alkaloids. The phytochemical investigation of the water-acetone extract led to the isolation of five flavonoids derivatives, namely: 5,7-dihydroxy-3,6,4'-trimethoxyflavone (1); 5,7,4'-trihydroxy-3,6,8,3'-tetramethoxyflavone (2); 3,3',4', 5,7- pentahydroxy flavanone (3); 3-glucosyl 3',4',5,7 tetrahydroxy flavonol (4); and kaempférol-3-digalactopyranoside (5). The structures of 1–5 were identified by comparison of their spectral data with those reported in the literature.

Conclusion: In this work, it was possible to isolate and identify five flavonoids after fractionation of the hydroacetone extract from the leaves of the medicinal plant *M. arvensis*.

Keywords: *Moricandia arvensis*, Brassicaceae, Spectral data, Medicinal plants, Sahara.

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INTRODUCTION

South Algeria with its rich floral resources and ethnobotanical history is an ideal place to screen plants for biological activity and as a source of new pharmacological compounds [1-3].

Saharan plants are known by their resistance to several stress factors. Under extreme climatic conditions, they could constitute a large reservoir of new natural, safe, and effective structural moieties which work together exhibiting a wide range of biological activities [1,3,4].

Moricandia arvensis belonging to Brassicaceae family is one of species native to the Mediterranean region and witch is cultivated as ornamental plants with purple, violet, and white flowers in Europe and America [5]. In traditional ethnopharmacopeae of Algerian Sahara, *M. arvensis*, known by the common name "*Krom Jmal,*" is used for treating various diseases as rheumatism, syphilis, and scorbut [4] and provides food for camels [6].

A limited phytochemical works were reported for this species, and an indole derivative, glucosinolates, fatty acids, essential oils, and anthocyanins derivatives have been isolated and characterized [7-10].

In the aim of valorization of Saharan medicinal plants growing in Algerian Sahara and following our phytochemical works [11-16], on the secondary metabolite and bioactive substances, now we are interested in isolating the natural compounds from *M. arvensis* [11].

METHODS

General experimental procedure

Column chromatography was performed over silica gel 60 (Merck, particle size 230-400 mesh). Thin-layer chromatography (TLC) was carried out on silica gel 60 F254 plates (Merck, Germany). Ultraviolet (UV) spectra were obtained in methanol (MeOH) solvent with Unicam UV 300 spectrophotometer and Specord 200 Plus spectrophotometer.

Infrared (IR) spectra were obtained with a Thermo Nicolet Avatar 320 Fourier-transform-IR spectrophotometer. The nuclear magnetic resonance (NMR) spectra were taken on a Bruker Avance GP 250 (^{1}H: 250 or 300 MHz; ^{13}C: 75 or 125 MHz) spectrometer.

Plant material

M. arvensis was collected during full blossom (December 2009–January 2010) from Oued Zouzfana (Algerian Sahara) and identified by Pr. A. Maarouf (University of Naama, Algeria). A voucher specimen is deposited in the herbarium of Phytochemistry and Organic Synthesis Laboratory under the number CA02/32.

Extraction and isolation

150 g of dried leaves (grounded into powder) of *M. arvensis* were extracted with acetone-water (80:20 [v: v]) using Soxhlet apparatus, and reflux for 4 h was performed. This extract solution is filtered and evaporated (80%) using a vacuum rotary evaporator. The aqueous extract was fractioned successively by three solvents: Diethyl ether (Et_2O), ethyl acetate (AcOEt), and butanol (BuOH) [17].

To purify and to identify the constituents of the diethyl ether fraction (2.83 g), ethyl acetate fraction (4.25 g), and butanol fraction (2.52 g), some separations by liquid chromatography were achieved using a column in glass packed with a stationary phase of silica gel (0.20 mm); the mobile phases chosen for those separations are: Petroleum ether/ethyl acetate 90/10, 80/20, 70/30, 60/40, 50/50, 40/60, and 20/80 for the chromatography analysis of the ethyl ether fraction; petroleum ether, ethyl acetate, mixture of chloroform-methanol for the analysis of the ethyl acetate fraction and finally hexan, ethyl acetate and MeOH for the butanol fraction analysis. All fractions were monitored by TLC. Fractions with spots of the same retention factor values were combined and chromatographed in appropriate solvent systems until pure compounds were obtained.

