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Full Length Research Paper

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

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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.

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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:

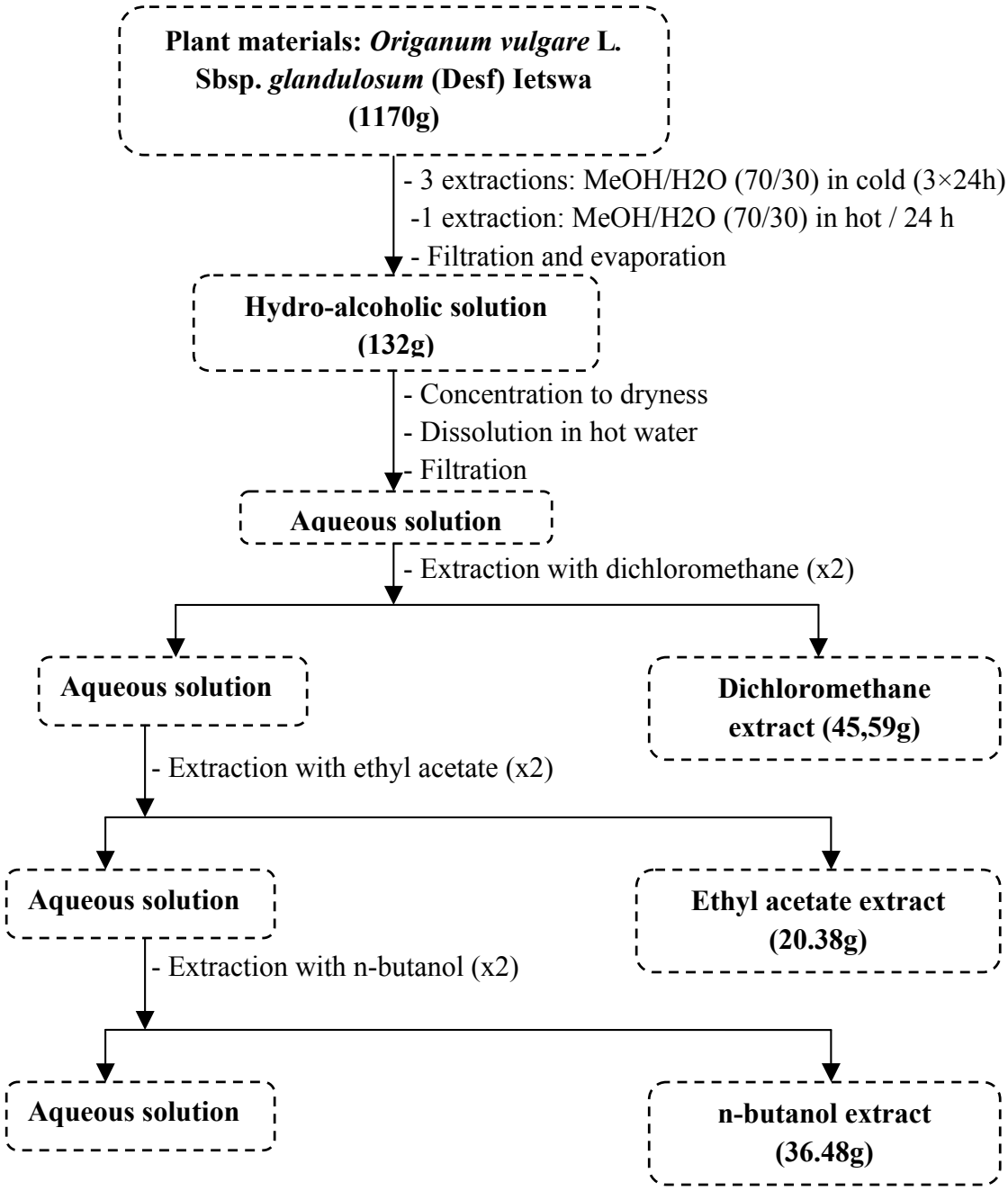

Fig. 2: Extraction Protocol of *Origanum vulgare* L. Sbsp. *glandulosum* (Desf) Ietswaart

