Journal Academica Vol. 5(1), pp. 3-12, November 14 2015 - Organic Chemistry - ISSN 2161-3338
online edition www.journalacademica.org

Full Length Research Paper

Flavonoid Compounds Composition and Antibacterial Activity of Origanum vulgare L. Sbsp. glandulosum (Desf) Ietswaart ethyl acetate extract

Farid Ait Kaki [a]*, Rachid Benkiniouar [a], Amar Zellagui [b], Saleh Rhouati [a], Farouk Zaidi [c]

[a] Laboratory of Natural Products and Organic Synthesis, Faculty of Exacts Sciences, Department of Chemistry, University Constantine 1 (Algeria)

[b] Laboratory of Bio-molecules and Plants Amelioration, Faculty of Exacts Sciences and Nature and Life Science, University Larbi ben M'hidi. Oum El Bouaghi (Algeria)

[c] Department of chemistry, University Ferhat Abbas, Setif (Algeria)

Received September 16 2015; Accepted October 27 2015

ABSTRACT

The main objective of this work is the separation and identification of secondary metabolism of the subspecies *Origanum vulgare* L. Sbsp. *glandulosum* (Desf) Ietswaart, belonging to the Lamiaceae family. The use of the different chromatographic methods (column, paper, thin layer) permitted the isolation of a flavonoid: 5, 4'-dihydroxy-7-O-α-D-glucoside flavone (Apigenin7-O-α-D-glucoside). This compound is isolated for the first time from *Origanum vulgare* L. Sbsp. *glandulosum* (Desf) Ietswaart subspecies and also in *Origanum* kind. The structure of this compound was well established using state-of-the-art spectroscopic methods (UV, ^{1}H NMR, ^{13}C NMR). Eventually, the antibacterial activity of ethyl acetate extract of the subspecies *Origanum vulgare* L. Sbsp. *glandulosum* (Desf) Ietswaart was tested with positive results, especially with Gram-negative bacteria *Pseudomonas aeruginosa* as well as with Gram-positive bacteria *Staphylococcus blanc.*

Key words: *Origanum vulgare* L. Sbsp. *glandulosum* (Desf) Ietswaart, Flavonoids, Ethyl Acetate

1. INTRODUCTION

Origanum is a genus of about 43 species of aromatic herbs in the family Lamiaceae [1,2], native from the Mediterranean Basin east of eastern Asia [1,3]. The genus includes some important culinary herbs, including *Origanum vulgare* L. Sbsp. *glandulosum* (Desf) Ietswaart.

Several species of this genus are used in traditional medicine in bitter stomachic. They are reported to have antiseptic, antispasmodic, as well as antioxidant effects [4-7]. Previous work on members of this genus revealed that the main constituents are flavonoids [8], essential oils [9], and terpenoids [10].

The main objective of this work is the separation and identification of secondary metabolism of the subspecies *Origanum vulgare* L. Sbsp. *glandulosum* (Desf) Ietswaart.

*Corresponding author: jskf56@yahoo.fr

2. MATERIALS AND METHODS

2.1. Plant material

Aerial parts (flowers and leaves) of *Origanum vulgare* L. Sbsp. *glandulosum* (Desf) Ietswaart were collected in April 2005 from Jijel (texana), Algeria.

Fig. 1: *Origanum vulgare* L. Sbsp. *glandulosum* (Desf) Ietswaart

2.2. Extraction

The crucial first step for analysis of medicinal plants is to extract the desired chemical components from the plant materials for further separation and characterization.

Air-dried aerial parts of *Origanum vulgare* L. Sbsp. *glandulosum* (Desf) Ietswaart (1170g) were macerated three times with 70% MeOH and 30% water solution by replacing the solution every day with fresh solvent and one time with hot solvent. The hydro-alcoholic solutions were concentrated under reduced pressure to dryness and the residue was dissolved in hot water being kept cold overnight. After filtration, the aqueous solution was successively extracted with dichloromethane, ethyl acetate, and n-butanol for two times for each solvent. Finally, the dichloromethane ethyl acetate and n-butanol extracts were concentrated to dryness. A brief summary of the extraction is described in Figure 2:

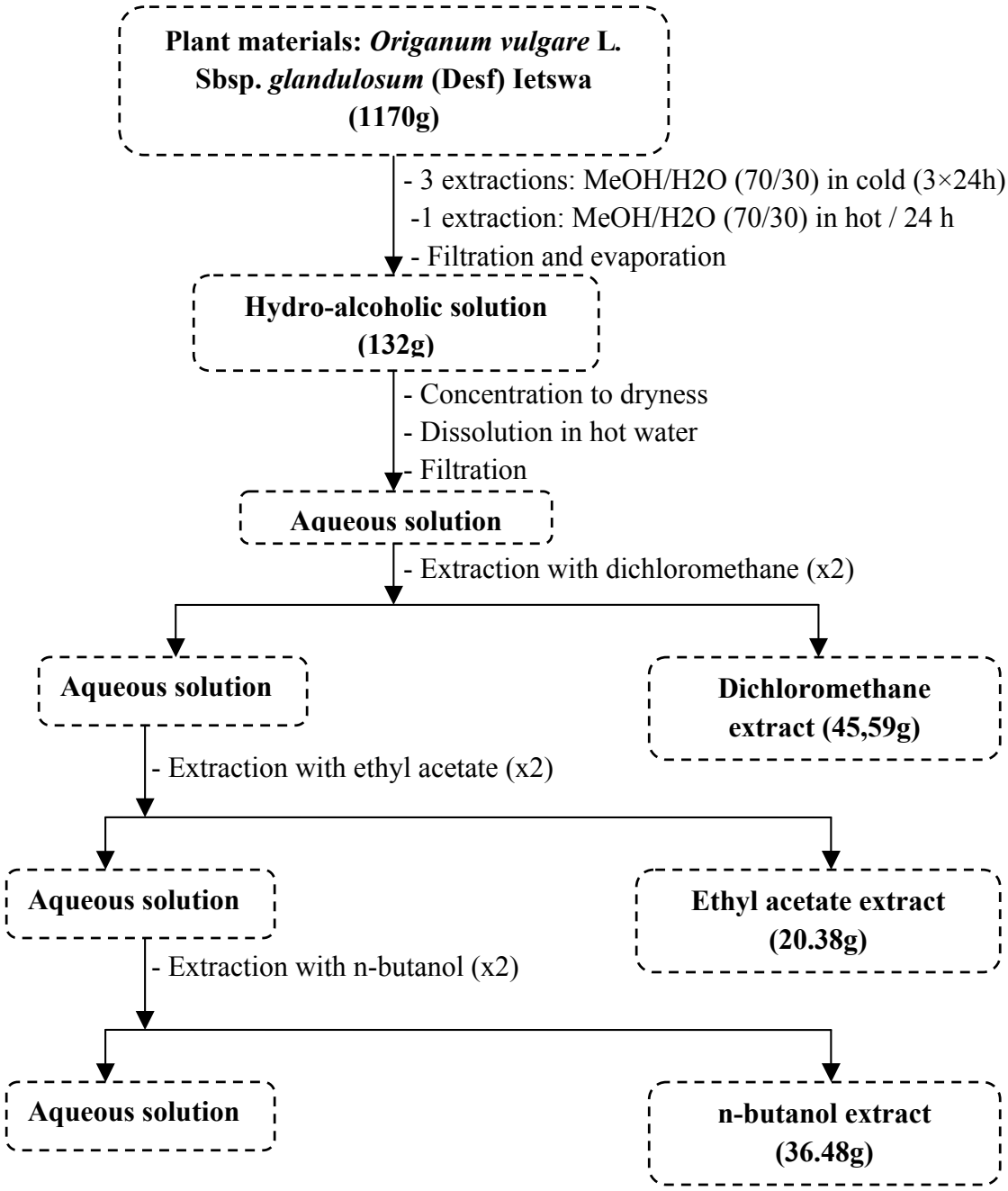

Fig. 2: Extraction Protocol of *Origanum vulgare* L. Sbsp. *glandulosum* (Desf) Ietswaart

2.3. Separation and purification of ethyl acetate extract compounds

The ethyl acetate extract (10.57g) was subjected to a SC_6 polyamide column chromatography being eluted with a gradient of Toluene/MeOH with increasing polarity. Twelve fractions were collected and compound *A* was obtained as a yellow precipitate from fraction 8 which was washed with methanol. This compound is identified for the first time in this species.

The structure of compound *A* was determined by the usual spectroscopic methods (UV, ^{1}H NMR, ^{13}C NMR).

2.4. Antibacterial activity

Moreover the antibacterial activities of the ethyl acetate extract of subspecies *Origanum vulgare* L. Sbsp. *Glandulosum* against four human pathogenic bacteria, including Gram-positive and Gram-negative bacteria, was carried out using disk diffusion method [11].

3. RESULTS AND DISCUSSION

3.1. Chromatographic comportment

Table 1: Chromatographic comportment of purified compound.

System	SI	SII
R_f	0.1	0.22
Spot color in ultraviolet light	Without NH_3 Dark purple	With NH_3 Yellow

SI: (CH2Cl2/MeOH)(5/1)
SII: AcOH (15%)
The dark purple fluorescence and the R_f shifts show that the compound is a mono glycosides, either a flavone 3-H or a flavonol 3-OR substituted.