RESULTS AND DISCUSSION

The experimental results of phytochemical screening of *M. arvensis* leaves revealed the presence of flavonoids, tannins, cardenolides, saponins, and alkaloids [4]. The presence of tannins is confirmed by positive reaction with ferric chloride ($FeCl_3$). Flavonoids test results showed a positive reaction in the presence of magnesium and HCl. In contrast, the study indicated that reducing compounds were absent.

TLC analysis of the samples separated by liquid chromatography on column revealed the existence of two products ([1] and [2] in AcOEt fraction), two products ([4] and [5] in diethyl ether fraction), and one product ([3] in Butanol fraction). After several separations and analysis chromatographic, we regrouped the results in Table 1.

Compound (1): - 5, 7-dihydroxy-3, 6, 4'-trimethoxyflavone

UV_{max}(MeOH): 356, 270 nm; (MeOH+NaOH): 357, 271, 280 nm; (MeOH+$AlCl_3$): 391, 272 nm; (MeOH+$AlCl_3$+HCl): 390, 271 nm; (MeOH+NaOAc): 355, 278 nm; (MeOH+NaOAc+H_3BO_3): 356, 276 nm. IR (KBr): 3410.02, 2918.36, 1732.93, 1508.95, 1465.25, 1383.31, 1071.93, 837.03 cm^{-1}. ^1H NMR (δ[ppm]): 6.53(H-8, s), 7.51(H-2',d, J=8.2Hz), 6.91(H-3',d, J=8.4Hz), 6.91(H-5', d, J=8.4Hz), 12.8 (OH-5,s), 7.51(H-6',d, J=8.4Hz), 3.97(OMe, s). ^{13}C NMR (DMSO-d6, δ (ppm)): 155(C-2), 138 (C-3), 178 (C-4), 152 (C-5), 105 (C-5a), 131 (C-6), 157 (C-7), 94 (C-8), 152 (C-8a), 122 (C-1'), 130 (C-2'), 114 (C-3'), 161 (C-4'), 114 (C-5'), 130 (C-6'), 56.40 (CH_3').

Compound (2): - 5, 7, 4'-trihydroxy-3, 6, 8, 3'-tetramethoxyflavone

UV_{max}(MeOH): 352, 272 nm; (MeOH+NaOH): 392, 271, 283 nm; (MeOH+$AlCl_3$): 383, 272 nm; (MeOH+$AlCl_3$+HCl): 382, 271 nm; (MeOH+NaOAc): 352, 276 nm; (MeOH+NaOAc+H_3BO_3): 352, 276 nm. IR (KBr): 3442.79, 2951.14, 1727.47, 1459.79, 1388.77, 1055.54 cm^{-1}. ^1H-NMR (δ (ppm)): 7.80 (H-2', s), 6.98(H-5', d, J=8.2Hz), 12.45(OH-5, s), 7.68(H-6', d, J=8.2Hz), 4.01(OMe, s), 3.96(OMe, s), 3.84 (OMe, s). ^{13}C-NMR (DMSO-d6, δ(ppm)): 150.95(C-2), 135.2 (C-3), 176 (C-4), 152.2 (C-5), 102.9 (C-5a), 93.6 (C-6), 157.0 (C-7), 131.0 (C-8), 144.9 (C-8a), 123.1 (C-1'), 114.8 (C-2'), 146.2 (C-3'), 147.2 (C-4'), 115.3 (C-5'), 121.2 (C-6'), 56,41 (CH_3'), 59.8 (CH_3'), 60.2 (CH_3').

Compound (3): -3, 3', 4', 5, 7- pentahydroxy flavanone

UV_{max}(MeOH): 351, 249 nm; (MeOH+NaOH): 391, 250, 325 nm; (MeOH+$AlCl_3$): 381, 249 nm; (MeOH+$AlCl_3$+HCl): 361, 251 nm; (MeOH +NaOAc): 265 nm; (MeOH+NaOAc +H_3BO_3): 361, 264 nm. IR (KBr): 3470, 2951, 2847, 1733, 1613, 1536, 1455.2, 1378, 1170, 1104, 620, 32 cm^{-1}. ^1H- NMR (δ [ppm]): 12.8 (OH-5,s), 6.17 (H-6,s, J=1.7Hz), 6.37 (H-8, s, J=1.7Hz), 7.55 (H-2', s, J=2.2Hz), 6.83 (H-5',d), 7.55 (H-6',d, J=2.2Hz). ^{13}C NMR (DMSO-d6, δ [ppm]): 79.8 (C-2), 27.54 (C-3), 160.10 (C-4), 67.34 (C-5), 99.8 (C-5a), 96.30 (C-6), 156.88 (C-7), 93.01 (C-8), 156.80 (C-8a), 130.85 (C-1'), 116.10 (C-2'), 145.78 (C-3'), 145.81 (C-4'), 115.01 (C-5'), 118.60 (C-6').