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

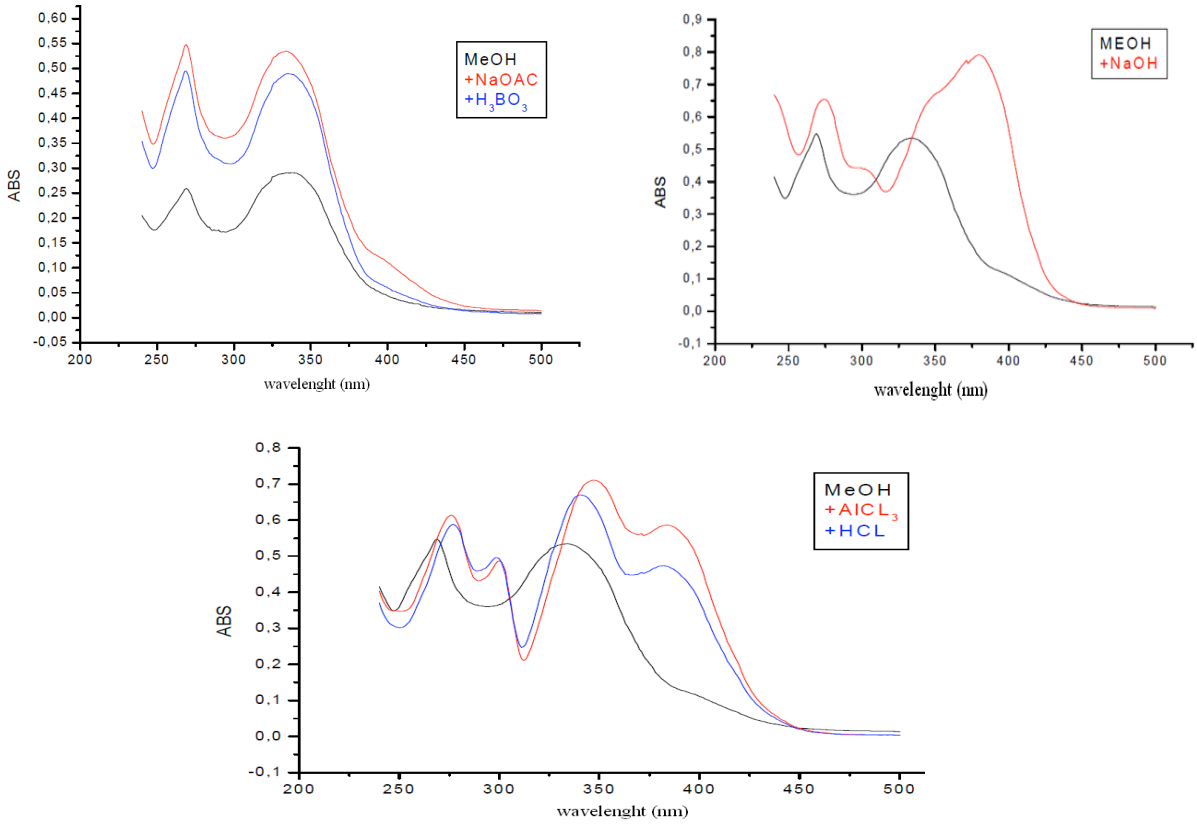

Fig. 3: The UV spectra of compound *A*

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.

