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RESEARCH ARTICLE

Compounds from the pods of *Astragalus armatus* with antioxidant, anticholinesterase, antibacterial and phagocytic activities

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ABSTRACT

Context: The phytochemical study and biological activities of *Astragalus armatus* Willd. subsp. *numidicus* (Fabaceae) pods, an endemic shrub of Maghreb, are reported.

Objective: This study isolates the secondary metabolites and determines the bioactivities of *Astragalus armatus* pods.

Materials and methods: The chloroform, ethyl acetate and *n*-butanol extracts of hydro-ethanolic extracts were studied. Antioxidant activity was investigated using DPPH and ABTS radical scavenging, CUPRAC and ferrous chelating assays at concentrations ranging from 3 to 200 µg/mL. Anticholinesterase activity was determined against acetylcholinesterase and butyrylcholinesterase enzymes at 50, 100 and 200 µg/mL. Antibacterial activity was performed according to minimum inhibitory concentration (MIC) method. Carbon clearance method in albino mice was used for the phagocytic activity at concentrations 50, 70 and 100 mg/kg body weight. Spectroscopic techniques were used to elucidate the compounds.

Results: Ethyl acetate extract afforded a flavonoid (**1**) while the *n*-butanol extract gave four flavonoids (**2–5**), a cyclitol (**6**) and a cycloartane-type saponin (**7**). The ethyl acetate extract exhibited highest antioxidant activity in DPPH (IC_{50}: 67.90 ± 0.57 µg/mL), ABTS (IC_{50}: 11.30 ± 0.09 µg/mL) and CUPRAC ($A_{0.50}$: 50.60 ± 0.9 µg/mL) assays. The chloroform extract exhibited the best antibacterial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa,* each with 80 µg/mL MIC values. The *n*-butanol extract enhanced phagocytic activity.

Discussion and conclusion: Isorhamnetin (**1**), isorhamnetin-3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-galactopyranoside (**2**), isorhamnetin-3-*O*-β-D-apiofuranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-galactopyranoside (**3**), kaempferol-3-*O*-(2,6-di-*O*-α-L-rhamnopyranosyl)-β-D-galactopyranoside (**4**), kaempferol-3-*O*-(2,6-di-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside (**5**), pinitol (**6**) and cyclomacroside D (**7**) were isolated whereas **1, 2, 6** and **7** are reported for the first time from *A. armatus*.

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Biological activity; flavonoids; saponins; structure elucidation

Introduction

Astragalus armatus Willd. (Fabaceae) locally known as "ketad" is an endemic species to Algeria. The *Astragalus* genus consist of ~3000 species worldwide (Ozenda 1991). In North African flora, there are ten *Astragalus* species that are endemic to Tunisia, Morocco and Algeria. In folk medicine, *Astragalus* species are used against chronic bronchitis, stomach ulcer, cough, hypertension, diabetes, gynecological disorders and venomous bites of scorpion (Bellakhdar 1997). *A. membranaceus* (Fisch.) Bge. var. *mongholicus* (Bge.) Hsiao has a wide attention in the traditional Chinese medicine among the *Astragalus* species. Its roots have long been used as an antiperspirant, antidiuretic, or tonic (Leung & Foster 1996; Guo 1997; Upton & Petrone 1999). In Ghardaia, its barks and seeds are frequently used to treat different types of wounds, and against pain, fever, constipation and stomach problems (Voisin 1987).

Several *Astragalus* species were studied for their antiviral, cardiotonic, antioxidant, cytotoxic, anticancer, immunostimulating, anti-inflammatory and analgesic activities (Li et al. 2001; Huang et al. 2009; Hong et al. 2011; Nalbantsoy et al. 2011; Shojaii et al. 2015). The biologically active sources of *Astragalus* species comprise polysaccharides, saponins, phenolics and the toxic compounds; such as nitrotoxins, imidazoline alkaloids and selenium derivatives (Pistelli 2002). However, researchers have mainly focused on the isolation and elucidation of cycloartane-type triterpenes (Polat et al. 2010; Savran et al. 2012), oleanane-type triterpenes (Gülcemal et al. 2013), flavonoids (Benchadi et al. 2013; Chaturvedula & Prakash 2013) and isoflavonoids (Zheng Zhong et al. 1998; Abd El-latif et al. 2003) from various *Astragalus* species.