3.2. UV spectral data

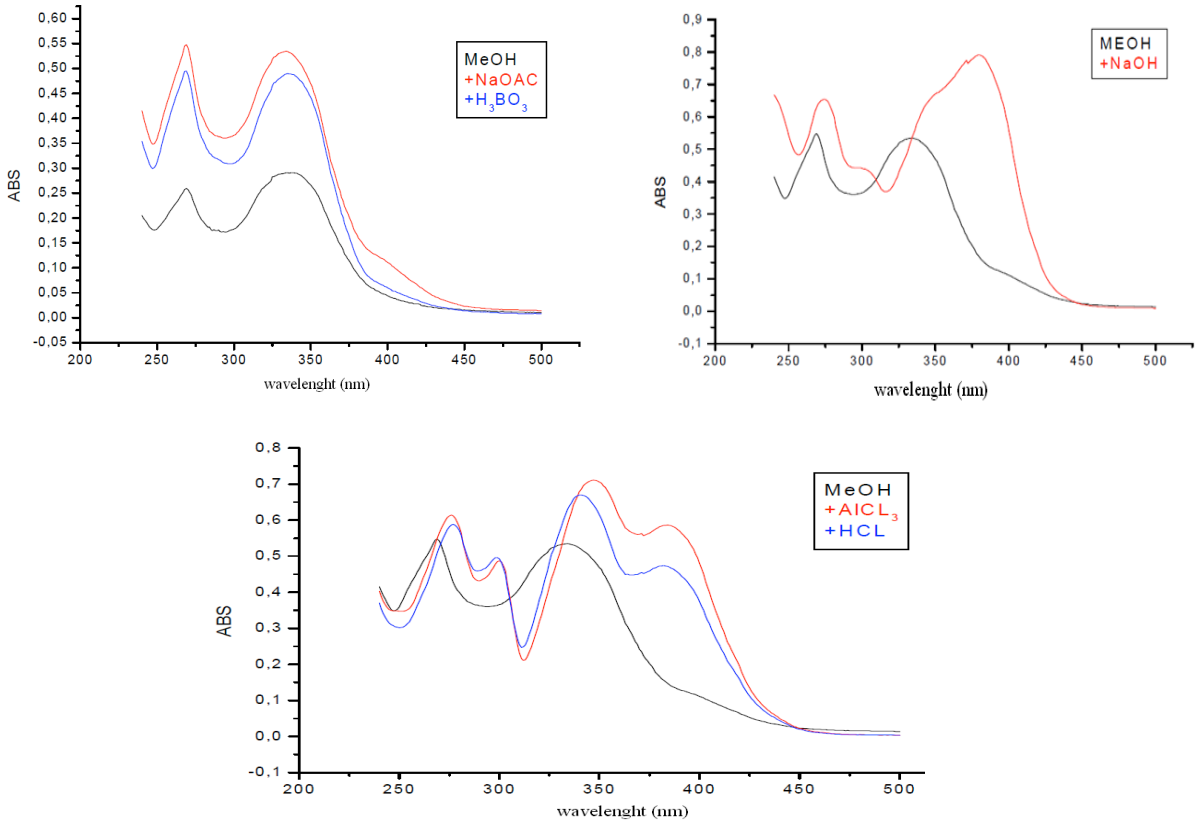

Fig. 3: The UV spectra of compound *A*

The UV spectra of compound *A* obtained in this study were as follows: (MeOH, λmax, nm) gave bands at 335 and 269nm for band I and II, in addition of NaOH; 380,272 and 303nm, $AlCl_3$; 384, 275 and 299-347nm, $AlCl_3$/HCl; 381, 276 and 298-340nm, NaOAc; 333 and 269nm while NaOAc/H_3BO_3; 336 and 269nm. The UV spectrum in methanol and its changes after the addition of the customary shift reagents suggested that the compound is a flavone 3H with free hydroxyl groups at positions C4', C5, and a substitution in position 7 (7-OR) while shift reagents AlCl3, AlCl3/HCl exhibited band I absorption suggesting the absence of ortho-dihydroxyl group in A and B-ring [12,13]. The band-shifts in the UV spectra of compound *A* is tabulated in Table 2:

Table 2: The shift of bands in the UV spectra

Chemical shift reagents	Band I (nm)	Band II (nm)	Shift of observed band (nm)
MeOH	335	269	-
NaOH	380	272	303
$AlCl_3$	384	275	347-299
$AlCl_3$+HCl	381	276	340-298
NaOAc	333	269	-
NaOAc+H_3BO_3	336	269	-

3.3. NMR Spectroscopy

NMR is one the most powerful research techniques used to investigate the structure and properties of molecules. One of the main applications of NMR in flavonoid research is the structural elucidation of novel compounds about which nothing is known.

3.3.1. The 1H NMR spectrum

The ^{1}H NMR spectrum of the purified compound is illustrated in Figures 4, 5, and 6.