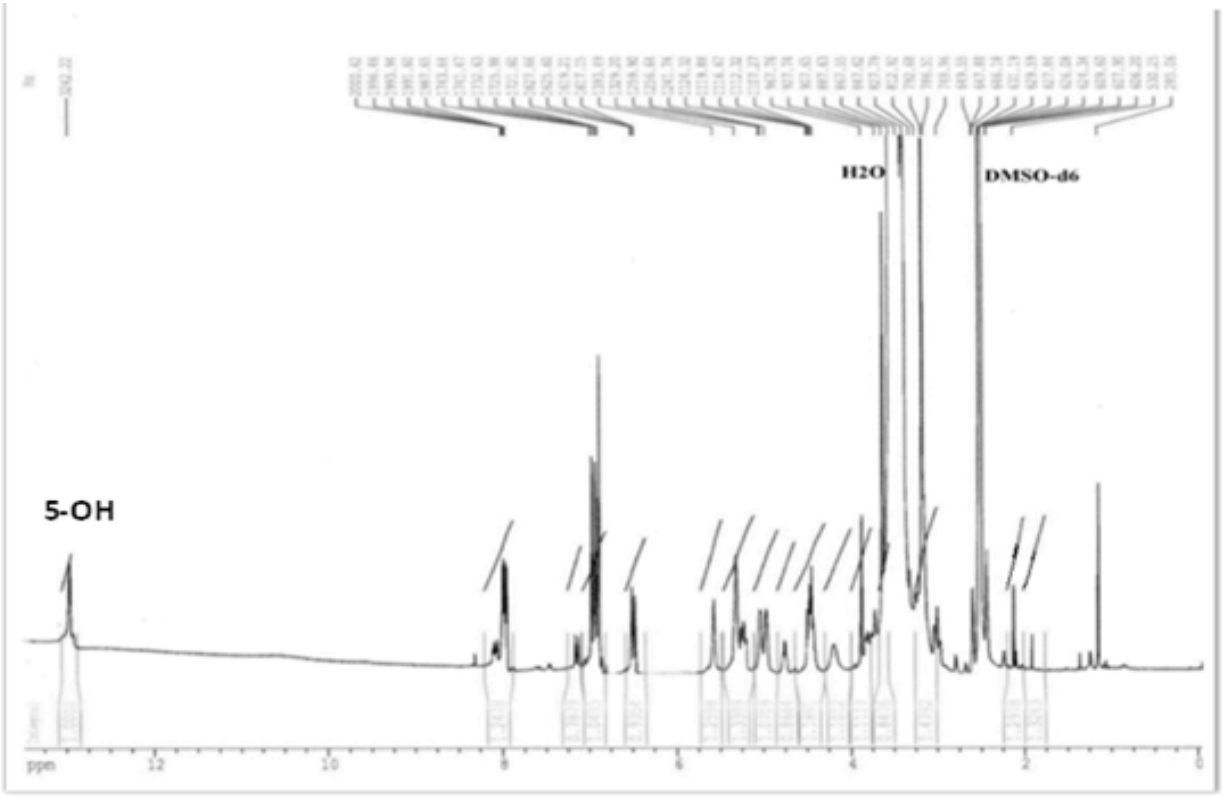

Fig. 4: ^{1}H NMR (DMSO-d_6; 250 MHz) spectrum of compound *A*

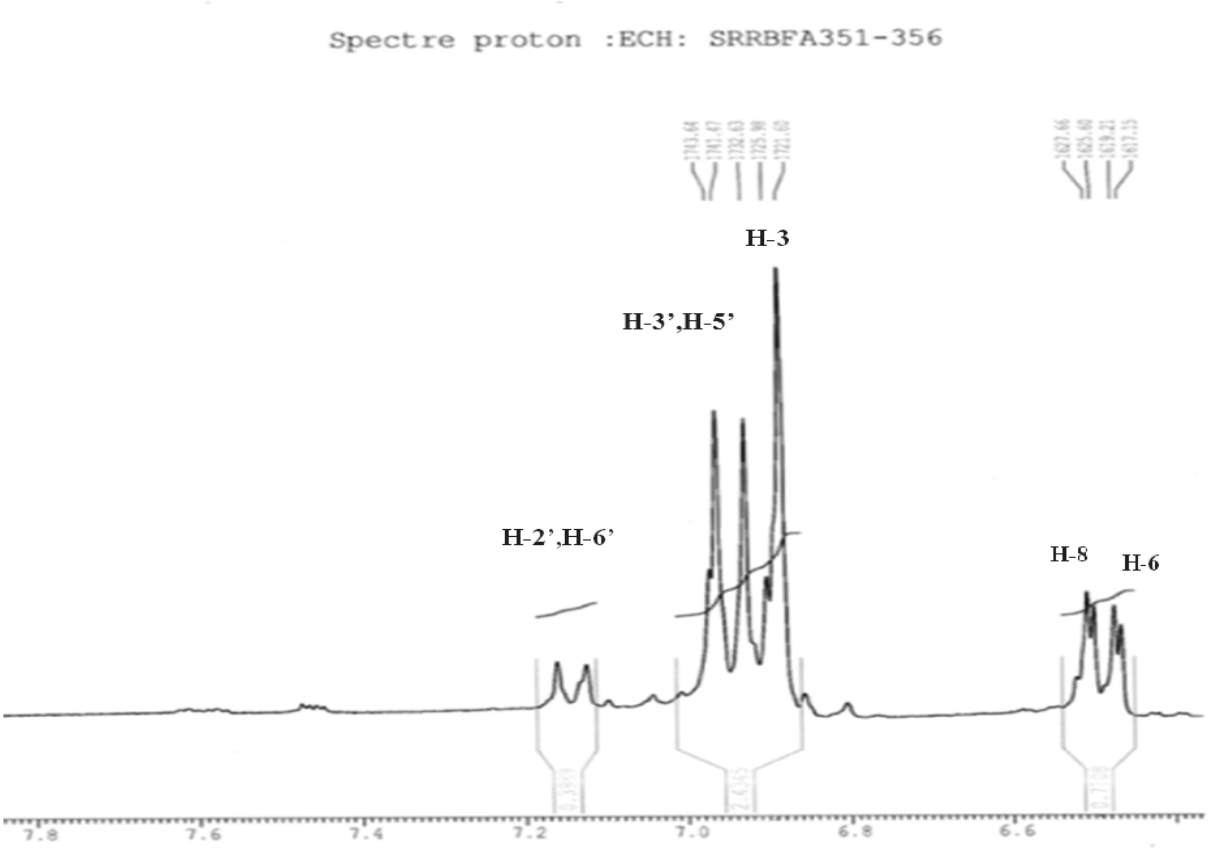

Fig. 5: [1]H NMR spectrum of compound *A* (enlarge the area 6.5- 8.00 ppm)

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].

