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Antioxidant, antibacterial activities and flavonoids of *Convolvulus fatmensis* G. Kunze

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ABSTRACT

*The secondary metabolites of butanol and ethyl acetate extracts of the endemic species Convolvulus fatmensis G. Kunze were separated by the use of different chromatographic techniques. The antioxidant activity of the butanol and ethyl acetate extracts was investigated and the antibacterial activity of the chloroform extract was tested against several Gram-positive and Gram-negative bacteria. Four known flavonols: quercetin (**1**), quercetin-3-O-β-glucoside (**2**), quercetin-3-O-β-galactoside (**3**), quercetin-3-O-rutinoside (**4**) were isolated from the ethyl acetate and butanol extracts. Compound **3** was new for the genus Convolvulus. A significant phenolic content (>33.74 g/100 g and >11.59 g/100 g of dry extracts) were found for the butanolic (BECF) and ethyl acetate (EACF) extracts, respectively. The EACF and BECF exhibited a good antioxidant activity (IC_{50} 5.5 µg/ml, IC_{50} 15 µg/ml) successively compared with the reference (rutin IC_{50} 3.01 ± 0.2 µg/ml). The best antibacterial activity of the chloroformic extract of C. fatmensis (CECF) was observed against Proteus mirabilis, Staphylococcus aureus, Staphylococcus aureus ATCC 43300, Pseudomonas aeruginosa ATCC 27853, Pseudomonas aeruginosa, with 14, 13, 13, 11, 12 mm, inhibition zone diameters, respectively.*

Keywords: *Convolvulus fatmensis* G. Kunze, .Flavonoids. Antioxidant. Antibacterial

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INTRODUCTION

Convolvulus is a fairly large genus with over 250 species. Many plants of this genus are traditionally used in the treatment of headache constipation, rheumatism, diabetes and skin diseases [1]. The species of this genus are problematic weeds but several members posses cytotoxic activity on some tumor cell lines.

Phytochemical studies on this genus showed the presence of terpenes [2], flavonoids [3, 4], phenolic compounds, sterols and resins, sugars [5-9]and alkaloids [10-11]. Various biological activities such as antinociceptive [12], antioxidant [13,14], antimicrobial [15,16] have been established for the genus. In continuation of our phytochemical and biological works on Algerian Saharian plants [17-32], we report here the antioxidant, antibacterial activities and flavonoids of the endemic species *Convolvulus fatmensis* G. Kunze.

MATERIALS AND METHODS

Plant extract

Convolvulus fatmensis was collected from Ghardaia (Septentrional Algerian Sahara) in March 2010, during the flowering stage and authenticated by Mr K. Kabouche. A voucher specimen has been deposited in the Herbarium of the laboratory of Therapeutic Substances (LOST), University Mentouri-Constantine (LOST Cf/03/10).

Extraction

Air-dried and powdered aerial parts (1000 g) of *C. fatmensis* were macerated in water/methanol (30/70 v/v) for 24 hours (three times). After filtration and concentration, the residue was dissolved in water (600 ml). The resulting solution was extracted successively with $CHCl_3$, EtOAc and *n*-butanol. Concentration in *vacuo* led to the chloroforme extract (CECF) (1.3g), ethyl acetate extract (EACF) (3.5g) and *n*-butanol extract (BECF) (21.3g).

Compound **1** crystallized from the EACF as yellow fine needles, it was identified as Quercetin. The BECF (10 g) was column chromatographed on polyamid SC6, eluted with a gradient of MeOH-Toluene with increasing polarity to give twelve fractions (1-12). The fraction F4 obtained, from toluene 75%, was submitted to silica gel column chromatography with an isocratic system of (AcOEt-MeOH-H_2O, 10:1:0.5) leading to six **s**ubfractions: f1 yielded compound **2**, which was obtained as a yellow precipitate, identified as Quercetin-3-*O*-glucoside.

The subfraction f6 yielded compound **3**, which was obtained as yellow fine crystals, identified as Quercetin-3-*O*-galactoside.

The fraction F7, obtained from toluene 75%, was submitted to silica gel column chromatography with an isocratic system of (AcOEt-MeOH-H_2O, 10:1:0.5), affording eight **s**ubfractions. f5 was separated by silica gel column chromatography being eluted with (AcOEt-MeOH-H_2O, 10:1:0.5), leading to compound **4**, which was identified as Quercetin-3-*O*-rutinoside.