Previously, two acylated tridesmosidic saponins (armatoside I & II) and two cycloartane type glycosides (trigonoside II & trojanoside H) were isolated from the roots of *A. armatus* (Semmar et al. 2010) while eight flavonoids from its flowering aerial parts (Khalfallah et al. 2014).

Regarding the chemical components and traditional usage of certain *Astragalus* species, it was aimed to investigate the secondary metabolites from pods of *A. armatus* subsp. *numidicus*

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together with antibacterial, antioxidant, anticholinesterase and phagocytic activities of the various extracts. Herein, we report the bioactivities of *A. armatus* pods for the first time.

Materials and methods

Chemicals and drugs

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE DRX 500 NMR spectrometer (Karlsruhe, Germany) (^{1}H at 500 MHz and ^{13}C at 125 MHz) and Bruker AVANCE DRX 600 NMR spectrometer (^{1}H at 600 MHz (Karlsruhe, Germany) and ^{13}C at 150 MHz). 2D-NMR experiments were performed using standard Bruker sequences. Anticholinesterase and antioxidant activity were performed on a 96-well microplate reader, Spectra Max 340PC^{384}, Molecular Devices (Sunnyvale, CA). Software PRO *v*5.2 software (Sunnyvale, CA) was used to calculate the measurements. The absorbance was measured on a Thermo scientific 300-UV Spectrophotometer (Waltham, MA). Thin Layer Chromatography (TLC) was carried out on precoated silica gel 60 F_{254} plates (Merck), and spots were visualized by heating after spraying with sulfuric acid vanillin.

Sephadex LH-20 and polyamide SC6 were purchased from Fluka (Steinheim, Germany). Ethanol, potassium persulfate, copper (II) chloride and ammonium acetate, were obtained from E. Merck (Darmstadt, Germany). Butylated-hydroxyl anisole (BHA), α-tocopherol, ethylene diamine tetra acetic acid (EDTA), galantamine, 2,2′-azinobis (3-ethyl-benzothiazoline-6-suphonic acid) diammonium salt (ABTS), 1,1-diphenyl-2-picrylhydrazyl (DPPH), neocuproine, 5,5′-dithiobis (2-nitrobenzoic) acid (DTNB), butyrylthiocholine chloride, acetylthiocholine iodide, horse serum butyrylcholinesterase (BChE, EC 3.1.1.8, 11.4 U/mg, Sigma), electric eel acetylcholinesterase (AchE, Type-VI-S, EC 3.1.1.7,425.84 U/mg, Sigma), were obtained from Sigma Chemical Co. (Sigma-Aldrich GmbH, Steinheim, Germany). All other chemicals used were of analytical grade.

Plant material

Astragalus armatus subsp. *numidicus* pods were collected from Bekira-Constantine (Eastern Algerian) during May 2007 and authenticated by Pr. Gérard De Bélair (University of Annaba). The voucher specimen was kept in the Herbarium of the Faculty of Sciences, University of Mentouri Constantine (specimen number LOST ZKAK Asar/05/07).

Extraction and isolation of compounds

The pods were air-dried under the shadow with ventilation. Dried pods were powdered (500 g) and extracted with 2 L ethanol:water (80:20) mixture for 24 h. at room temperature. The experiment was repeated three times. The hydro-ethanolic extract was dried under vacuum using rotary evaporator. The dried extract (42.7 g) was dissolved in water and kept overnight. After filtration, the aqueous solution was subjected to liquid–liquid extraction with chloroform ($CHCl_3$), ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH) for three times with each solvent, successively. The organic phases were filtered and concentrated to dryness under reduced pressure to obtain $CHCl_3$ (CA) (2.19 g), EtOAc (EA) (2.0 g) and *n*-BuOH (BA) (35 g) extracts.