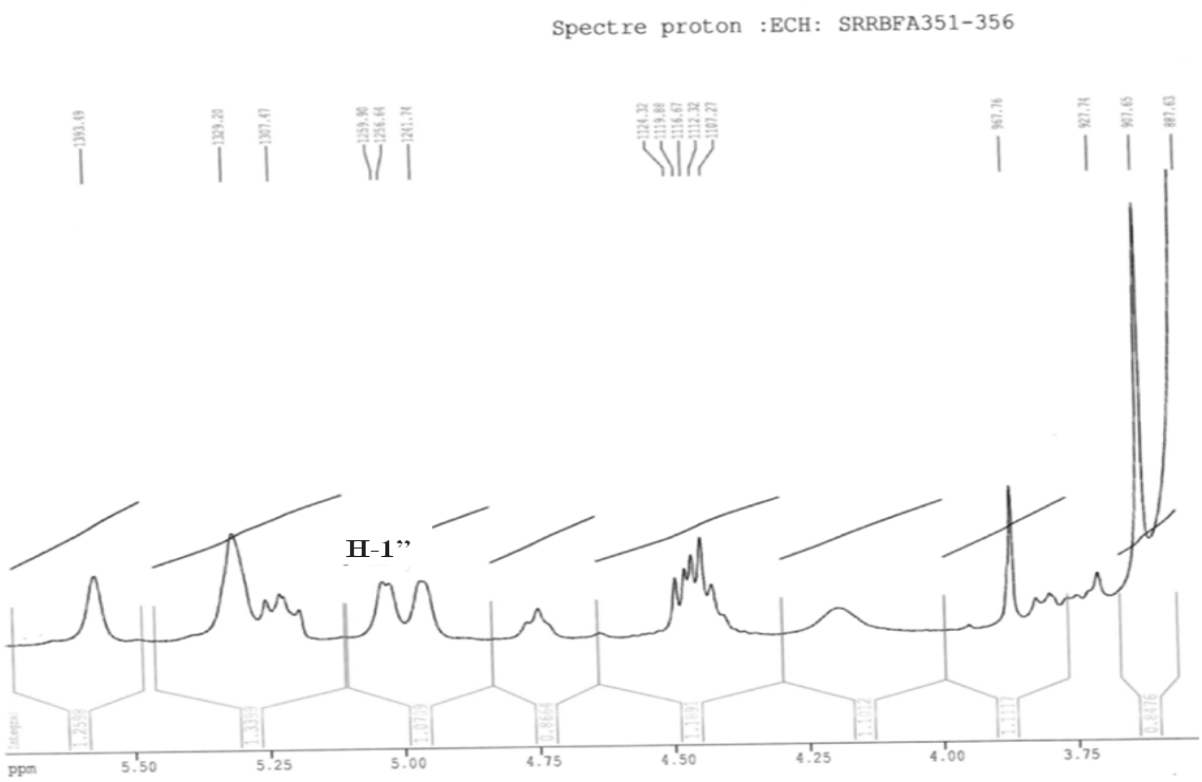

Fig. 6: [1]H NMR spectrum of compound *A* (Enlarge the area 3.75- 5.50 ppm)

