**BIOASSAY-GUIDED ISOLATION OF THE MAJOR COMPOUND WITH ANTIOXIDANT ACTIVITY FROM THE ALGERIAN MEDICINAL PLANT BUBONIUM GRAVEOLENS**

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**ABSTRACT**

**Objective:** The objective of this research was to isolate and identify the major compound from the antioxidant extract obtained from the leaves of *Bubonium graveolens*.

**Methods:** A bio-guided fractionation was done to isolate and identify the major compound responsible of the antioxidant activity. The chemical structure of the natural compound was established by spectroscopic means including UV, Fourier transform infrared, 1H NMR, and 13C NMR. The purified compound was accurately tested for its antioxidant activity by the 2, 2-diphenyl-1-picrylhydrazyl assay at 517 nm.

**Results:** The major compound named myricetin 3-O-rhamnoside was isolated for the first time from the leaves of *B. graveolens* a medicinal specie of Algerian Sahara belonging to the Asteraceae family. The study showed that the myricetin 3-O-rhamnoside has an antioxidant potential 59% when compared with standard ascorbic acid with an IC₅₀=0.916 mg/ml.

**Conclusion:** In the present work, it was possible to isolate and identify for the first time the major compound from the antioxidant fraction of *B. graveolens*.

**Keywords:** *Bubonium Graveolens*, Antioxidant activity, Asteraceae, Medicinal plants, Sahara.

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**INTRODUCTION**

South Algeria with its rich floral resources and ethnobotanical history is an ideal place of new pharmacological compounds [1,2]. One of these floral resources is *Bubonium graveolens* (Forssk.). Maire locally known as "Tafs" belongs to the Asteraceae family, it is considered synonymous with *Asteriscus graveolens* (Forssk.), *Buphthalmum graveolens* (Forssk.), and *Nauplius graveolens* (Forssk.) subsp [3]. This Saharan medicinal plant is widely represented in south-west of Algeria and south-east of Morocco [3], in Sahara folk medicine, this species is used as stomachic, treating fever, gastrointestinal tracts, cutaneous, bronchitis, and as anti-inflammatory [4,5] and also previous studies revealed that the extracts from this species have an antifungal effect against *Fusarium oxysporum* [6], antioxidant [7], and antimicrobial activity [8,9].

Phytochemical investigations on the species have led to the isolation of numerous and varied secondary metabolites; monoterpenes [4,10], sesquiterpene [11-13], and flavonoids [14]. It is well reported that polyphenol compounds have attracted considerable attention due to their pharmacological properties like flavones which were previously revealed a high biological activity [15-18].

The study of chemicals compositions of plants is on the basis of isolation and identification of compounds by various chromatographic methods (TLC, GC, high-performance liquid chromatography, gas chromatography) and spectrosocpic [19] such as the UV-Vis, infrared (IR) spectroscopy, mass spectrometry, and nuclear magnetic resonance (NMR) spectroscopy that offers the most useful and valuable information about the structure of natural products. These techniques are all important tools for a proper identification of the components of an extract [20]. In this context, we include a research on the Saharan medicinal plant *Bubonium graveolens*.

**Experimental**

The chemicals used were in analytical grade and purchased from the Sigma-Aldrich. UV spectra measurement (Unicam UV 300 and Spectord 200 Plus), IR spectra (Thermo Nicolet Avatar 320 FT-IR spectrophotometer), NMR spectra (Bruker Avance GP 250, 1H: 250 MHz; 13C: 63 MHz), TLC was performed on silica gel coated aluminum plates (Merck kieselgel 60 F254, Germany) and column chromatography was performed over silica gel 60 (Merck, particle size 230–400 mesh).

**METHODS**

Leaves of *B. graveolens* were collected during the flowering period March 2015 in south-western Algeria. A voucher specimen has been deposited in the herbarium of POSL Laboratory (UTMB, Algeria) under Accession No CA 00/14.

**Extraction and isolation**

Air-dried and powdered leaves of *B. graveolens* (240 g) were extracted with water/acetonitrile (30/70) under reflux and the hydroacetone solution was concentrated by evaporation up to 1/3 of the initial volume [21]. The extract was fractionated by the following solvents with increasing polarity: Hexane (3×150 ml), diethyl ether (3×150 ml), ethyl acetate (3×150 ml), and butanol (3×150 ml). The diethyl ether fraction was concentrated to dryness by rotary evaporator providing 2.86 g of residue which was divided into fractions to isolate a pure compound by a successive column chromatography on silica gel, eluting with hexane/acetonitrile/ethyl acetate/formic acid (5/2/2.9/0.1).

**Scavenging 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical test**

The free radical scavenging activity of the major compound was measured *in vitro* by DPPH assay according to the method described earlier [22,23]. Briefly, the DPPH methanolic solution (25 μg/100 ml) was mixed with samples in four concentrations and left to stand at room temperature in a dark place for 30 min. Absorbance was read using a spectrophotometer at 517 nm. The ability of pure compound to scavenge DPPH was calculated using the following equation:

\[
\text{Radical scavenging activity}\% =\frac{\text{Control OD} - \text{Sample OD}}{\text{Control OD}} \times 100
\]

Where, OD: optic density.
Reference standard compound being used was ascorbic acid and experiment was done in triplicate. The 50% inhibition (IC$_{50}$) of antioxidant activity was calculated as the concentrations of samples that inhibited 50% of scavenging activity of DPPH radical activity [24].

**General procedure**

UV spectra were obtained in MeOH solvent with Unicam UV 300 spectrophotometer and Spectord 200 Plus spectrophotometer. IR spectra were obtained with a Thermo Nicolet Avatar 320 Fourier transform-IR spectrophotometer. The NMR spectra were taken on a Bruker Avance GP 250 (H: 250 MHz; 13C: 63 MHz) Spectrometer. Column chromatography was performed over silica gel 60 (Merck, particle size 230–400 mesh).

