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DOI: 10.2478/auoc-2021-0005

**Ovidius University Annals of Chemistry** Volume 32, Number 1, pp. 33 - 39, 2021

# Phytochemical properties, antibacterial and anti-free radical activities of the phenolic extracts of *Retama raetam* (Forssk) Webb. & Berthel. collected from Algeria Desert

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**Abstract.** This study aimed to evaluate the phytochemical properties, antibacterial and anti free-radical activities of *Retama raetam* extracts which is growing in the South-East of the Algeria Desert. The chemical screening showed the presence of many secondary metabolites such as tannins catechin, sterols and terpenes, and the absence of gallic tannins compounds. The obtained results demonstrated that the methanolic extract has shown moderate total phenolic and flavonoids contents (31.59 ± 2.82 mg AG E/g extract and 14.35 ±1.02 mg Qu E/g extract respectively). In the free radical DPPH test, the values of $IC_{50}$ were converging in all extracts of *R. raetam*. The antibacterial activity of extracts has been tested against ten bacterial strains, were registered as the best inhibition zones with *Vibrio cholera*, *Micrococcus luteus* and *Serratia marcescen*. *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 25923 strains showed high resistance against most of all concentrations of extracts, and we noted the Gram-negative bacteria strains are the most sensitive to the different extracts of the plant. The qualitative analysis of extracts by using HPLC showed the contrast in presence of the phenolic compounds, such as in ethyl acetate extract registered absence of chlorogenic acid, also the absence of caffeic acid in 1-butanol extract. These results confirmed of the phenolic extracts of this plant are a source natural alternative to antibiotics and antioxidants.

***Keywords*:** *Retama raetam*; anti-free radical activity; antibacterial activity; HPLC.

## 1. Introduction

The abundance of active ingredients gives the plant remarkable pharmacological properties, which could justify its multiple therapeutic indications and for which it is used in traditional therapy [1].

*Retama raetam* (Frosk) Webb. & Berthel. (Fabaceae) is a perennial plant widely distributed in the North Africa and Mediterranean area [2-4]. In folk medicine of Algeria, the *R. raetam* used to treat stomach malady, inflammation and diabetic disease [5]. The antibacterial, antifungal and cytotoxic activities of two flavonoids licoflavone C and derrone isolated from *Retama raetam* flowers were evidenced. They were active against *Pseudomonas aeruginosa* and *Escherichia coli* and showed important antifungal activity as for example found with derrone. The tested compounds also showed strong cytotoxicity against Hep-2 cells [6]. Also, Edziri *et al.* [7] confirmed the essential oils from the flowers of *Retama raetam* collected from Tunisia possesses compounds with antibacterial, antifungal and antioxidant capacities.

The chemical screening of a plant reveals the importance of the secondary metabolites, which play very important roles in the plant, *i.e.* against microbial and fungal attacks, antioxidants against various environmental stresses, attract pollinators, reserves and alienation of herbivores [8].

For identifies and quantification of some metabolites secondary and research a new source natural alternative to antibiotics and antioxidants in plants desertic, this study aims to estimate the phytochemical properties, antioxidant and antibacterial activities of crude, flavonoids and tannins extracts of *R. raetam* growing in the Algeria Desert.

## 2. Experimental

### *2.1. Materials*

*Plant material.* The aerial part of *R. raetam* was collected from the Oued Souf region (South-East of Algeria Sahara).

### *2.2. Chemicals*

Methanol (MeOH) and dichloromethane were purchased from Biochem Chemophara (Montreal, Quebec, Canada); ethyl acetate, 1-butanol, acetone, DMSO, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, aluminum chloride ($AlCl_3$), sodium carbonate ($Na_2CO_3$), gallic acid, quercetin, and ferric chloride ($FeCl_3$) were purchased from Sigma Aldrich, Chemicals Co (St. Louis, MO, USA).

### *2.3. Methods*

*2.3.1. Chemical screening.* The plant was tested for the presence of bioactive compounds such as flavonoids, tannins, anthocyanins, alkaloids, saponins, sterols and terpenes following standard procedures [9-11].

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*2.3.2. Preparation of the extracts*

*Methanolic extract.* 50 g of the dried plant were macerated in 500 mL of MeOH at room temperature in dark for 24 h. The solvent was evaporated under reduced pressure at 60 °C by rotary evaporator type Buchi R-200 [12].