3.3.2. The ^{13}C NMR spectrum

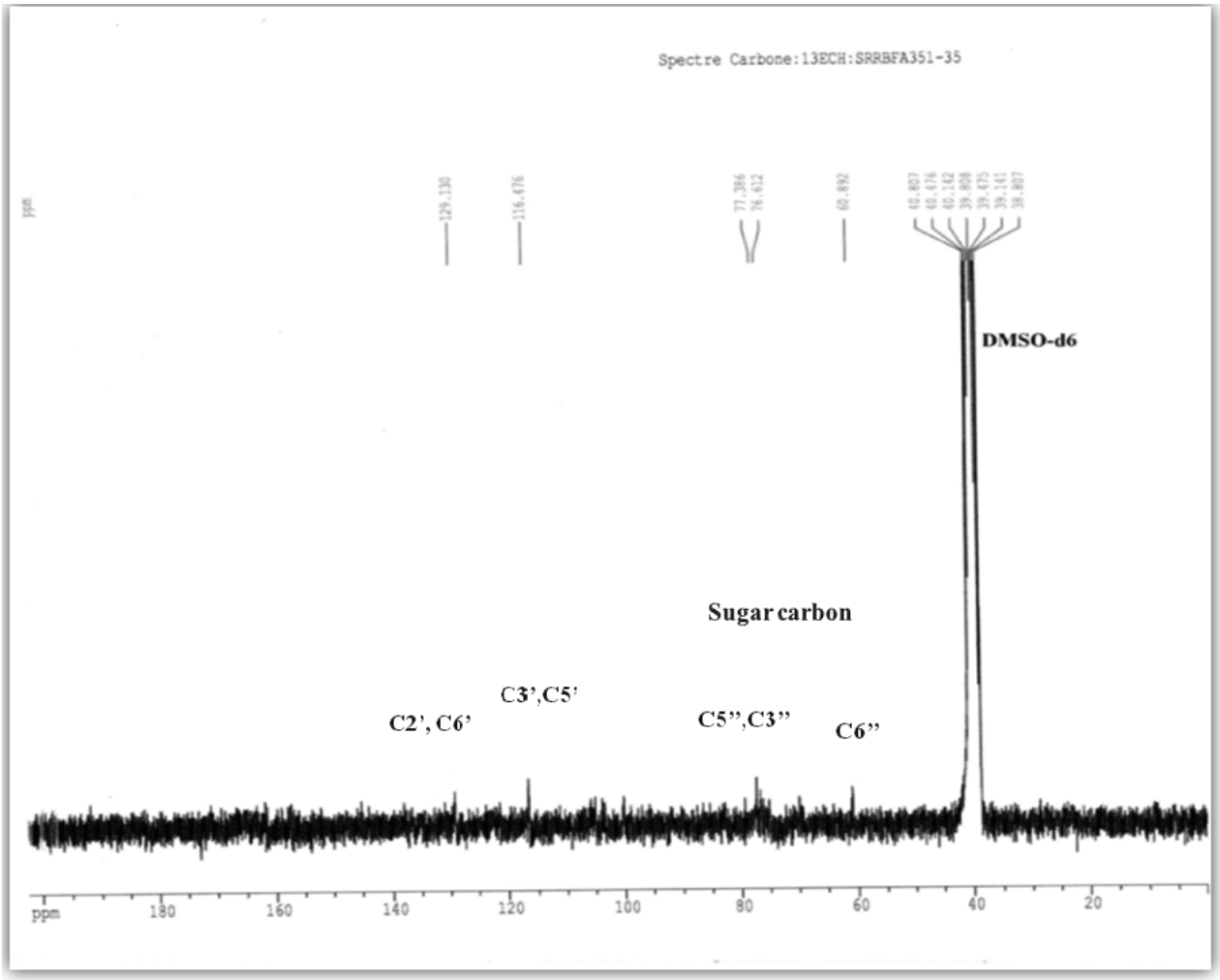

Fig. 7: [13]C NMR (DMSO-d_6; 250 MHz) spectrum of compound *A*

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:

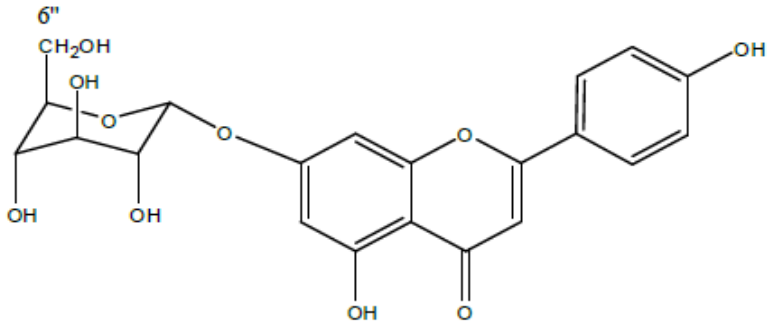

Fig. 8: Structural formulas of the compound 5,4'-dihydroxy-7-O-α-D-glucoside flavone (Apigenin 7-O-α-D-glucoside)

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.