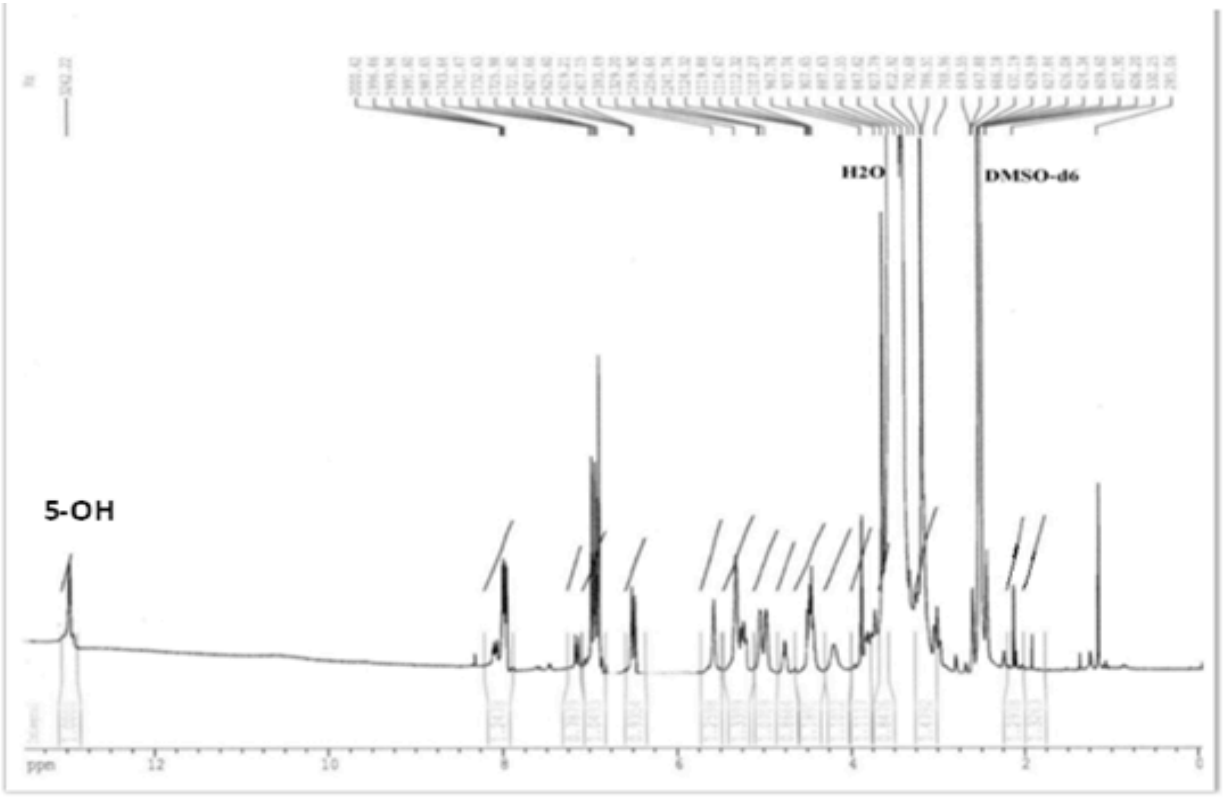

Fig. 4: ^{1}H NMR (DMSO-d_6; 250 MHz) spectrum of compound *A*

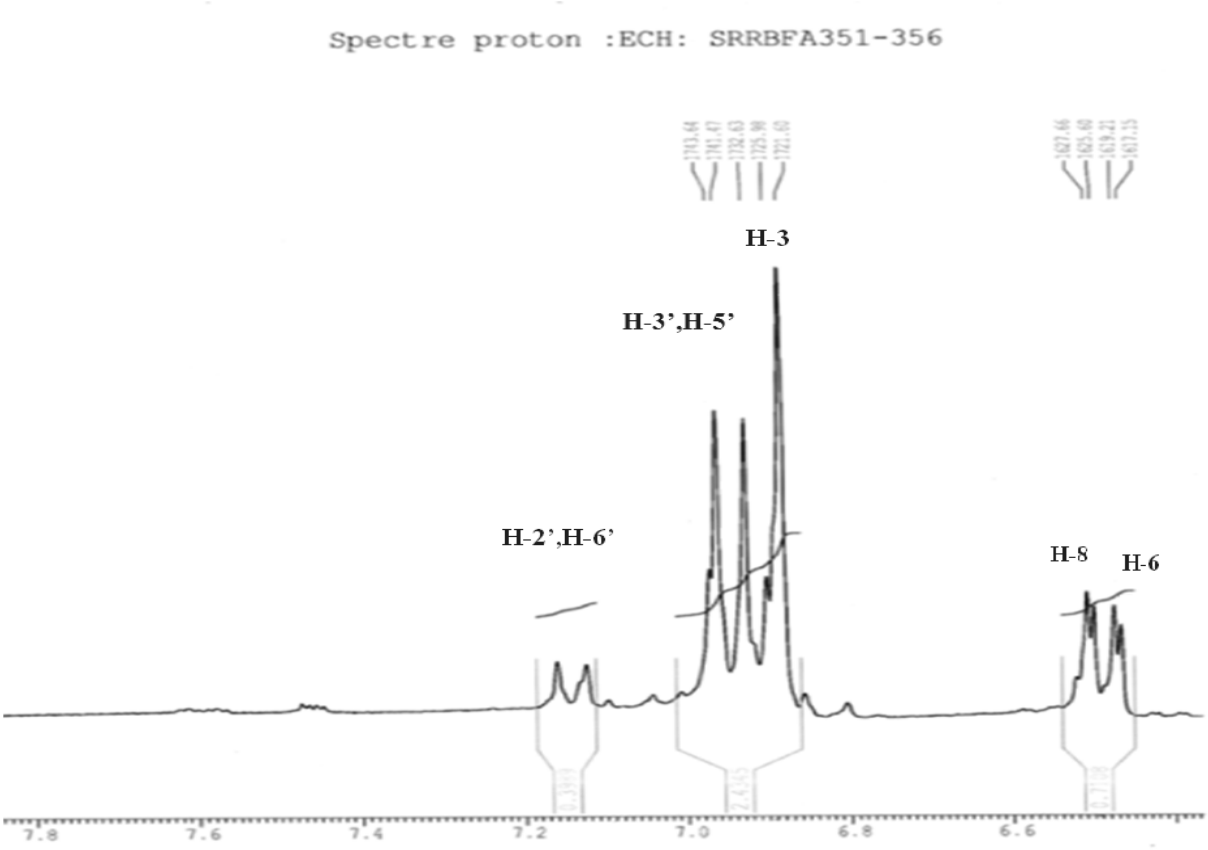

Fig. 5: [1]H NMR spectrum of compound *A* (enlarge the area 6.5- 8.00 ppm)

The spectral details of the purified compound are tabulated in Table 3.

Table 3: [1]H NMR chemical shifts

Chemical moving δ_H (ppm)	Integration	Multiplicity *J*(Hz)	Attribution
6.48	1H	d (2.06)	H6
6.52	1H	d (2.06)	H8
6.9	1H	s	H3
6.95	2H	d (8.84)	H3', H5'
7.15	2H	d (8.84)	H2', H6'
13	1H	s	5-OH

The 1H NMR Spectra showed the presence of a singlet at δ = 13 ppm, it was attributed to 5-OH

Two doublets at δ= 6.95ppm (2H, *J*=8.84 Hz) and δ= 7.15ppm (2H, *J*=8.84 Hz) was ascribed to H3', H5'and H2', H6'respectively, while the singlet at δ= 6.9ppm (1H) was attributed to H3.

Two doublets at δ= 6.48ppm (1H, *J*=2.06 Hz) and δ= 6.52ppm (1H, *J*=2.06 Hz) was ascribed to H6 and H8 respectively.

Sugar moiety at δ= 5.03ppm (1H, d, *J*=3.26 Hz, H1" glucose, α liaison to aglycon) [14].