*Extraction of flavonoids.* According to Bekkara *et al*. [13], 30 g of the plant were macerated in 300 mL of MeOH for 24 h. After filtration and evaporation of the solvent, the first extraction was obtained with 150 mL of hot water and 150 mL of ethyl acetate (2 times). In the second extraction were added 150 mL of 1-butanol (2 times) in the aqueous phase. The two organic phases (ethyl acetate and 1-butanol) were evaporated in a rotary evaporator device to obtain two phases of flavonoids, *i.e.* ethyl acetate and 1-butanol.

*Extraction of tannins.* According to the method citing in Zhang *et al.* [14], 30 g of the dried plant were macerated in 300 mL of the water/acetone (7V/3V) in dark and room temperature for 3 days. After the filtration, the acetone was evaporated and the aqueous layer was extracted respectively with dichloromethane and ethyl acetate (2×180 mL). The organic phase was dried to give the tannins extract.

*2.3.3. Estimation of total polyphenols content.* The total polyphenols in the crude extracts were determined by using Folin-Ciocalteau reagent with the method of [15]. 200 µL of extract were mixed with 1 mL of Folin-Ciocalteu reagent (10 %) and 800 µL of $Na_2CO_3$ (7.5 %). The mixtures were incubated for 30 min at room temperature and protected from light. They were read at 765 nm in a spectrophotometer (type Shimadzu). The calibration curve was prepared with gallic acid solutions in the concentration range of 0.02-0.12 mg/mL. The total polyphenols content was expressed in mg equivalent of gallic acid (GA) per gram of extract.

*2.3.4. Quantification of flavonoids.* The flavonoids content was determined by using $AlCl_3$ according to the method of Ordonez *et al.* [16]. 0.5 mL of crude extract were mixed with 0.5 mL of $AlCl_3$ (2 %). The mixture remained at room temperature for 15 min; the absorbance was measured at 420 nm with a spectrophotometer. The calibration curve was prepared with quercetin solutions in the concentration range of 0.03-0.1 mg/mL. The content was expressed in mg equivalent of quercetin (Qu) per gram of extract.

*2.3.5. HPLC analysis.* In this work, we used a High-Performance Liquid Chromatography (HPLC) system, type Shimadzu LC 20 AL equipped with an universal injector (Hamilton 25 µL). The analytical column used was a Shim-pack VP-ODS C18 (4.6 mm×250 mm, 5 µm), type Shimadzu. An UV-VIS detector SPD 20A (Shimadzu) was used. The mobile phase was a mixture of acetonitrile and acetic acid 0.1 %. The contents of the mobile phase were filtered before use through a 0.45 µm membrane filter, sonicated and pumped from the solvent reservoir to the column at a flow rate of 1 mL/min. according to Chouikh *et al.* [17], and 20 µL of plant extracts solution were injected into the flow of the mobile phase. We adjusted the high pressure that drives the mobile phase by using a pump. The separated compounds shall be determined using the column for 40-50 min with the mobile phase in the effluent detected at λ = 268 nm and to the computer which records the results as chromatographic curves. In this study, the quantification of some peaks was compared by calibration of standards: ascorbic acid, gallic acid, chlorogenic acid, caffeic acid, vanillin, *p*-coumaric acid and rutin (Figure 1).

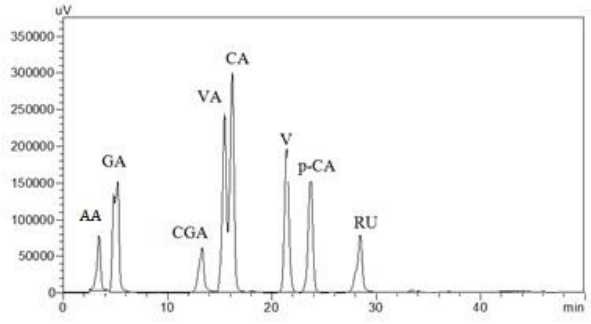


**Figure 1.** The High Performance Liquid Chromatography (HPLC) chromatogram of standard phenolic compounds. AA— ascorbic acid; GA—gallic acid; CGA—chlorogenic acid; VA—vanillic acid; CA—caffeic acid; V—vanillin; p-CA—*p*-coumaric acid; RU—rutin.