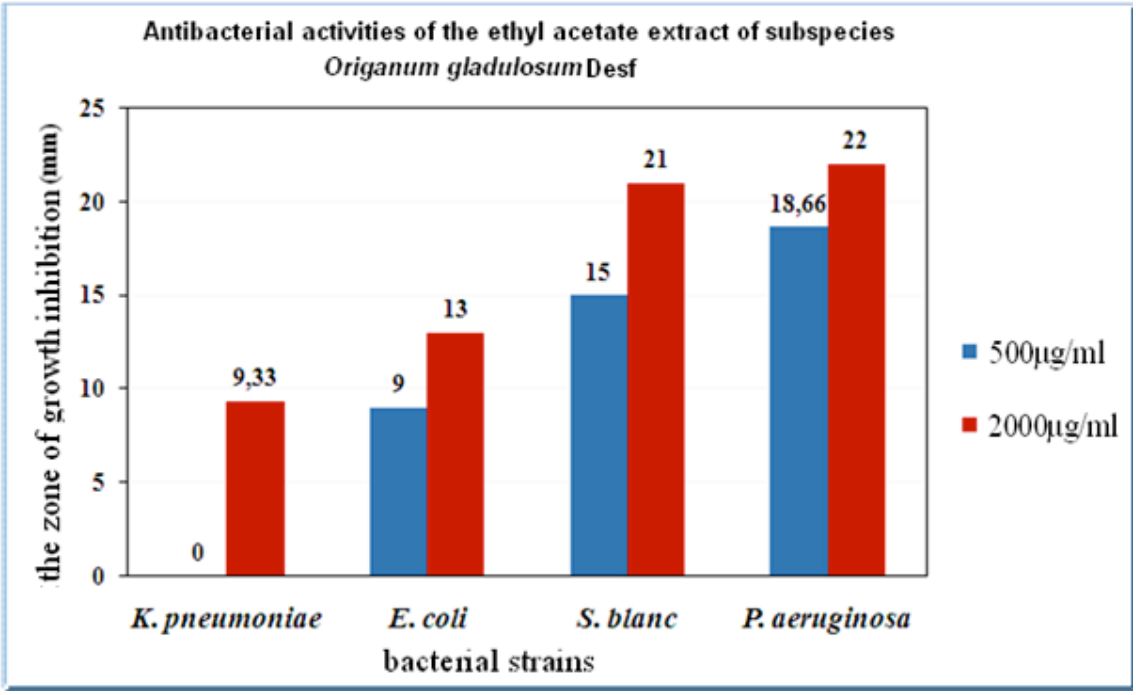

Fig. 9: Antibacterial activities of the ethyl acetate extract of subspecies *Origanum vulgare* L. Sbsp. *glandulosum.*

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.*

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Full Length Reproduction

Friendship - various

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Toronto (Canada)

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Key words: literature, poetry, linguistics, friendship, metaphysics

Hidden personality

A sudden mirage was thy call
Showering me with dreams and wishes
Articulated by your beautiful words
I shone each day dazzled by thee
Eager to see a glimpse of your image
When I knew not this image was far from real
Where a dark shadow lurks hiding reality
Leaving none but memories that will forever sail

November 15, 2010

*Corresponding author: seeking_illm@yahoo.com

Mysterious friend

Moments ago I was sitting alone in agony
Humble with my face dipped into my palms
Out in the cold hugging my trembling tucked knee
All I was longing for was a loving arm
To embrace my bleeding heart and banish every bit of misery
Numbness was a veiled form of my suicide… but
Mysterious morning I woke and was a day of glory
A day when you revealed thyself no more to hide
I never imagined a great friend like thee existed
Never have I seen anyone as good as thyself
None will ever show the goodness your heart has offered

November 11, 2007

Nature smiled

Many have touched my heart and passed by
Hurting and leaving scar where they rested
Out in darkness I was left each moment
After moments of such sweet affection
Each nature had spoken and showed me signs
That none was true and that I had not yet found that friend
Mountains stared back at me in silence
All the stars seemed so apart proving my loneliness
Inks that stroke perfect words seemed to dry out
Now that I have reached out to a mysterious friend
None would dare show sad signs but smiles of approval

November 11, 2007

Sweet September

When the mountains stayed still
Staring back into my depressed eyes
They proved they had frozen out of panic
When rivers and fountains run down
They kept reminding me their cries along with my tears
When trees moved harshly by the blowing wind
They show their intoxicated moods for my hurting
When the wind blew brushing me away
It tried to clear away the agony drowning me
When the rain fall and washed my body
It helped me spare my tears for the next day
So I won't dry it out and be tearless
But when the time went by
And when months passed by
It planted a great hope in my heart
That I finally came to reach
A blissful moment when I met you
And a moment that made me whisper
I will always smile and remember
The day I smiled back in that precious September

November 11, 2007

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