Compound (4): -3-glucosyl 3', 4', 5, 7 tetrahydroxyflavonol or 3-glucosyl quercetin

UV_{max}(MeOH): 340, 281 nm; (MeOH+NaOH): 382, 283, 328 nm; (MeOH+$AlCl_3$): 370, 281 nm; (MeOH+$AlCl_3$+HCl): 345, 282 nm; (MeOH+NaOAc): 297 nm; (MeOH+NaOAc+H_3BO_3): 358, 296 nm. IR (KBr): 3437.33, 2956.6, 2847.34, 1732.93, 1486, 1465.25, 1383.31, 1366.92, 1168, 1104, 625.3 cm^{-1}. ^1H NMR (δ[ppm]): 7.1 (H-2',s, J=2.1Hz), 6.98(H-5', d, J=8.2Hz), 12.7 (OH-5,s), 6.15 (H-6,s, J=1.5Hz), 6.33 (H-8, s, J=1.5Hz), 6.80 (H-5',d, J=8.4Hz), 7.10 (H-6',d, J=2.1Hz), 7,55 (H-1'', d, J=5.1Hz), 2,59 (H-2'', d, J=7.1Hz), 2,70 (H-4'', t, J=3.2Hz)., 3.20 (H-5'', d, J=2.0Hz). ^{13}C NMR (DMSO-d6, δ[ppm]): 146.9 (C-2), 135.9 (C-3), 176 (C-4), 160.9 (C-5), 103.2 (C-5a), 98.3 (C-6), 164 (C-7), 93.5 (C-8), 156.3 (C-8a), 122.2 (C-1'), 115.3 (C-2'), 145.2 (C-3'), 147.8 (C-4'), 115.8 (C-5'), 120.1 (C-6').

Compound (5): Kaempférol-3-digalactopyranoside

UV_{max}(MeOH): 355, 265 nm; (MeOH+NaOH): 375, 283, 327 nm; (MeOH+$AlCl_3$): 398, 281 nm; (MeOH+$AlCl_3$+HCl): 398, 282 nm; (MeOH+NaOAc): 358, 280 nm; (MeOH +NaOAc+H_3BO_3): 358, 296 nm. IR (KBr): 3371.78, 2940.21, 2874.65, 1667.37, 1601.82, 1503.49, 1459.79, 1377.85, 1268.59, 1071.93 cm^{-1}. ^1H-NMR (δ [ppm]): 8.06 (H-2',d, J=7.1Hz), 6.88 (H-3', d, J=7.1Hz), 6.88 (H-5', d, J=7.1Hz), 12.7 (OH-5,s), 6.17 (H-6,d, J=1.5Hz), 6.39 (H-8, d, J=1.5Hz), 6.80 (H-5',d, J=8.4Hz), 8.06 (H-6',d, J=7.1Hz), 5.34 (H-1'', d, J=5.1Hz), 2.91 (H-2'', m), 4.06 (H-6'', m). 3.77 (H-1''', d, J=6.5Hz), 2.84 (H-2''', m), 3.95(OH-5''', m), 3.38 (H-6''', d, J=3.0Hz). ^{13}C- NMR (DMSO-d6, δ [ppm]): 159.3 (C-2), 133.2 (C-3), 177.32 (C-4), 161.16 (C-5), 103.63 (C-5a), 98.97 (C-6), 165.15 (C-7), 93.85 (C-8), 156.50 (C-8a), 120.84 (C-1'), 130.94 (C-2'), 115.08 (C-3'), 159.08 (C-4'), 115.08 (C-5'), 130.94 (C-6'), 102.89 (C1''), 71.11 (C-2''), 73.75 (C-3''), 69.87 (C-4''), 76.59 (C-5''), 67.15 (C-6'').

The analysis of the ^1H-NMR spectrum of compound (1) indicates the presence of three methoxyls at 3.96, 3.97, and 3.99 ppm, four aromatic protons: Two at 7.51 (H-2 ' and H-6') and two others at 6.91 ppm (H -3 ' and H-5'), and an ethylenic acid at 6.53 ppm (H-8). There is a signal at 12.8 ppm relative to hydroxyl (5-OH).