*2.3.6. Free radical DPPH scavenging activity.* According to the method of Brand-Williams *et al.* [18], free radical scavenging activity was measured with DPPH. 1 mL of different concentrations of extracts was mixed with 1 mL methanol containing DPPH ($10^{-4}$ M) and incubated in the dark for 15 min. The absorbance was measured at 517 nm with an UV-Vis spectrophotometer. The ascorbic acid standards were prepared for positive comparison. The percentage of inhibition was calculated by using the following formula:

$$I\,\% = \frac{A_c - A_s}{A_c} \times 100$$

$A_c$ = absorbance of the control;
$A_s$ = absorbance of the sample.

The $IC_{50}$ values were calculated from the linear equation of scavenging activity against the concentrations of the samples. $IC_{50}$ is defined as the total antioxidant necessary to decrease by 50% DPPH free radical [19].

*2.3.7. Antibacterial activity*. Ten strains of bacteria were used in antibacterial activity. The six strains Gram-negative are: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Enterobacter cloacae*, *Salmonella enterica*, *Serratia marcescens* and *Vibrio cholerae*, and the four strains Gram-positive: *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis*, *Staphylococcus epidermidis* and *Micrococcus luteus*. For positive comparison, three antibiotics were used: Gentamicin $HLG_{120}$ (120 µg/dish), Nitroxoline (30 µg/dish) and Cephalexin $CN_{30}$ (30 µg/dish).

The antimicrobial activity of the extracts of *R. raetam* was evaluated by using the disc diffusion method as reported by [20, 21]. Each strain of bacteria was inoculated in Mueller-Hinton agar. Sterile paper discs of 6 mm diameter were impregnated with 10 µL of various concentrations (0.25, 0.5, 1, and 2 mg/mL) of each extract [22]. Empty sterile discs and discs

impregnated with DMSO were used as negative controls. The Petri dishes were incubated at 37 °C for 24 h. After this, the diameter of the inhibition zone of each disc was measured [23].

## 3. Results and discussion

### *3.1. Chemical screening*

The results of the phytochemical screening of the aerial parts of *R. raetam* are given in Table 1. Preliminary chemical screening showed most secondary metabolites with the recording of the absence of gallic tannins.

**Table 1.** Phytochemical screening of the aerial parts of *R. raetam*

| Secondary metabolites | Observation | Results |
|---|---|---|
| Flavonoids | Red color | + |
| Tannins catechin | Bluish-green color | + |
| Tannins gallic | Not appear black color | - |
| Anthocyanins | Pink color | + |
| Alkaloids | White precipitate | + |
| Sterols and Terpenes | Purple ring | + |
| Saponosides | Forms foam in all tubes | + |

(+) Presence; (-) Absence.

Phytochemical screening with color reaction method was conducted to determine the group of secondary metabolites in the sample. These tests facilitate their quantitative estimation and qualitative separation. Phytochemical screening experiments are commonly performed to promote a guidance of substantial phytochemicals that may be involved in the biological functions of plants [24]. On the other hand, this variation can be linked to the distribution of secondary metabolites, which changes during plant development, environmental stress (drought, poor soil nutrients) as well as strong sunlight [25].

### *3.2. Yield and properties of extracts*

Among the extracts of *R. raetam*, the methanol extract (8.80 %) has a higher yield compared to other extracts (Table 2). The extraction solvents influence the extraction yield and the content of bioactive compounds [26]. In this study, the different yield of extracts can return to solvents types and their polarity, a successful determination of biologically active compounds from plant material being largely dependent on the type of solvent used in the extraction procedure [27].

**Table 2.** Properties of the extracts of *R. raetam* aerial part

| Extracts | Nature | Color | Yield (%) |
|---|---|---|---|
| Methanol extract | Dough solid | Blackish green | 8.80 |
| Extract of flavonoids phase ethyl acetate | Dough | Dark brown | 2.43 |
| Extract of flavonoids phase 1-butanol | Viscous | Umber | 3.07 |
| Tannins Extract | Dough solid | Blackish green | 0.60 |

### *3.3. Quantification of polyphenols and flavonoids in the crude extract*

31.59 ± 2.82 mg gallic acid equivalents per gram of extract for polyphenols and 14.35 ± 1.02 mg of quercetin equivalents per gram of extract for flavonoids were determined for crude extract.

