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Phytochemical Study of Two Algerian Plants *Origanum vulgare* L. Sbsp. *glandulosum* (Desf) Ietswaart and *Thymus algeriensis* (Boiss. and Reut)

FARID AIT KAKI[1,2,*], R. BENKINIOUAR[2,3], I. DEMIRTAS[4], A. MERZOUG[5,6] and A. TOUIL[2]

[1]Department of Chemistry, Faculty of Exacts Sciences, Mentouri Brothers University, Constantine 1, Algeria
[2]Laboratory of Natural Products and Organic Synthesis, Department of Chemistry, Faculty of Exacts Sciences, Mentouri Brothers University, Constantine 1, Algeria
[3]Department of Industrial Chemistry, Mentouri Brothers University, Constantine 1, Algeria
[4]Department of Chemistry, Faculty of Science, Çankiri Karatekin University, Çankiri, Turkey
[5]Laboratory of Applied Biochemistry, Department of Biochemistry and Cellular and Molecular Biology, Faculty of Natural Sciences, Mentouri Brothers University, Constantine 1, Algeria
[6]Abdelhafid Boussouf University Center, Mila, Algeria

*Corresponding author: E-mail: jskf56@yahoo.fr

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In phytochemical study of two Algerian plants, the ethyl acetate and *n*-butanol extracts from aerial parts of *Origanum vulgare* L. Sbsp. *glandulosum* (Desf) ietswaart and *n*-butanol extract from aerial parts of *Thymus algeriensis* (Boiss. and Reut), one flavonoid (8,4'-dihydroxy-7-*O*-β-D-arabinopyranosideflavone) was isolated for the first time from *Origanum vulgare* L. Sbsp. *glandulosum* ethyl acetate extract using chromatographic methods. The structure was identified on the basis of spectral analysis including UV-visible, HPLC-TOF/MS, IR, ^{1}H NMR and ^{13}C NMR. The HPLC-TOF/MS analysis of the two *n*-butanol plants extracts showed the presence of important compounds such as phenolic acids and flavonoids. The antibacterial assay revealed that the different *n*-butanol extracts have a weak activity against the tested bacteria.

Keywords: *Origanum vulgare* L. Sbsp. *glandulosum*, *Thymus algeriensis*, Flavonoids, Antibacterial.

INTRODUCTION

Considered the areas bordering the Mediterranean basin are rich in therapeutic plants, especially those belonging to the Lamiaceae family [1,2]. In this study we selected two types of Algerian plants belonging to this family *i.e.*, *O. vulgare* L. Sbsp. *glandulosum* (Desf) ietswaart and *Thymus algeriensis* (Boiss. and Reut). These plants are aromatic herbs and usually used as cooking spices and in folk medicine. It has been reported that they have pharmacological activities like antibacterial, stomachic, antispasmodic, antioxidant and diaphoretic [3-5]. These biological properties have been attributed to the presence of several bioactive compounds in its secondary metabolites.

Previous phytochemical studies of *O. vulgare* L. Sbsp. *glandulosum* and *Thymus algeriensis* plants have been specialized in the extraction of their volatile oils [6,7]. The aim of this work is the continuation of our research on flavonoid compounds from Algerian *O. vulgare* L. Sbsp. *glandulosum* (Desf) Ietswaart ethyl acetate [8] and *n*-butanol extracts and from *n*-butanol extract of *Thymus algeriensis* [9].

EXPERIMENTAL

Extraction protocol: In continuation of our phytochemical study, the ethyl acetate (20.38 g) and *n*-butanol (36.48 g) extracts of *O. vulgare* L. Sbsp. *glandulosum* (Desf) Ietswaart [8] and *n*-butanol extract (8 g) of *Thymus algeriensis* (Boiss. and Reut) [9] were extracted with chromatography protocols.

HPLC-TOF/MS analysis: HPLC-TOF/MS analysis of *O. vulgare* L. Sbsp. *glandulosum* and *T. algeriensis* extracts was carried out as described previously [10]. It was used to evaluate the phenolic acids and flavonoids in those plants extracts by comparing retention times and spectral data with those of the standard mixture chromatogram. An Agilent 6210 HPLC TOF-

MS instrument with Zorbax SB-C18 column (3.5 µm, 4.6 mm × 100 mm) and an injection volume of 10 µL was used. The ultra-pure water with 0.1 % formic acid (eluent A) and acetonitrile (eluent B) were used as mobile phases. The following linear gradient was applied: 0-1 min 10 % B; 1-20 min 50 % B; 20-23 min 80 % B; 23-25 min 10 % B; 25-30 min 10 % B. The flow rate used was 0.60 mL min^{-1} at 35 °C and TOF analyses were executed in negative ion mode.