The ^{13}C-NMR spectrum shows three signals at 56.10, 56.40, and 59.01 ppm, relating to primary attached oxygen (methoxyl) carbons, three signals at 94, 114, and 130 ppm corresponding to tertiary carbons (sp^2), and ten quaternary carbons including a carbonyl type at 178 ppm characteristic of a flavonoid. From the above spectral data, the structure is probably 5, 7-dihydroxy-3, 6, 4'-trimethoxyflavone (Fig. 1).

The study of the ^1H-NMR spectrum of compound (2) gives the following indications: A signal as singlet at 12.45 ppm with ^1H integration confirms the presence of a free OH in position 5. A singlet at 7.80 ppm with ^1H integration is attributable to H-2', and two doublets at 7.68 ppm and 6.98 ppm (J=8.2 Hz) of ^1H integration each are attributable to H-6 'and H-5', respectively. These signals indicate that it is a disubstitution of the B ring. Four signals as singlets at 4.01, 3.96, 3.84, and 3.83 ppm with ^3H integration, attributable to the methoxyl groups.

The data reported above are in agreement with those of the literature [18-20] and lead to the structure: 5,7,4'-trihydroxy-3,6,8,3'tetramethoxyflavone (Fig. 1).

The IR spectrum of compound (3) shows an O-H band in the vicinity of 3470 cm^{-1}, and an absorption band at 1104 cm^{-1} which is characteristic of the C-O group of aliphatic ether. The vibration at 620 cm^{-1} notes the presence of a benzene ring, and an additional confirmation comes from the elongation bands of aromatic C-H located at the limit of the high frequencies of the aliphatic C-H elongations.

The examination of the ^1H-NMR spectrum shows: Two signals in the form of singlets at 6.17 and 6.37 ppm (J=1.7 Hz) with ^1H integration, characteristic of the H-6 and H-8 protons, respectively; a singlet at 12.8 ppm, attributable to OH at position C-5; and two other signals (singlet and doublet) at 7.55 ppm (J=2.2 Hz) attributable to H-2' and H-6' [21]. The doublet at 6.83 ppm (J=7.1Hz) of 1H integration is attributable to H-5'.

Therefore, the most probable structure, based on the above spectral data, is 3, 3 ', 4', 5,7-pentahydroxy flavanone (Fig. 1).

The investigation of the ^1H-NMR spectrum of compound (4) shows the presence of two singlets at 6.15 and 6.33 ppm (J=1.5 Hz) with ^1H integration each, characteristics of the H-6 and H-8 protons, respectively; a singlet at 12.7 ppm, attributable to OH at position C-5; and two signals at 7.10 ppm (J=2.1 Hz) in the form of a singlet and doublet with ^1H integration, attributable to H-2' and H-6' [22]. A doublet at 5.22 ppm (J=5.1 Hz) is attributable to the anomeric proton of a sugar bonded to the aglycone by a C-O bond (at position 3) because all other positions are occupied by protons; three doublets with 1H integration each at 3.70 ppm (J=7.1 Hz), 3.50 ppm (J=3.1 Hz), and 3.61ppm (J=2.0 Hz).

It is noted that the values of the coupling constants are characteristic of vicinal couplings of protons of hydroxyl groups with C-H groups. These observations indicate the presence of three hydroxyl groups in the sugar entity.

Table 1: Results of liquid column chromatography

Compound	Rf (color in TLC) (eluent)*		
Compound (1)	0.32 (yellow) (I)	0.93 (yellow) (II)	0.86 (yellow) (IV)
Compound (2)	0.43 (yellow) (I)	0.09 (yellow) (II)	0.84 (yellow) (IV)
Compound (3)	0.80 (blue) (I)	0.04 (blue) (II)	0.32 (blue) (IV)
Compound (4)	0.90 (yellow) (I)	0.43 (pale yellow) (II)	0.32 (purple) (V)
Compound (5)	0.59 (yellow) (I)	0.43 (pale yellow) (III)	0.20 (yellow) (V)

(Eluent)*: (I) B/A/W (4/1/5), (II) $CHCl_3$/MeOH/H_2O (9/1/0.5), (III) MeOH/H_2O/AcOH (20/1/1), (IV) $(CH_3)_2CO$/H_2O (1/1), (V) Toluene/MeOH/EtOH/Petroleum ether (2/1.5/1/0.5). Rf: Retention factor, TLC: Thin-layer chromatography