The amount of phenolic compounds and flavonoids changes from one extract to another according to the type of compounds, as their behavior varies according to their chemical structure and the medium in which they are present [28, 29]. The total content of phenolics or flavonoids is controlled by many parameters and conditions, one of them being the environmental factors in which plant grows such as the season and date of collecting, soil composition, climate, temperature, light, humidity and stress [30, 31].

### *3.4. HPLC analysis*

The HPLC chromatograms of different extracts of *R. raetam* are presented in Figures 2-4. The comparison of the retention time with standards resulted in a difference in the number and concentration of compounds per extract.

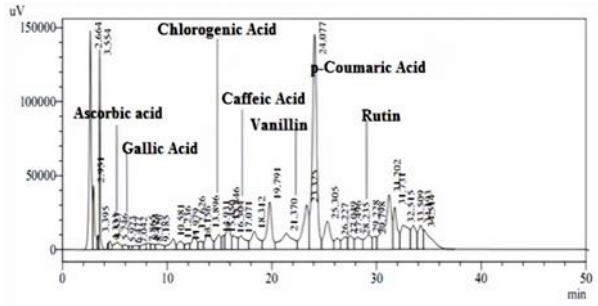


**Figure 2.** HPLC chromatogram of methanol extract of *R. raetam*

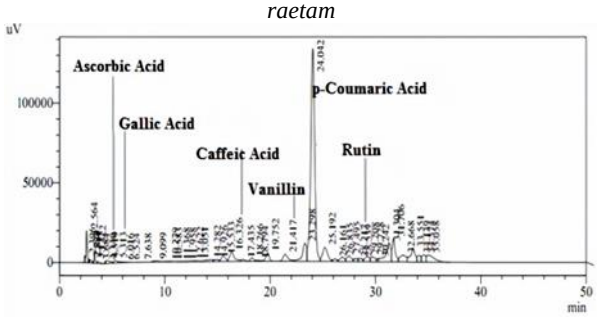


**Figure 3.** HPLC chromatogram of flavonoids extract phase ethyl acetate of *R. raetam*

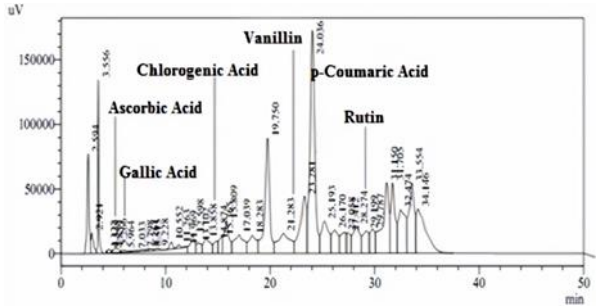


**Figure 4.** HPLC chromatogram of flavonoids extract phase 1-butanol of *R. raetam*

The concentrations of phenolic compounds in studied extracts are shown in Table 3. We managed the identification of seven compounds in methanol extract (ascorbic acid, gallic acid, chlorogenic acid, caffeic acid,

vanillin, *p*-coumaric acid, and rutin), but in extract ethyl acetate was showed the absence of chlorogenic acid, and also the absence of caffeic acid in 1-butanol extract.

**Table 3.** The concentration (µg/mg extract) of major phenolic compounds identified by HPLC in extracts of *R. raetam*

| | Methanol extract | Flavonoids extract phase ethyl acetate | Flavonoids extract phase 1-butanol |
|---|---|---|---|
| Ascorbic acid | 14.705 | 10.286 | 8.436 |
| Gallic acid | 0.949 | 0.385 | 0.563 |
| Chlorogenic acid | 1.209 | - | 1.333 |
| Caffeic acid | 0.422 | 0.497 | - |
| Vanillin | 1.070 | 0.340 | 1.531 |
| *p*-Coumaric acid | 2.644 | 4.257 | 3.356 |
| Rutin | 1.467 | 0.410 | 4.035 |

The methanol extracted a higher number of compounds when compared to other extracts. According to Marco *et al.* [32] and Visioli *et al.* [33], it was demonstrated that ethyl acetate is somewhat selective towards low and medium molecular mass phenolic compounds. Onother hand, the caffeic acid is absent in the flavonoids extract phase 1-butanol, this being in accordance with a study of [34].

### *3.5. Free radical DPPH scavenging activity*

The $IC_{50}$ values (Figure 5) showed converging values of radical scavenging activity at extract of tannins (0.223 mg/mL), methanol extract (0.232 mg/mL) and flavonoids phase ethyl acetate extract (0.247 mg/mL), whereas the less scavenging activity was showed for extract of flavonoids phase 1-butanol (0.344 mg/mL).