The EA (1.8 g) was submitted to a vacuum liquid chromatography (VLC) on silica gel and eluted with $CHCl_3$-MeOH with increasing polarity (gradient $CHCl_3$-MeOH: 100:0 to 0:100). A total of 30 fractions were gathered and combined into eight main fractions (G-1 to G-8), using thin layer chromatography. The fractionation of G-1 on Sephadex LH 20 using methanol as a solvent gave nine sub-fractions (f-1 to f-9). Fraction f-4 was obtained as pure product **1** (5 mg).

The BA (20 g) was subjected to polyamide SC6 VLC using gradients of toluene-methanol solvents (100:0 to 0:100). Fractions having similar TLC profiles were combined to afford 16 fractions (B-1 to B-16). The combined fractions B-8 and B-9 (2 g) were applied to silica gel column chromatography and eluted with $CHCl_3$-MeOH-H_2O (10:0:0 to 4:6:1) to obtain 16 fractions. The fraction S-8 eluted with $CHCl_3$-MeOH-H_2O (80:20:2) (80 mg) was purified on silica gel preparative TLC to provide 6 mg of **7** using $CHCl_3$-MeOH-H_2O (28:6:1) mixture as a solvent system. Fraction B-13 (1.19 g) containing a major component was subjected to silica gel column chromatography eluted with an isocratic system EtOAc-MeOH-H_2O (20:2:1) to afford 15 fractions. The fraction F-6 (72 mg) was submitted to silica gel column chromatography eluted with an isocratic system EtOAc-MeOH-H_2O (9:2:1) to afford compound **2** (30 mg). Fraction B-14 (70 mg) was submitted to silica gel column chromatography that was eluted with an isocratic system EtOAc-MeOH-H_2O (18:2:1) to afford compound **3** (3 mg). Fraction B-15 (50 mg) was submitted to silica gel column chromatography that eluted with an isocratic system EtOAc-MeOH-H_2O (20:2:1) to afford 7 fractions. Fraction C-7 studied over silica gel column chromatography using an isocratic system EtOAc-MeOH-H_2O (18:2:1) gave compounds **4** (1.8 mg), **5** (1.2 mg), and **6** (3.6 mg).

Phytochemical screenings on the chloroform extract (CA) showed that fatty acids were predominant. Therefore, it was not submitted to chromatographic separations.

Determination of antioxidant activity

DPPH free radical scavenging assay

The spectrophotometric method developed by Blois was used to determine the free radical-scavenging activity (Blois 1958; Ay et al. 2007) at concentrations of 1, 2.5, 5, 10, 25, 50 and 100 µg/mL concentrations. BHA and α-tocopherol were used as positive standards. The results were given as IC_{50} (µg/mL) corresponding the concentration of 50% inhibition. Following equation was used to calculate scavenging of DPPH radical.

$$\text{DPPH radical scavenging activity } (\%) = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

where $A_{control}$ and A_{sample} are the absorbencies of the reference and sample obtained from the 96-well microplate reader, Spectra Max 340PC^{384}, Molecular Devices (Sunnyvale, CA).

Metal chelating activity assay

The ferrene-Fe^{2+} complexation method was selected to assay the metal chelating activity (Decker & Welch 1990). EDTA was used as a positive standard. The test was carried at 1, 2.5, 5, 10, 25, 50 and 100 µg/mL concentrations. The calculation was performed according to the following equation.

$$\text{Metal chelating activity } (\%) = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

ABTS cation radical decolorization assay

The spectrophotometric method developed by Re et al. (1999) was used to determine the radical-scavenging activity of the samples with concentrations of 1, 2.5, 5, 10, 25, 50 and 100 µg/mL. BHA and α-tocopherol were used as positive standards. The results were given as IC_{50} (µg/mL) corresponding the concentration of 50% inhibition. Following equation was used to calculate scavenging of ABTS cation radical.

$$\text{ABTS}^{\bullet+}\text{scavenging activity } (\%) = \frac{A_{Control} - A_{Sample}}{A_{Control}} \times 100$$

Cupric reducing antioxidant capacity (CUPRAC) assay

CUPRAC was determined according to the method developed by Apak et al. (2004). BHA and α-tocopherol were used as positive standards. The test was carried out at 1, 2.5, 5, 10, 25, 50 and 100 µg/mL concentrations. The results were given as $A_{0.5}$ (µg/mL) corresponding the concentration indicating 0.50 absorbance intensity.