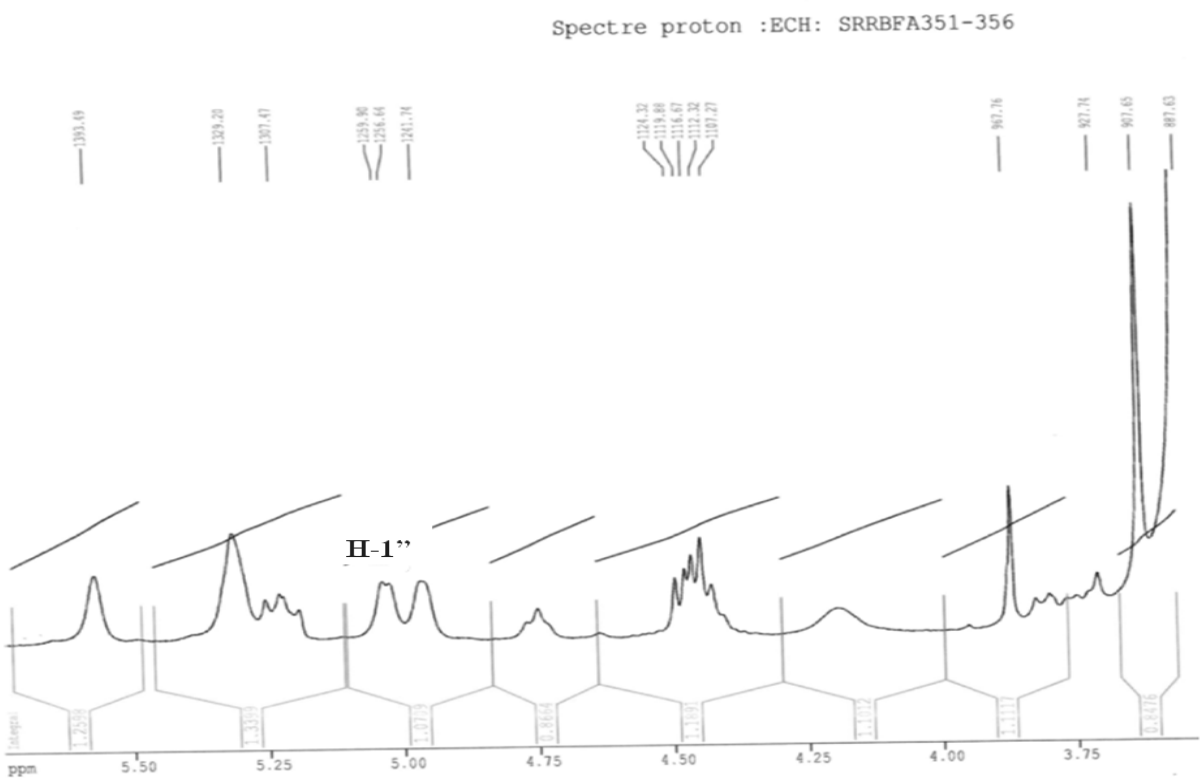

Fig. 6: [1]H NMR spectrum of compound *A* (Enlarge the area 3.75- 5.50 ppm)