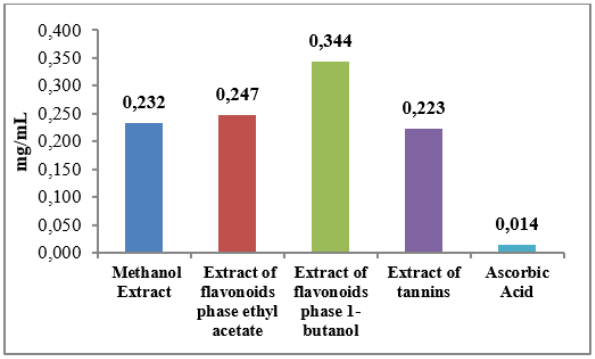


**Figure 5.** $IC_{50}$ values (mg/mL) of DPPH radical scavenging activity of the different extracts of *R. raetam* and ascorbic acid

The secondary metabolites are characterized by the presence of several phenol groups in their structure. Most of these groups are active in scavenging free radicals due to their ability to donate a hydrogen atom or an electron [35, 36]. In this study, the results indicate a direct relationship between the total content of polyphenols and flavonoids, and the antioxidant capacity; this was confirmed by [37]. Besides, the DPPH test revealed that the tannins extract possessed a good antioxidant capacity. This suggests that extract contains secondary metabolites with strong antioxidant activity [16] and medium molecular mass phenolic compounds.

### *3.6. Antibacterial activity*

The antibacterial activity of the different extracts of *R. raetam* was determined against 10 bacterial strains (Figure 6).