Determination of anticholinesterase activity

The spectrophotometric method developed by Ellman et al. (1961) was performed for AChE and BChE inhibitory activities. In the assay, AChE and BChE were used as enzymes while acetylthiocholine iodide and butyrylthiocholine chloride were used as their substrates. DTNB was employed as a color developer to measure the activity. In this assay, galantamine was the positive standard drug. The test was carried out at 5, 10, 25, 50, 100 and 200 µg/mL concentrations.

Antibacterial activity

The antibacterial activity was evaluated against tested organisms according to the Clinical and Laboratory Standards Institute (NCCLS 1993; CLSI 2007). Freshly cultured bacterial suspensions in Mueller–Hinton Broth were standardized to a cell density of 1.5×10^8/mL (McFarland No. 0.5). These extracts were tested against nine microorganisms including reference strains which were obtained from the Pasteur Institute (Algiers), namely *Staphylococcus aureus* ATCC 43300, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922. Others were clinical isolates, namely, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Morganella morganii* and *Klebsiella pneumoniae*. The stated microorganisms were obtained using conventional methods from the Bacteriology Laboratory of Benbadis Hospital, Constantine (NCCLS 1993; CLSI 2007).

Determination of minimum inhibitory concentration (MIC)

Susceptibility of the bacterial strains to *A. armatus* extracts was investigated using the MIC method. The nine tested bacteria were susceptible to the extract; MICs of different extracts were determined using agar dilution method at different concentrations of the three extracts included in Mueller–Hinton agar plates. MIC is the concentration at which, no colony can be observed after incubation (CLSI 2007). The essays were performed in triplicate and the results were expressed as their average. Gentamicin and ampicillin were used as a positive reference standard.

Immunomodulating activity

Animals

Adult albino male mice (32–40 g, 2–2.5 months old) were procured from central pharmacy Algeria. The animals were kept in polyacrylic cages and maintained under standard housing conditions (room temperature 25 ± 1 °C with 12:12 light: dark cycles). Food was provided in the form of dry pellets (SARL Production Locale, Bouzareah, Algeria) and water *ad libitum*. The clearance of the animal studies obtained from Institutional Animal Ethics Committee. The experiments were performed according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Phagocytic activity

The phagocytic activity of the butanol extract (BA) was evaluated *in vivo* by the carbon clearance rate test (Biozzi et al. 1953). Animals were divided into four groups, GI, GII, GIII and GIV. NaCl (0.5 mL/mouse) was injected 0.9% intraperitoneal to GI (Control). Groups GII, GIII and GIV were administered with 50, 70 and 100 mg/kg of BA, prepared in 0.9% NaCl. After 48 h of intraperitoneal injection, carbon ink suspension was injected via the tail vein to each mouse at a dose of 0.1 mL/10 g. The mixture comprised of 3 mL black carbon ink, 4 mL saline and 4 mL 3% gelatin solutions. Blood samples were taken at 5 and 15 min using glass capillaries from the retro orbital vein and mixed with 4 mL of 0.1% Na_2CO_3 solution for the lysis of erythrocytes. Then, the absorbance was read at 675 nm.

The phagocytic activity is expressed by the phagocytic index K that measures all the reticuloendothelial system function in the contact with the circulating blood. The clearance rate is expressed as the half-life period of the carbon in the blood ($t_{1/2}$, min). Calculations were performed by the following equations:

$$K = \frac{LnOD1 - LnOD2}{t2 - t1}, \quad t1/2 = 0.693/K$$

Where, OD_1 and OD_2 are the optical densities at times t_1 and t_2, respectively.