Separation and purification of extract compound: A part of *O. vulgare* L. Sbsp. *glandulosum* ethyl acetate extract (10.57 g) was subjected to a SC6 polyamide column chromatography (55 × 3 cm), being eluted with a gradient of (toluene/MeOH) with increasing polarity [8]. Twelve fractions were collected and the separated compound was obtained from fraction 8 using paper chromatography (Whatmans® No. 3) (57 × 46 cm) eluted by AcOH (15 %) to give 7 bands. The 4th main band with dark purple colour was purified by preparative thin layer chromatography ($CHCl_3$/MeOH) (9/1) to isolate this compound. It is identified for the first time in this species.

Antibacterial activity test: The different bacteria strains; *Escherichia coli* (ATCC25922), *Pseudomonas aeruginosa* (ATCC27853), *Staphylococcus aureus* (ATCC25923) and *Enterococcus faecalis* (ATCC29212) were obtained from Abdelhafid Boussouf University Center, Mila, Algeria. The inoculums were prepared in nutrient broth from 24 h old bacterial cultures at 37 °C. The bacterial suspension was adjusted to 10^8 UFC/mL with sterile saline and inoculated on the surface of Mueller-Hinton agar plates. The antibacterial activity of *n*-butanol extracts of *O. vulgare* L. Sbsp. *glandulosum* (Desf) Ietswaart and *T. algeriensis* was tested by the paper disc diffusion method against the test bacteria. The extracts were dissolved in DMSO to prepare solutions having concentration 500 µg/mL and 2000 µg/mL and then 20 µL of each solution were impregnated onto sterile 6mm diameter filter paper discs, ciprofloxacin (5 µg) was used as positive control and DMSO (20 µL) as negative control. Growth inhibition activity was evaluated after one night incubation at 37 °C. The experiments were performed in duplicates.

RESULTS AND DISCUSSION

HPLC-TOF/MS analysis: HPLC-TOF/MS analysis showed the presence of phenolic acid and flavonoid compounds in *O. vulgare* L. Sbsp. *glandulosum* and *T. algeriensis* extracts (Table-1 and Fig. 1). However, *n*-butanol extract of *T. algeriensis* was richer than the extract of *O. vulgare* L. Sbsp. *glandulosum*. Where the first extract of the plant *T. algeriensis*, was rich with flavonoids and phenolic acid. Scutellarin was detected as a major flavonoid (2725.66 ng/mL) and 4-hydroxybenzoic acid was detected as major phenolic acid (326.67 ng/mL). Several compounds (fumaric acid, gentisic acid, naringin, diosmin, hesperidin, neohesperidin, baicalin) were detected with important concentrations. By comparing this results of *n*-butanol extract of *T. algeriensis* with the results of the analyzes obtained for the same extract [11]. It is rich in polyphenolic compounds especially in flavonoids, due to the difference in region of collection (east and central of Algeria) between the two plants and variation in extraction method and the technical method of analysis [9,11]. In the second plant extract of *O. vulgare* L. Sbsp. *glandulosum,* fumaric acid was detected as major constituent (901.01 ng/mL) and baicalin was detected as a major flavonoid (429.20 ng/mL). Several compounds (gentisic acid, syringic acid, protocatechuic acid, scutellarin, diosmin, morin) were detected with different concentrations.

Identification of separated compounds: Chromatography of *O. vulgare* L. Sbsp. *glandulosum* yielded only one flavonoid. The chemical structure of flavonoid was established by spectroscopic methods and comparison to literature data. The chromatographic compartment by R_f shifts and the retention time value