Antioxidant activity

The radical scavenging activity of the EACF and BECF was measured by the slightly modified method of Hatano (1988) [33, 34]. One milliliter of a 0.2 mM DPPH methanol solution was added to 4 mL of various concentrations of the extract in methanol. The mixture was shaken vigorously and left to stand at room temperature. After 30 min, the absorbance of the solution was measured at 517 nm and the antioxidant activity calculated using the following equation:

Scavenging capacity % = [(Ab of sample – Ab of blank) × 100/ Ab of sample].

Methanol (1 mL) plus plant extract solution (4 mL) were used as a blank, while DPPH solution plus methanol was used as a negative control. The positive control was DPPH solution plus 1 mM rutin. Extract concentration providing 50% inhibition (IC50) was calculated from the plot of inhibition percentage against extract concentration.

Antibacterial activity

Susceptibility of the bacterial strains to the CECF was investigated using the disk diffusion method and by comparing their antibiogram inhibition zones to those reported by the National Committee for Clinical Laboratory Standards (NCCLS). Disks containing freshly prepared CECF were used for antibacterial activity assays. The diameters of inhibition zones were measured and compared with those suggested by NCCLS (sensitive P≥15 mm). The susceptibility of the strains to the extract was further evaluated by agar dilution method; different concentrations of the essential oil were included in Mueller-Hinton agar plates (sensitive MIC ≤ 32 lg/ml). The minimum inhibitory concentration (MIC) was defined as the concentration at which no colony was observed after incubation (NCCLS 1997) [35]. Approved Standard M2-A6, Wayne, PA. The agar plates were prepared and inoculated with bacterial suspension. After incubation at 37 °C for 18–24h, the inhibition zones were measured and averaged. The essays were performed in triplicate. MICs of the CECF were also determined by an agar dilution method. The antibacterial activity of the CECF was tested against a range of microorganisms, namely *Escherichia coli ATCC 25922*, *Escherichia coli, Staphylococcus aureus* ATCC 43300, *Staphylococcus aureus, Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa, Klebsiella pneumonia Enterobacter sp* and *Proteus mirabilis Strep.* The reference strains were obtained from the Pasteur Institute (Algiers). The other strains were obtained from the laboratory of bacteriology, Benbadis Hospital, Constantine, using conventional methods (clinical isolation).

RESULTS AND DISCUSSION

Identification of components

Compound 1. $C_{15}H_{10}O_7$, C, UV (MeOH, λ_{max}, nm): 255, 368; +NaOH: 280, 325, 417, +$AlCl_3$: 269, 450; +$AlCl_3$/HCl: 267, 418; NaOAc: 269, 397. +H_3BO_3: 261, 386. ^{1}H NMR (500 MHz, CD_3DO, δ, ppm, J/Hz): 7.72 (1H, d, J = 2.0, H-2'), 7.62 (1H, dd, J = 8.5-2.0, H-6'), 6.88 (1H, d, J = 8.5, H-5'), 8), 6.39 (1H, d, J = 2.5, H-8), 6.16 (1H, d, J = 2.5, H-6). This compound was characterized as quercetin.

Compound 2. $C_{21}H_{20}O_{16}$, UV (MeOH, λ_{max}, nm): 258, 357; +NaOH: 271, 326, 410, +$AlCl_3$: 267, 431; +$AlCl_3$/HCl: 270, 403; NaOAc: 268, 392. +H_3BO_3: 261, 380 ^{1}H NMR (400 MHz, CD_3DO, δ, ppm, J/Hz): 7.74 (1H, d, J = 2.0, H-2'), 7.49 (1H, dd, J = 8.4-2.0, H-6'), 6.77 (1H, d, J = 8.4, H-5'), 6.30 (1H, d, J = 2.0, H-8), 6.10 (1H, d, J = 2.0, H-6).. 5.07 (1H, d, J = 7.1, H-1" glucose), 3.20-4.00 (sugar protons).

Acid hydrolysis of compound **2** produced Quercetin and Glucose. This compound was characterized as quercetin -3-O-*β*-glucoside.

Compound 3. $C_{21}H_{20}O_{16}$, UV (MeOH, λ_{max}, nm): 257, 357; +NaOH: 271, 324, 409, +$AlCl_3$: 268, 425; +$AlCl_3$/HCl: 269, 401; NaOAc: 273, 392. +H_3BO_3: 263, 381. ^{1}H NMR (250 MHz, CD_3DO, δ, ppm, J/Hz): 7.66 (1H, d, J = 2.0, H-2'), 7.50 (1H, dd, J = 8.0-2.0, H-6'), 6.80 (1H, d, J = 8.0, H-5'), 6.32 (1H, d, J = 2.0, H-8), 6.15 (1H, d, J = 2.0, H-6). 5.16 (1H, d, J = 7.4, H-1" galactose), 3.20-4.00 (sugar protons)

Acid hydrolysis of compound **3** produced Quercetin and Galactose. This compound was characterized as Quercetin -3-O-*β*-galactoside.