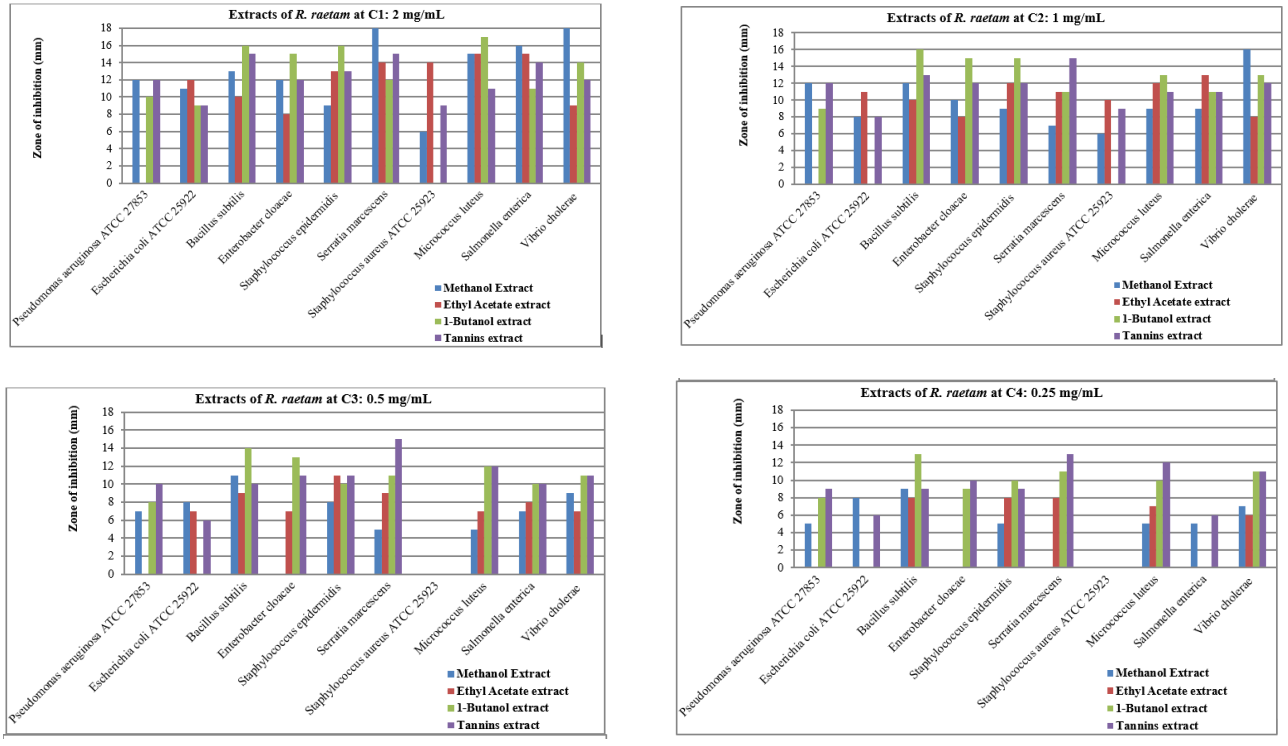


**Figure 6.** Antibacterial activity of the different concentrations of extracts of *R. raetam*

The inhibition zone, measured in millimeters, was reported after 24 h of incubation time, showing that the tested extracts could be classified according to their activity. The strongly active compounds included the

extract of tannins with a mean antimicrobial growth, the second most active extract against bacteria strains beings that in methanol extract. The third active extract was an extract of flavonoids phase 1-butanol, whereas the flavonoids extract phase ethyl acetate was less active.

The antibiotics used in antibacterial activity showed the best activity than all extracts of *R. raetam*, as the inhibition diameters from 0-35 mm (Figure 7).

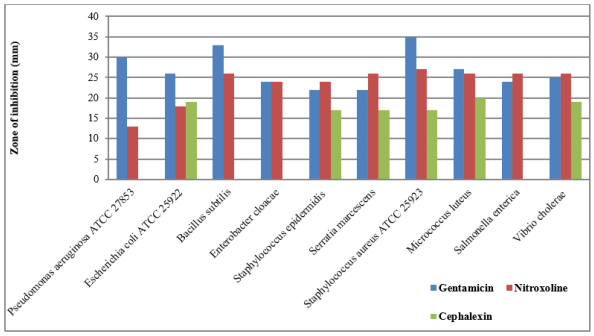


**Figure 7.** Antibacterial activity of the used antibiotics

Generally, these data indicate that Gram-negative bacteria are the most sensitive to the different extracts compared to Gram-positive bacteria (Figure 8).

In the antibacterial activity of the extracts, they can be associated with major constituents of all extracts. The research on the antimicrobial activity of phenolic compounds suggests that they diffuse into and damage cell membrane structures [38]. Whereas, the difference between bacteria of Gram-negative and Gram-positive can return to the structure, composition and nature of the walls [39].

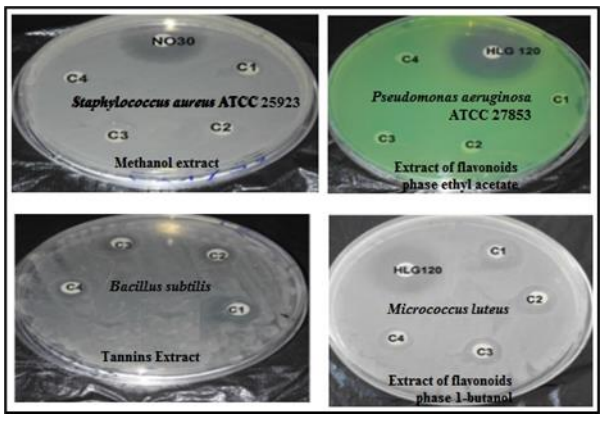


**Figure 8.** Antibacterial activity of the different concentrations of extracts of *R. raetam* against some bacteria strains

## 4. Conclusions

In this work, we evaluated the phytochemical properties, antibacterial and anti-free radical activities of phenolic extracts of *Retama raetam*. The chemical screening showed the presence of many secondary metabolites, the analysis of extracts by HPLC showed the appearance of six phenolics compounds and the absence of chlorogenic acid in ethyl acetate extract and caffeic acid in 1-butanol extract. In the free radical DPPH test, the values of $IC_{50}$ were converging in all extracts of *R. raetam*, and from the results of the antibacterial activity we noted the Gram-negative bacteria strains are the most sensitive to the extracts of the plant. These results confirmed that the plant is an alternative source of natural antibiotics and antioxidants.

## Conflict of interest

The authors declare no conflict of interest.

## Acknowledgment

The authors would like to gratefully thank Dr. REBIAI Abdelkrim (VTRS Laboratory, El Oued University, Algeria) for HPLC Chromatographic analysis.

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*Received:* 11.11.2020
*Received in revised form:* 20.02.2021
*Accepted:* 22.02.2021