TABLE-1
QUANTITATIVE CONTENT ANALYSIS OF PHENOLIC COMPOUNDS OF SEPARATED PHASES

Compounds	*O. vulgare* L. Sbsp. *glandulosum*. *n*-BuOH extract		*T. algeriensis*. *n*-BuOH extract	
	Final concentration (ng/mL)	Retention time (min)	Final concentration (ng/mL)	Retention time (min)
Fumaric acid	901.0128	2.350	191.3905	2.366
Gentisic acid	79.4779	4.500	94.9105	4.596
Chlorogenic acid	60.5573	5.527	71.0966	5.543
Catechin	Traces	Traces	Traces	Traces
4-Hydroxybenzoic acid	49.7497	6.698	326.6758	6.730
Protocatechuic acid	78.2697	7.340	77.8083	6.987
Caffeic acid	1.6487	7.629	52.7915	7.677
Vanillic acid	36.4609	7.677	50.0778	7.917
Syringic acid	89.0487	8.174	89.5627	8.014
Rutin	Traces	Traces	11.7684	9.137
Polydatine	Traces	Traces	Traces	Traces
Scutellarin	160.7569	9.811	2725.6664	9.795
Quercetin-3-β-D-glucoside	Traces	Traces	30.8126	9.795
Naringin	36.9331	10.389	328.3164	10.132
Diosmin	164.7343	10.565	750.9427	10.533
Hesperidin	Traces	Traces	627.1417	10.661
Neohesperidin	1.7189	10.950	406.4840	10.661
Baicalin	429.2042	10.950	608.3731	10.934
Salicylic acid	Traces	Traces	96.5690	13.228
Morin	52.6467	13.871	52.3562	13.357
Apigenin	2.4428	15.491	69.9619	15.523

(tr = 12.08 min) using HPLC/(TOF-MS) showed that the compound is polar (glycoside), it is a monosaccharide, R_f on TLC silica 0.27 (SI:CH_2Cl_2/MeOH) (9/1), R_f on TLC cellulose 0.09 [SII: AcOH (15 %)]. The dark purple of spot colour under UV light changing to yellow when fumed with ammonia showed that the compound is a flavone 3-H or a flavonol 3-OR substituted. However the UV spectra in methanol and after addition of classical shift reagents suggested that the compound is a flavone with 4'-OH, substituted in position 7, without *ortho*-dihydroxyl in A and B-ring and lacking a free 5-OH [12], UV (MeOH, λ_{max}, nm): 268, 326; + NaOH: 276, 393; + $AlCl_3$: 296, 385; + $AlCl_3$/HCl: 296, 385; + NaOAc: 268, 387; + NaOAc/H_3BO_3: 268, 338 (Fig. 2).

The ^{1}H NMR analysis display [12,13] a singlet signal at 6.82 ppm attributed to H3, two doublets at δ 7.06, 2H and δ 7.48, 2H attributed to H3′, H5′ and H2′, H6′, respectively, where the coupling constant (J = 8.5 Hz) suggested an *ortho*-related between the protons, two doublets at (δ 6.96, 1H) and (δ 7.94, 1H) with 8.8 Hz of *ortho*-coupling constant was ascribed to H5 and H6 respectively. The using of DMSO-d_6 suggested that the position C5 is not substituted with hydroxyl [12,13] so a singlet at 9.06 is attributed to 8-OH.

From the ^{13}C NMR analysis, the presence of C3 signal at 108.46 ppm confirmed that the separated compound is a flavone [14-16], Other apparent signs are ^{13}C NMR (DMSO-d_6; 250 MHz): δ_C 165.53 (C2), 108.46 (C3), 147.27 (C9) [14,15,17,18].

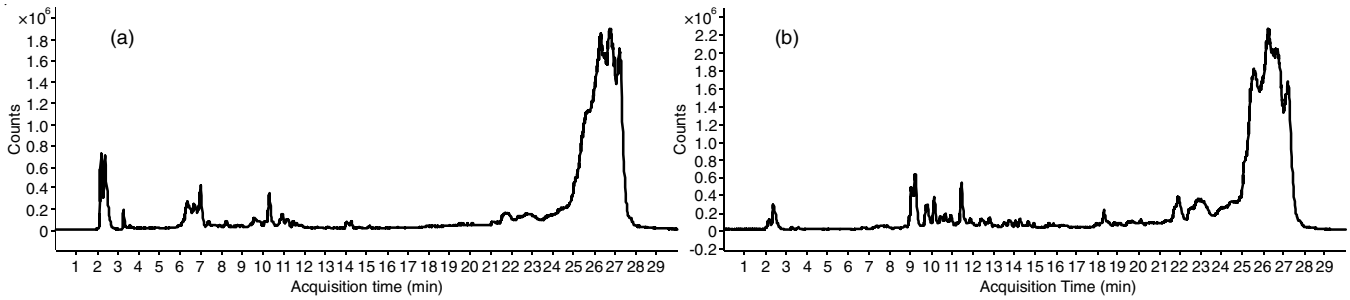

Fig. 1. HPLC chromatography for analysis of phenolic quantitative compounds in *n*-butanol extracts of *O. vulgare* L. Sbsp. *glandulosum* (a) and *T. algeriensis* (b)