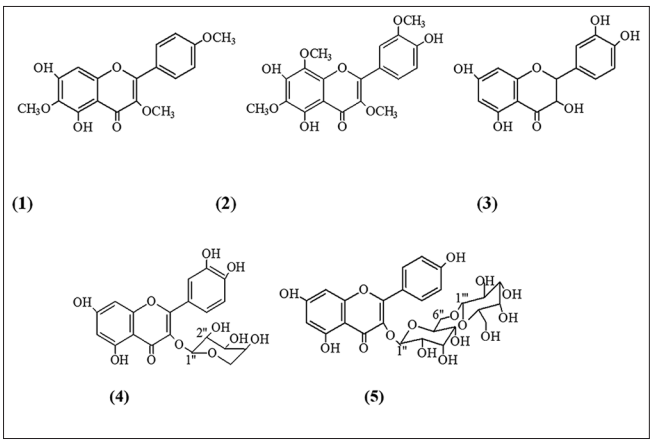

Fig. 1: Structures of flavonoids isolated from leaves of *Mor1icandia arvensis*

This hypothesis is supported by the presence of a triplet of doublet with ^{1}H integration at 2.59 ppm (J=7.1 Hz) and two doublets at 2.48 ppm (J=7.1 Hz) and 2.70 ppm (J=3.2 Hz). From the values of the coupling constants of this ring, it is clear that these signals are attributable to the three protons of the three CH groups of the three hydroxyls.

Also we noted a doublet at 3.20 ppm (J=2.0 Hz), with 1H integration, thus from the previous results, this C-H must be part of the sugar entity ring.

The IR spectrum of compound (5) shows absorption bands at 3371, 1667, and 1503 cm^{-1} corresponding to the presence of hydroxyl, carbonyl, and a double bond.

The ^{1}H-NMR spectrum of the compound (5) shows some signals characterizing a flavonoid skeleton of the flavonol type. In the fact, the doublets at 6.88 and 8.06 ppm (J=7.1Hz) with 2H integration each are attributable to H-3′, H-5′ and H-2′, H-6′. The spectrum shows two signals at 6.17 and 6.39 ppm (J=1.5 Hz) in the form of two singlets with ^{1}H integration, attributable to the H-6 and H-8 protons of A ring, respectively.

The proton signals of the sugar portion appear at 5.34 ppm (H-1″), 2.91 ppm (H-2″), 3.44 ppm (H-3″), 3.32 ppm (H-4′), and 3.45 ppm (H- 5″).

The ^{13}C-NMR spectrum shows signals which allow us to attribute all secondary carbons (C-H) of the flavonol skeleton: 93.85 (C-8), 98.97 (C-6), 115.08 (C-3 ′, 5′), and 130.94 (C-2 ′, 6′) and the carbons of the sugar portion at 2.89 (C-1 ″), 71.11 (C-2 ″), and 73.75 (C-3 ′ ″). The comparison of the ^{13}C-NMR spectral data of this compound with those reported in the literature suggests that the glucose is attached to the C-3 carbon insofar as the carbon C-3 and C-2 are at 136.1 and 147.5, respectively, if the carbon C-3 is a carrier of a free OH, and at 132 and 159.5, if the C-3 carbon is substituted [23-25]. Thus, all this spectral analysis makes it possible to say that the compound (5) is kaempferol-3-digalactopyranoside [23] (Fig. 1).

CONCLUSION

Plants of traditional medicine constitute a medical and economic potential of natural resources that provide the raw materials necessary for the production of drugs. Our works on ethnopharmacology of medicinal plants are complemented by phytochemical studies (screening, extraction, purification, and identification), for the valorization of medicinal species. In this context, it was possible to isolate and identify five flavonoids after fractionation of the hydroacetone extract from the leaves of the medicinal plant *M. arvensis*, hoping that our work allows a technical contribution to study and value plant by integrating it into the health and economic system of the country.

IN MEMORIAM

Article dedicated to the memory of Cheriti Bouhafs died on November 25th, 2017.

AUTHOR'S CONTRIBUTIONS

The experimental work was done by A. Berreghioua under the supervision of Pr. A. Cheriti.

CONFLICTS OF INTERESTS

The authors do not have any conflict of interest to declare.

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