3.3.2. The ^{13}C NMR spectrum

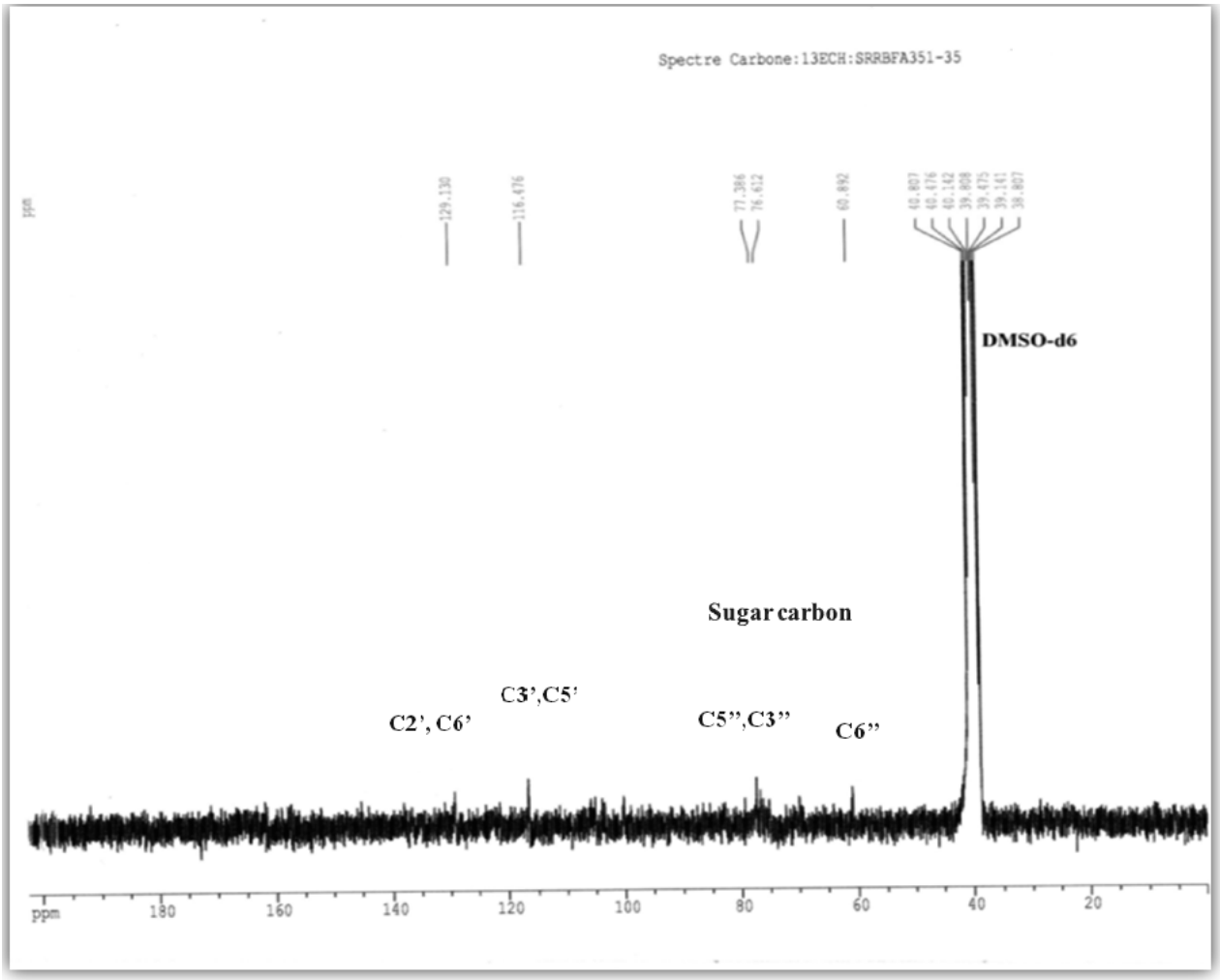

Fig. 7: [13]C NMR (DMSO-d_6; 250 MHz) spectrum of compound *A*

The ^{13}C NMR Spectra disclosed carbon signals, the results summarized in Table 3:

Table 3: ^{13}C NMR chemical shifts

Carbon No.	^{13}C NMR chemical shifts (δ)
C2', C6'	129.13
C3', C5'	116.47
C5'', glucose	77.38
C3'', glucose	76.61
C6'', glucose	60.89

The structure of this compound is:

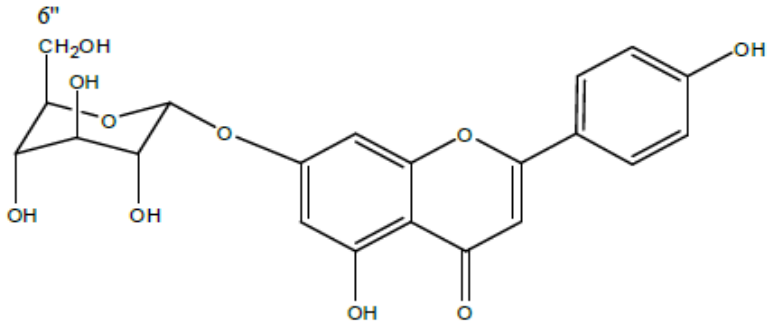

Fig. 8: Structural formulas of the compound 5,4'-dihydroxy-7-O-α-D-glucoside flavone (Apigenin 7-O-α-D-glucoside)

3.4. Antibacterial activity

The bacterial strains were first grown on Muller Hinton medium (MHI) at 37°C for 24 hours prior seeding on the nutrient agar. A sterile 6mm diameter filter disk (Whatman paper # 3) was placed on the infusion agar seeded with bacteria and the extract suspended in water was dropped onto each paper disk (40 µL per disk) for all of the prepared concentrations (2mg/mL, 0.5mg/mL). The treated Petri disks were incubated at 37°C for 24h. Antibacterial activity was assessed by measuring the growth-inhibition zone surrounding the disks. Each experiment was carried out in triplicate.

The results presented in Figure 9 show that the medium diameter of inhibition zone increases proportionally with the increase of flavonoids concentration. The obtained inhibition zone varied from 9.00 to 22.00mm.

The results were positive, especially with Gram negative bacteria *Pseudomonas aeruginosa* and Gram positive bacteria *Staphylococcus blanc.* However, no activity against *Klebsielle pneumoniae* at low concentration (0.5mg/ml) has been observed.