Statistical analyses

All data obtained from the activities were the averages of triplicate analyzes. The data were recorded as means ± standard error meaning (S.E.M). Significant differences between means were determined by Student's *t*-test; p values <0.05 were regarded as significant. ANOVA test and Tukey's multiple comparison tests were used to analyze data of phagocytic activity using SPSS Version 9 (IBM Corporation, Armonk, NY).

Results and discussion

Identification of compounds 1–7

The EtOAc (EA) and *n*-BuOH (BA) extracts of *A. armatus* pods were selected to isolate compounds due to showing good antioxidant activity (Table 1). Phytochemical studies of both extracts afforded seven compounds (**1**–**7**) including flavonoids (**1**–**5**), one cyclitol (**6**) and one saponin (**7**) (Figure 1). The compounds were elucidated as isorhamnetin (**1**) (Lee et al. 2008), isorhamnetin-3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-galactopyranoside (**2**)

Table 1. Antioxidant activity of *A. armatus* pods by the DPPH, ABTS, CUPRAC and metal chelating assays.[a]

	Antioxidant activity			
Extracts	DPPH assay IC_{50} (μg/mL)	ABTS assay IC_{50} (μg/mL)	CUPRAC assay $A_{0.50}$ (μg/mL)	Metal chelating assay % Inhibition at 100 μg/mL
Ethyl acetate extract	67.90 ± 0.57	11.30 ± 0.09	50.60 ± 0.9	17.4 ± 0.3
n-Butanol extract	80.37 ± 0.81	90.58 ± 0.74	>100	NA
α-Tocopherol[b]	7.31 ± 0.17	4.31 ± 0.10	25.50 ± 0.0	NT
BHA[b]	45.37 ± 0.47	4.10 ± 0.06	10.9 ± 0.1	NT
EDTA[b]	NT	NT	NT	96.3 ± 0.1

[a]IC_{50} and $A_{0.50}$ values represent the means ± S.E.M. of three parallel measurements ($p < 0.05$).
[b]Reference compounds; BHA: butylatedhydroxyl anisole; EDTA: Ethylenediaminetetraacetic acid.
NT: not tested; NA: not active.

(Burasheva et al. 1975), isorhamnetin-3-*O*-α-L-apiofuranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-galactopyranoside (**3**) (Bedir et al. 2000), kaempferol-3-*O*-(2,6-di-*O*-α-L-rhamnopyranosyl)-β-D-galactopyranoside (mauritanin) (**4**) (Yasukawa & Takido 1987), and kaempferol-3-*O*-(2,6-di-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside (**5**) (Leite et al. 2001), pinitol (**6**) (Pistelli et al. 1998), and 24R-cycloartan-1α, 3β, 7β, 24, 25-pentaol-3-*O*-α-L-rhamnopyranosyl-24-*O*-β-D-xylopyranoside (**7**) (Iskenderov et al. 2009) using the 1D-, and 2D-NMR, and MS techniques.

Antioxidant properties

The extracts have a combination of compounds of different chemical groups. Therefore, dependents on the test employed, the combination having different polarity could lead to scattered results. Thus, several antioxidant methods are used to determine the activity. In this study, ABTS cation radical scavenging activity, DPPH free radical scavenging activity, metal chelating activity and cupric reducing power assays were used. The differences between the extracts and the control were statistically significant in all antioxidant activity tests ($p < 0.05$).

ABTS cation radical and DPPH free radical absorb at 734 nm and 517 nm in their radical forms, respectively. When these radicals are reduced by an antioxidant, their absorptions decrease. Thus, lower absorbance indicates higher antioxidant activity of the molecule. In ABTS assay, the EA (IC_{50} 11.30 ± 0.09 μg/mL) exhibited higher activity than BA, but lesser activity than those of BHA (IC_{50} 4.10 ± 0.06) and α-tocopherol (IC_{50} 4.31 ± 0.10 μg/mL). In DPPH assay, EA (IC_{50} 67.90 ± 0.57 μg/mL) also demonstrated better activity than BA (IC_{50} 80.37 ± 0.81 μg/mL).