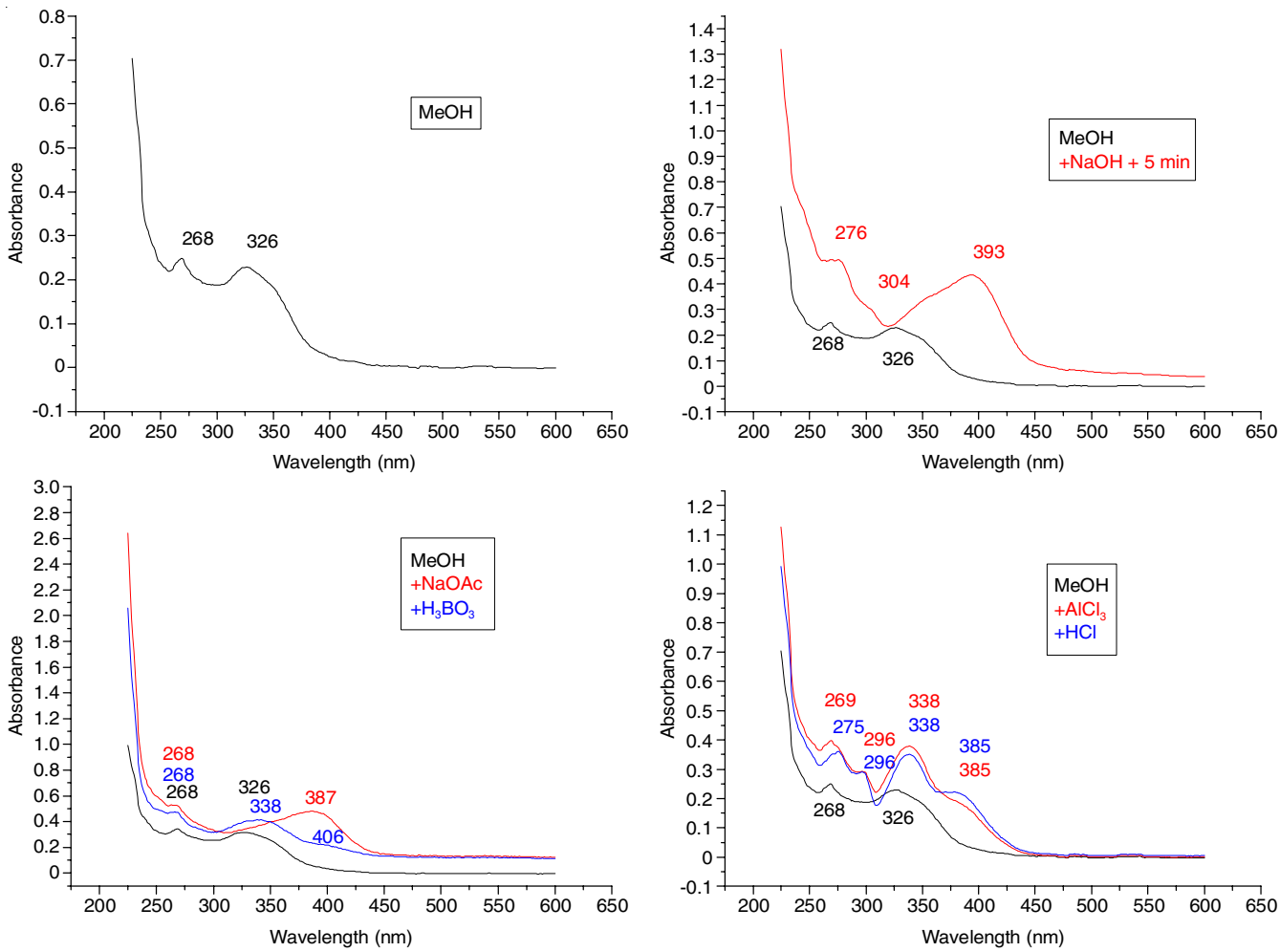

Fig. 2. UV spectra of separated compound

TABLE-2
ANTIBACTERIAL ACTIVITY OF *O. vulgare* L. Sbsp. *glandulosum* AND *T. algeriensis* *n*-BUTANOL EXTRACTS

Tested extracts	Zone of inhibition (mm)							
	E. coli		*S. aureus*		*E. faecalis*		*P. aeruginosa*	
	500 µg/mL (5 µg/disc)	2000 µg/mL (20 µg/disc)	500 µg/mL (5 µg/disc)	2000 µg/mL (20 µg/disc)	500 µg/mL (5 µg/disc)	2000 µg/mL (20 µg/disc)	500 µg/mL (5 µg/disc)	2000 µg/mL (20 µg/disc)
n-butanol extract (*O.g*)	–	7.75 ± 0.35	–	8.5 ± 0.7	7	8	–	7
n-butanol extract (*T.a*)	–	7	7	8	–	7	–	6.5 ± 0.7
Ciprofloxacin (5 µg)	30.5 ± 0.7		32 ± 2.82		34 ± 0.7		29.5 ± 0.7	

– Indicates no zone of inhibition. The negative control did not show any activity.