Compound 4. $C_{27}H_{30}O_{16}$, UV (MeOH, λ_{max}, nm): 257, 358 ; +NaOH: 272, 325, 414, +$AlCl_3$: 267, 431; +$AlCl_3$/HCl: 270, 402; NaOAc: 273, 386. +H_3BO_3: 263, 382. ^{1}H NMR (250 MHz, CD_3DO, δ, ppm, J/Hz): 7.78 (1H, d, J = 2.0, H-2'), 7.51 (1H, dd, J = 8.4-2.0, H-6'), 6.77 (1H, d, J = 8.4, H-5'), 6.30 (1H, d, J = 2.0, H-8), 6.10 (1H, d, J = 2.0, H-6), 5.06 (1H, d, J = 7.0, H-1" Glucose), 4.50 (1H, d, J = 2.0, H-1'" Rhamnose), 1.1 (3H, d, J = 6.2, H-6'" Rhamnose) 13C NMR (400 MHz, CD_3OD, δ, ppm, J/Hz):: 177.9 (C-4), 164.6 (C-7), 161.5 (C-5), 157.9 (C-2), 157.06 (C-9),148.4 (C-4'), 144.4 (C-3'), 134.2 (C-3), 122.1 (C-6'), 121.6 (C-1'), 116.2 (C-5'), 114.6 (C-2'), 104.17 (C-10), 103.3 (C-1"), 100.9 (C-1'"), 98.5 (C-6), 93.4 (C-8), 76.7 (C-5"), 75.7 (C-3"), 74.3 (C-2"), 72.5 (C-4'"), 70.7 (C-4"), 70.6 (C-2"), 69.9 (C-3'"), 68.3 (C-5'"), 67.1 (C-6"), 16.4 (C-6'").

Acid hydrolysis of compound **4** produced Quercetin and Glucose + Rhamnose. This compound was characterized as quercetin-3-*O*-rutinoside.

Table 1. Polyphenol quantification and antioxidant activity of the AECF and BECF extracts

	Phenolic compounds (g/100 g equiv. Pyrogallol)	IC_{50} DPPH (µg/ml)
AECF	**33.74 ± 1.29**	**5.5**
BECF	**11.59 ± 0.68**	**15**
Picnogenol rutin	**30.7**	**12**
Vitamin E		**3.01**

Table 2. Antibacterial activity of the CEET (Inhibition zones and MIC values)

Microorganism	Inhinhibition Zone [a]	MIC (µg/ml)
Escherichia coli ATCC 25922	/	80
Escherichia coli	/	80
Staphylococcus aureus ATCC 43300	14	80
Staphylococcus aureus	13	80
Pseudomonas aeruginosa ATCC 27853	13	80
Pseudomonas aeruginosa	11	80
Klebsiella pneumoniae	/	80
Enterobacter sp	/	80
Proteus mirabilis Strep	12	80

[a]: *(128 µg/ml)*

Repeated column chromatographies of EACF and BECF resulted in isolation of four flavonoids: Quercetin (**1**), Quercetin-3-*O*-glucoside (**2**), Quercetin-3-*O*-galactoside (**3**), Quercetin-3-*O*-rutinoside (**4**), The structures of these four flavonoids were elucidated by extensive UV analyses and NMR spectroscopic analyses as well as by comparing their spectroscopic data with those reported in the literature [36-42].

Table1. Total phenolic compounds determined as pyrogallol equivalents in grams per 100 g of dry material by Folin-Ciocalteu method and Radical Scavenging Activity against DPPH [43,44] from *C. fatmensis* (IC_{50} of the AECF, BECF and the references);

CONCLUSION

Four flavonoids: Quercetin (**1**), Quercetin-3-*O*-glucoside (**2**), Quercetin-3-*O*-galactoside (**3**), Quercetin-3-*O*-rutinoside (**4**) were isolated from ethyl acetate and n-butanol extracts of *Convolvulus fatmensis*. A significant phenolic content in agreement with good radical scavenging activity were found for these extracts. The best antioxidant activity of the Ethyl acetate could be explained by the main presence of quercetin. The chloroformic extract was tested against a panel of Gram + and Gram – bacteria but the growth of the used strains was weekly inhibited.

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