The CUPRAC assay is based on absorbance measurement at 450 nm, after the formation of a stable complex between neocuproine and cuprous ions. Cuprous ions in the media are produced by the reduction of cupric ions by antioxidants. A higher absorbance indicates higher reducing capacity of antioxidant. Therefore, $A_{0.5}$ values corresponding to the concentration of 0.50 absorbance were calculated from the absorbance versus concentration graph of each sample to compare the activity data. In this assay, The EA ($A_{0.5}$ 50.6 ± 0.9 μg/mL) also exhibited better activity than that of BA (Table 1).

The transition metals in biological fluids accelerate the radicalic degradations via the Fenton reaction. The metal chelating molecules are called secondary antioxidants, and their roles are to chelate the metals which lead to radical degradation. Therefore, the EA and BA were also tested for their metal chelating activity. However, only EA showed a slight activity (17.4 ± 0.3% at 100 μg/mL) (Table 1).

The higher antioxidant activity of the EA is probably due to the presence of isorhamnetin (**1**). The flavonoids isolated from BA are glycosylated in position 3. Therefore, the lesser antioxidant activity of BA can be explained due to the lack of –OH group in C-3 position.

Anticholinesterase activity

AChE and BChE inhibitory activities were studied for the extracts. Galantamine was used as a positive standard. The BA was inactive against both enzymes. On the other hand, EA exhibited weak inhibitory activity against AChE and BChE (10.4 ± 0.2% and 20.5 ± 0.4% at 200 μg/mL, respectively). The table was not drawn since they indicated almost no activity.

Antibacterial activity

Since *A. armatus* is used in traditional medicine, CA, EA and BA were tested for their antibacterial activity against a range of pathogens, namely, *Enterobacter aerogenes*, *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae* and *Morganella morganii*. As mentioned in Table 2, the CA exhibited the best antibacterial activity against *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 43300 and *Pseudomonas aeruginosa* ATCC 27853 with the MIC values of 80 μg/mL. CA also exhibited activity against other microorganisms with MIC values of 160 μg/mL. The EA extract was more susceptible to *Morganella morganii* (MIC 160 μg/mL) among the others. The BA extract exhibited higher activity against *Staphylococcus aureus* ATCC 43300 (MIC 160 μg/mL).

Evaluation of three extracts indicated that the CA inhibited selectively the growth of these three bacterial strains similarly to the references gentamicin and ampicillin. As CA was rich in fatty acids, it was not submitted to chromatographic separations to identify compounds responsible for this activity.

Phagocytic activity

The phagocytic activity of the BA was evaluated by *in vivo* carbon clearance rate test. Three concentrations of BA were evaluated, 50 mg/kg in GII, 70 mg/kg in GIII and 100 mg/kg in GIV. The present data showed the differences in the means for the phagocytic index (K) between groups (GI, GII, GIII and GIV) $p = 0.053$. Figure 2 demonstrates that the phagocytic index increased significantly in GII, GIII and GIV (0.0538, 0.081 and 0.112, respectively) compared with GI (0.046). Thus, the phagocytic index indicates that the BA enhanced the phagocytic activity by stimulating the ultranationalistes system at a dose of 100 mg/kg. The reticuloendothelial system is clearing particulate substances, such as bacteria and altered endogenous materials, such as fibrin aggregates. Phagocytosis is the mechanism by which