**RESULTS AND DISCUSSION**

Bioguided fractionation was been done on a mass (2.86 g) of diethyl ether extract. A successive column chromatography was used to separate the major compound. After several chromatographic analyses, we regrouped the major compound from the fractions 36 to 43 with RF 0.43 and yellow color (Fig. 1).

UV: 250, 359 nm. IR (cm$^{-1}$) (KBr): 3227, 2929, 2874, 1733, 1640, 1515, 1369, 1298, 976, 913 and 864 cm$^{-1}$. $^{1}H$ NMR: H2' (δ 7.17, d, 1H); H6' (δ 6.64, d, 1H); H6 (δ 6.44, m, 1H); H8 (δ 6.38, m, 1H); H3' (δ 4.29, q, 1H); H2 (δ 4.02, m, 2H); H3 (δ 4.07, m, 1H); H4 (δ 3.26, m glucose proton) 5.02 (m, glucosyl 1H).

C NMR (DMSO-d$_6$): 183 (C-3′), 180 (C-5′), 171 (C-6″), 170 (C-6), 166 (C-7),151 (C-9), 148(C-8),128(C-1′),129(C-2′),129(C-6′),138(C-3),139(C-4′),139(C-5),136(C-6′),135(C-3′),134(C-5′),133(C-5),132(C-6),131(C-5′),130(C-6′),129(C-3′),128(C-2′),127(C-6′),126(C-5),125(C-5′),124(C-3′),123(C-5′),122(C-4′),121(C-5′),120(C-4′),119(C-5′),118(C-6),117(C-7). The major compound (flavonol glycoside) isolated from *B. graveolens* as a yellow amorphous powder [a]$_{D}$ = 2.5 with a melting point 120°C. The IR spectra showed frequency at 3227 cm$^{-1}$ indicating the presence of hydroxyl group in conjugation [25], the valence vibration of the cyclic ketones (C = O) is located at 1640 cm$^{-1}$ and phenyl groups at 1515 cm$^{-1}$; and the aromatic links C-H are presented in the IR spectra at 2929 cm$^{-1}$ [26]. The frequency of vibration (976 and 913 cm$^{-1}$) corresponds to the distortion vibration out of the plan of the unsaturated hydrocarbons [27].

An examination of the NMR data and a comparison with the literature suggested that the isolated compound was a flavonol glycoside. Thus, the $^{1}H$-NMR spectrum revealed characteristic resonances of aromatic protons such as H2' (δ 7.17, d, 1H); H6' (δ 6.64, d, 1H); H6 (δ 6.44, m, 1H); H8 (δ 6.38, m, 1H); H3' (δ 4.29, q, 1H); H2 (δ 4.02, m, 2H); H3 (δ 4.07, m, 1H); H4 (δ 3.26, m glucose proton) and the aromatic links C-H are presented in the IR spectra.

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The big similarity of myricetin 3'-O-rhamnoside data with literature myricetin 3-O-rhamnoside data led us to conclude that the unique difference between the two compounds derives from the $H$ signal of the B ring. While H-2' and H-6' of myricetin 3-O-rhamnoside appear at δ 6.97 as a singlet [32] in myricetin 3'-O-rhamnoside, they are displayed as two doublets at δ 6.90 and 6.70 [29,33]. Therefore, it was evident from the above observations that the rhamnoside was attached to the C-3′ oxygen of ring B.

**DPPH radical scavenging activity**

The study of the hydrogen atom or electron donation ability of major compound from *B. graveolens* against DPPH free radical was precisely measured from the bleaching of violet-colored DPPH at 517 nm. Lower absorbance of the reaction mixture indicated higher free radical activity [34].

Fig. 2 shows the radical scavenging activity at different concentrations of myricetin 3′-O-rhamnoside (0, 0.25, 0.5, 0.75, and 1.0 mg/ml).

According to the results found, the myricetin 3′-O-rhamnoside has an antioxidant potential 59% compared to the standard ascorbic acid (86%) and IC$_{50}$ = 0.916 mg/ml.

Several studies have been done to investigate the various therapeutic and biological effects of myricetin, including its use as a potent antioxidant was shown to possess significant radical scavenging activity against DPPH [35], by comparing the radical scavenging activity of our compound myricetin 3′-O-rhamnoside and myricetin-3-O-galactoside and other flavonol glycoside reported in literature showed that myricetin 3-O-rhamnoside was a very potent radical scavenger with IC$_{50}$ value of 1.4 μg/ml and myricetin-3-O-galactoside showed a scavenging activity with an IC$_{50}$ value of 2.3 μg/ml [36] while the myricetin 3′-O-rhamnoside showed an IC$_{50}$ = 0.916 mg/ml. We noted that the presence of rhamnoside in 3′ position of B ring decreases the antioxidant activity; this result is an agreement with our early study that the presence of hydroxyl groups on B ring is the most significant structural parameter for the antioxidant activity [37].

**CONCLUSION**

The result of the study showed that the leaves of *B. graveolens* contain flavonol glycoside. The structure of the isolated compound was characterized by UV, IR, $^{1}H$NMR, and $^{13}CNMR. The antioxidant evaluation by DPPH method showed that the isolated compound has a moderate antioxidant activity when compared to standard ascorbic acid.
acid. This flavonol glycoside (myricetin 3′-O-rhamnoside) was isolated for the 1st time from B. graveolens.

AUTHORS’ CONTRIBUTION

The experimental work was done by R. Messaoudi and Y. Bourmita under the supervision of Pr. A. Cheriti.

CONFLICTS OF INTEREST

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

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