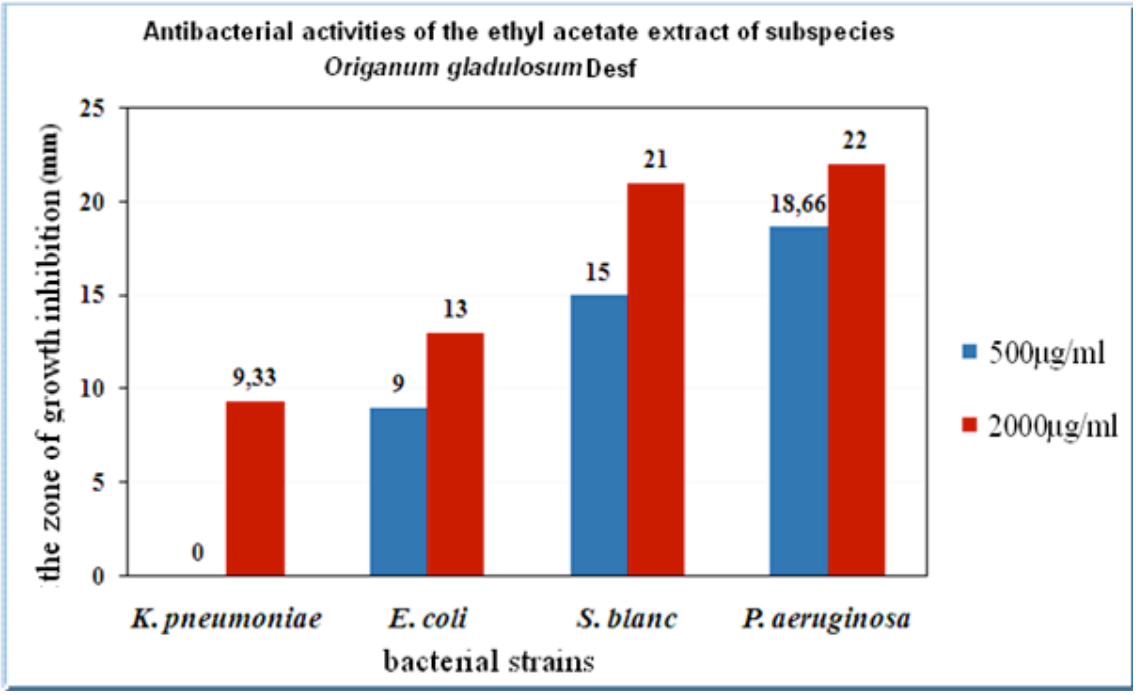

Fig. 9: Antibacterial activities of the ethyl acetate extract of subspecies *Origanum vulgare* L. Sbsp. *glandulosum.*

4. CONCLUSION

Antimicrobial activity of the ethyl acetate extract was reported. For the first time, one flavonoid has been isolated and identified from *Origanum vulgare* L. Sbsp. *glandulosum.*

5. REFERENCES

[1] Ietswaart JH. A taxonomic revision of the genus origanum (labiatae), Folia. *Geobotanica et Phytotaxonomica.* 1981; **16** (4): 390.

[2] Skoula M, Harborne JB. The taxonomy and chemistry of *Origanum*. In: Kintzios (Eed.), S.E. (Ed.), Oregano, the genera *Origanum* and *lippia*-Medicinal and Aromatic Plants Series-Industrial Profiles, vol. 25. Taylor & Francis, London, 2002, pp. 67–108.

[3] Ruberto C, Barratta MT, Sari M, Kaabache M. Chemical composition and antioxidant activity of essential oils from Algerian *Origanum glandulosum* Desf. *Flavour and Fragrance Journal.* 2002; **17**: 251-254.

[4] Baba Aissa F. Encyclopédie des plantes utiles, flore d'Algérie et du Maghreb, Librairie moderne-Rouiba, 1999, pp. 194-231.

[5] Sijelmassi A. Les plantes médicinales du Maroc. 2e éd, Le Fennec, 1991, pp.199.

[6] Chiej R. Macdonald encyclopedia of medicinal plants, Ed Macdonald, London, 1984, pp. 212-217.

[7] Mechergui K, Coelho JA, Serra MC, Lamine SB, Boukhchina S, Khouja ML. Essential oils of Origanum vulgare L. subsp. glandulosum (Desf.) Ietswaart from Tunisia: chemical composition and antioxidant activity. *J Sci Food Agric.* 2010; 15; 1745-1749.

[8] Wollenweber E. The systematic implication of flavonoids secreted by plants. In: Rodriguez, E., Healey, P.L., Mehta, I. (Eds.), Biology and Chemistry of Plant Trichomes Plenum Press, New York, 1984, pp. 53–69.

[9] Belhattab R, Larous L, Figueiredo CA, Pedro Santos AG, Barroso JG, Pedro LG. *Origanum glandulosum* Desf Grown Wild in Algéria : Essential oil composition and glycosidic bound volatiles, *Flaveour Fragr J*. 2005; **20**: 209-212.

[10] Skoula M, Gotsiou P, Naxakis G, Johnson CB. A chemosystematics investigation on the mono - and sesquitrepenoids in the genus *origanum* (Labiatae). *Phytochemistry*. 1999; **52**: 649-657.

[11] Carbonnelle BF, Denis A, Marmonier G, Rivargues P, Bacteriologie medicale-techniques usuelles, 1987, pp. 224-243.

[12] Ducrey B, Wolfender JL, Marston A. Analysis of flavonol glycosides of thirteen *Epilobium* species (onagraceae) by LC-UV and thermospray LC-MS, *Phytochemistry*. 1995; **38**: 129–137.

[13] Markham KR. Techniques of Flavonoid Identification, Academic Press, London, 1982, pp. 251–262.

[14] *Harborne JB*, *Marbry TJ, Marbry H*. The flavonids. In: *T.J. Marbry* and *K.R. Markham*. (Eds.), Mass Spectrometry of *Flavonoids*, *Chapman and Hall*, *London*, *1975*, pp. 78–126.