The molecular formula of the separated compound was determined as $C_{20}O_9H_{18}$ by negative ion mode ESI mass spectra (*m/z* 401 $[M-H]^-$), it showed that the compound is a monosaccharide and the corresponding sugar is arabinose $C_5O_5H_{10}$.

Referring to the ^1H NMR analysis, the chemical shifts of protons sugar signals increased to the range [δ_H 3.7-5.1 ppm] due to the effect of solvent (DMSO-d_6 + H_2O) [13], where the anomeric proton H1″ signal for this sugar is shown as a doublet at 5.05 ppm and 7.1 Hz resulting of (axial-axial) coupling with H2″, which indicate that sugar is β-linked to aglycone [19]. Other protons of sugar moiety are δ_H 4.19 (1H, d-d, *J* = 8, H2″), 3.75 (1H, d-d, *J* = 6, H3″), 4.11 (1H, t, *J* = 6.2, H4″) and 3.85 (2H, d, H5″).

By comparing the carbons chemical shifts of sugar δ_C 70.45 (C2″), 70.45 (C3″), 70.12 (C4″), 63.08 (C5″) with those published by Agrawal [14] and taking the previous sugar mass, the final formulation being β-D-arabinopyranoside.

The comparison between the IR data with published by Parker and Tipson [20] and Agrawal [14], showed the presence of absorption bands for hydroxyl flavonoid or phenolic group [20,21] 3444.6 cm^{-1}, carbonyl flavone [14] and conjugated double bond for C2 and C3 1650 cm^{-1} [20], the strong absorption band at 825.5 cm^{-1} was indicative of (Ctri-H aromatic) for aromatic cycle *para*-disubstituted [20,21], it's for (H2', H3') and (H5', H6') of B-ring, another at 763.8 cm^{-1} due to (Ctri-H aromatic) for aromatic cycle *ortho*-disubstituted [20,21], it's for H5, H6 of A-ring. Most flavonoid glycosides is characterized by broad bands at 3250 and 1060 cm^{-1} [14], however the glycoside part of this compound we discover it by two bands at 1041.5 cm^{-1} for (C-O-C) [20] and 1010.6 cm^{-1} for (C-C) [21]. Various spectral data demonstrated that the structure of this compound should be the 8,4'-dihydroxy-7-O-β-D-arabinopyranosideflavone.

Antibacterial assay: The antibacterial property was tested against two Gram-positive bacteria, which are *S. aureus* and *E. faecalis* and two Gram-negative bacteria namely *E. coli* and *P. aeruginosa*, with DMSO as a negative bacteria growth inhibition test and ciprofloxacin as standard drug.

According to Table-2, the inhibition zone produced ranged between 6.5 and 8.5 mm, which indicate that the extracts of *O. vulgare* L. Sbsp. *glandulosum* and *T. algeriensis* showed weak activity against the tested bacteria. The growth inhibition activity of *T. algeriensis* (Boiss. and Reut) essential oils have been investigated before by others authors [7,22], but in present study we tested the antibacterial activity of *n*-butanol extract from the aerial parts of this plant [9].

The antibacterial activity of *n*-butanol extract of *O. vulgare* L. Sbsp. *glandulosum* (Desf) Ietswaart was studied in continuation of our investigation carried out on this medicinal plant [8]. Our results showed that the *n*-butanol extract had less antibacterial activity than the ethyl acetate extract [8]. An introspection of this result revealed that the antibacterial effect depend upon the components in these two different extracts.

Conclusion

One compound, 8,4'-dihydroxy-7-*O*-β-D-arabinopyranoside flavone, was isolated for the first time from *O. vulgare* L. Sbsp. *glandulosum* (Desf) Ietswaart ethyl acetate extract, the HPLC-TOF/MS analysis of *O. vulgare* L. Sbsp. *glandulosum* and *T. algeriensis* *n*-butanol extracts showed the presence of new and important flavonoids. The Antibacterial assays showed that the different *n*-butanol extracts have a weak activity.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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