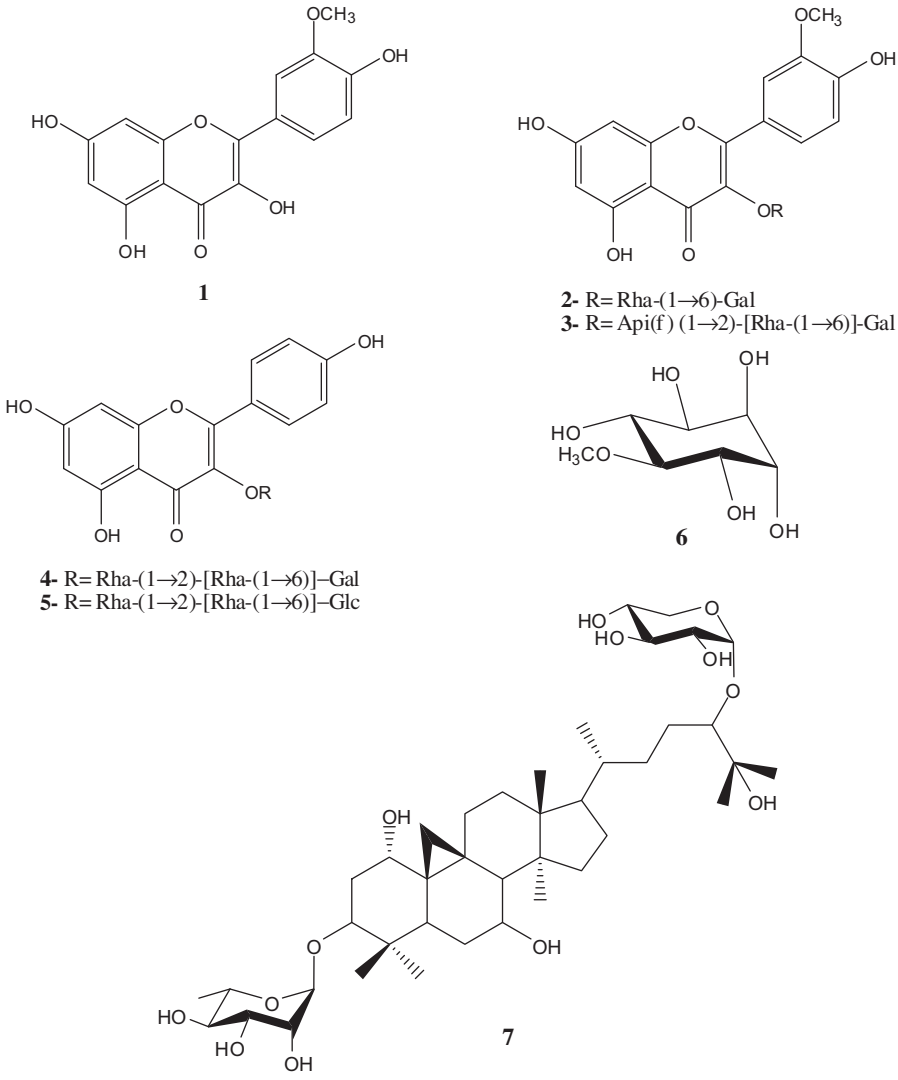

Figure 1. Structures of isolated Compounds (1–7).

Table 2. Antibacterial activity of the CA, EA and BA of *A. armatus* by the MIC.

	MIC (µg/mL)				
Microorganisms	CA	EA	BA	AMP	GEN
Escherichia coli ATCC 25922	80	640	320	10	10
Escherichia coli	160	640	640	–	–
Staphylococcus aureus ATCC 43300	80	640	160	5	–
Staphylococcus aureus	160	640	320	–	15
Pseudomonas aeruginosa ATCC 27853	80	320	640	–	–
Pseudomonas aeruginosa	160	640	640	–	5
Enterobacter aerogenes	160	320	320	–	–
Klebsiella pneumoniae	160	640	320	10	5
Morganella morganii	160	160	640	–	–

MIC: minimum inhibitory concentration; AMP: Ampicillin; GEN: Gentamicin (Reference compounds) (10 µg/mL).

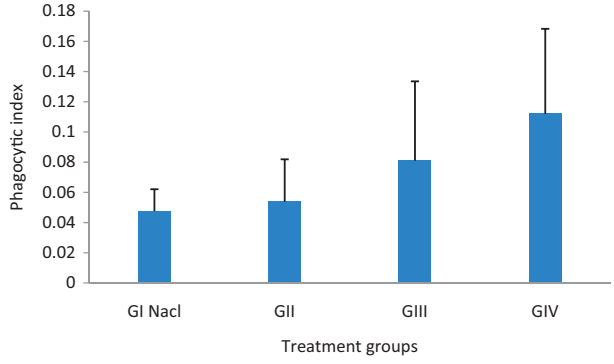

Figure 2. Effect of BA on phagocytic activity GI, GII, GIII and GIV were administered with 0, 50, 70 and 100 mg/kg of BA prepared in 0.9% NaCl.

microorganisms and foreign bodies, dead or injured cells are removed. Measurement of the activity of the reticuloendothelial system depends upon estimation of the rate of clearance from the blood of foreign materials, such as colloidal carbon (Normann & Benditt Earl 1965).

From Figure 3, the rate of carbon clearance from the blood increased after the treatment of the BA as seen in GII, when the concentration increased the half life of carbon clearance. This is an indication of nonspecific immunity in which foreign substances opsonized with complement C3b and antibodies resulting a more rapid clearance rate, and macrophage activity (Furthvan & Bergvanden 1991). These results are in agreement with the studies by Aribi et al. (2013) and Benmebarek et al. (2013) who reported the same activity of the Argan oil and the extract of *Stachys mialhesi*, respectively. Briefly, increasing of the concentrations increased the activity of the phagocytic index and decreased the half time of carbon clearance rate. The BA appeared

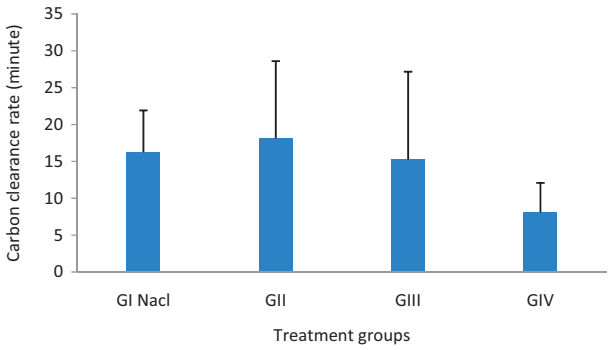

Figure 3. Effect of BA on the carbon clearance rate ($p = 0.046$).

to stimulate the phagocytic activity by increasing the clearance rate of carbon by the cells of the reticuloendothelium system. This is probably due to its flavonoid and saponin content.

Conclusion

Isorhamnetin (**1**) was obtained from EA which indicated the highest antioxidant activity. Other compounds; namely, isorhamnetin-3-*O*-α-L-rhamnopyranosyl-(1 → 6)-β-D-galactopyranoside(**2**), isorhamnetin-3-*O*-α-L-apiofuranosyl-(1 → 2)-[α-L-rhamnopyranosyl-(1 → 6)]-β-D-galactopyranoside (**3**), kaempferol-3-*O*-(2,6-di-*O*-α-L-rhamnopyranosyl)-β-D-galactopyranoside(mauritanin) (**4**), kaempferol-3-*O*-(2,6-di-*O*-α-L-rhamnopyranosyl)-β-D-glucopyranoside (**5**), pinitol (**6**), and 24R-cycloartan-1α, 3β, 7β, 24, 25-pentaol-3-*O*-α-L-rhamnopyranosyl-24-*O*-β-D-xylopyranoside (**7**) were isolated from BA which also indicated good antioxidant activity. Compounds **1**, **2**, **6** and **7** are reported here for the first time from the pods of *A. armatus*. In several fractions of the BA compound **2** (20% yield) was detected in major quantity. The abundance of **2** will make this plant an interesting biological source. The CA exhibited the highest antimicrobial activity. The EA and BA, however, had a mild antibacterial activity against tested organisms. Increments of the BA dose increased the clearance rate of carbon by the cells of the reticuloendothelium system; which also means that BA stimulated the phagocytic activity. This may be due to its flavonoid and/or cycloartane content. However, further studies on the biological activities of isolated compounds are needed to find the basis of the activity. Moreover, the phytochemical investigation by activity guided fraction of CA is also desired to isolate the antimicrobial components.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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