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GC/MS analysis, and antioxidant and antimicrobial activities of alkaloids extracted by polar and apolar solvents from the stems of *Anabasis articulata*

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Abstract

Anabasis articulata is a plant widely used in popular medicine to treat several pathologies in Algerian Sahara, such as diabetes. It is also associated with two other plants to cure certain types of cancer. Few research studies have been conducted on phytochemical analysis and in vivo biological activities of this species. However, no report is available on chemical elucidation of alkaloids and their antioxidant and antimicrobial properties. This study aims to characterise alkaloids in *A. articulata* stems and to evaluate their antioxidant and antimicrobial effects. Alkaloids extraction was carried out using polar (E1; E2.1; E2.2) and apolar solvents (E3), while chemical characterisation was performed by GC/MS. In vitro antioxidant activity was assessed by tree tests: total antioxidant capacity (TAC), DPPH scavenging assay and β-carotene–linoleate inhibition. For antimicrobial activity, eight different microorganisms were tested. The results suggest that E2.2 extract had the highest TAC value (14.742 ± 0.224 mg AAE/g DM), but E3 extract presented the highest DPPH scavenging activity ($EC_{50} = 1.242 \pm 0.168$ mg/mL) and a strong β-carotene–linoleate inhibition ($EC_{50} = 0.943 \pm 0.027$ mg/mL). In addition, E3 extract had the strongest inhibitory effects against all tested microorganisms and produced inhibition zones ranging between 9 and 20 mm, while the MICs of this extract ranged from 0.781 to >100 mg/mL and the MBCs ranged between 1.562 and >100 mg/mL. Forty-nine compounds belonging to 16 families, namely cyclopeptide alkaloids, steroid alkaloids, quinoline alkaloids, camptothecin alkaloids, quinazoline alkaloids, quinazoline alkaloids, isoquinoline alkaloids, isoquinolone alkaloids, indole alkaloids, terpene indole alkaloids, pyridine/pyrrolidine alkaloids, piperidine alkaloids, pyrrolizidine alkaloids, purine alkaloids, acridone alkaloids, benzazonine alkaloids and homolycorine-type Amaryllidaceae alkaloids were identified. Therefore, *A. articulata* can be considered as a source of antioxidant and antimicrobial agents.

Keywords *Anabasis articulata* · Alkaloids · GC/MS · Antioxidant activity · Antimicrobial activity

Introduction

Amaranthaceae (former denomination: Chenopodiaceae) family contains approximately 102 genera and 1400 species that are essentially halophytes (Kokanova-Nedialkova et al. 2009). The main characteristic of these plants is that they are particularly able to withstand salt conditions and environmental stresses. They are also known for their richness in bioactive molecules, such as betalain pigments, flavonoids, phenolic acids, glycosides, glycuronides, essential oils, sesquiterpenes, diterpenes, triterpenes and saponins (Mroczek 2015).

However, *Anabasis* genus has received the attention of the scientific community because of its characteristic biologically active secondary metabolites. To date, only a few scientific

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works have described the composition of *Anabasis* species extracts.

Thus, five glucosidic and isoflavonoid compounds were isolated from butanol-extract of *Anabasis salsa* and chloroform-extract of *Anabasis brevifolia*, such as 2-*O*-*β*-D-glucopyranosyloxy-4,6-dimethoxy phenylenthanone, 5,6,7,2′-tetramethoxy isoflavonoid, 2-*O*-(2)-*β*-D-glucopyranosyloxy-4,6-dimethoxy phenylenthanone, 3-methyl-but-2-enoic acid-[2-(4-methoxy phenyl)-ethyl]-amide and 2′-hydroxy-5,6,7-trimethoxyisoflavonoid (Chen et al. 2005).

Then, several types of alkaloids from *Anabasis aphylla*, like anabasine (neonicotine), anabasamine, lupinine, aphylline, aphylline *N*-oxide, aphyllidine, oxaphylline, lupinine methylanaphyllinate *N*-methylanabasine and isonicoteine, were isolated and characterised in other researches (Chopra et al. 1960; Tilyabaev et Abduvakhabov 1998; Du et al. 2008). In addition, five new molecules were isolated and identified in *n*-BuOH extract of *A. salsa* (tortoside A, phytolaccagenicacid-3-*O*-*β*-D-glucopyranuronide-28-*β*-D-glucopyranosyl ester, picraquassioside C, syringin and piceoside) (Pei et al. 2014).

In *Anabasis setifera*, oleanolic and 2-α-hydroxy ursolic acids were the most abundant sapogenins that are isolated in this species. They were detected usually as glycosylated with rhamnose or galactose (Alsofany 1998). Finally, jaxartinine was another alkaloid isolated from *A. jaxartica* (Segal et al. 1969).

Anabasis articulata (Forssk) Moq. species, locally named as 'ajrem', is a wild plant widely used in Algerian popular medicine to treat diabetes, kidney infections, fever, headache and skin diseases, such as eczema (Hammiche and Maiza 2006). It is used in decoctions, alone or mixed with other medicinal plants (Kambouche et al. 2009). In addition to its antidiabetic activity, it is also valued for its larvicidal property (Sathiyamoorthy et al. 1997) and in vivo hepatoprotective, and therapeutic effects against dimethylnitrosamine (DMN)-induced liver injury (Mohamed et al. 2014).

Several metabolites, including saponins, triterpenoid sapogenins, coumarins, flavonoids, phenolic acids, tannins, alkaloids, were reported, in general terms, in this species (Eman 2011; Benhammou et al. 2013; Mohammed et al. 2013; Ghembaza et al. 2016) and only rare evidence was described in detail about these compounds. For example, in addition to β-sitoglucoside saponin, which was initially considered absent in this species (Kambouche et al. 2011).

Four other well-known saponins, 3-*O*-glucopyranosyl of (stigmasterol, ß-sitosterol, sitostanol), 3-*O*-[ß-D-glucopyranosyl] oleanolic acid, 3-*O*-[ß-D-glucopyranosyl-28-*O*-ß-D-xylopyranosyl] oleanolic acid and proceric acid, were identified in hydro alcoholic extracts of *A. articulata* aerial districts (Metwally et al. 2012).

Anabasis' phytocomplex has not yet clearly defined and therefore this study will improve this knowledge. In literature, there is no survey that reports the chemical structures of *A. articulata* alkaloids. As a result, the main objective of this study is the isolation of alkaloids from *A. articulata*, using mixtures of polar and apolar organic solvents in order to obtain four different extracting fractions (E1; E2.1; E2.2 and E3). The evaluation of the antioxidant and antimicrobial activities of these extracts was carried out including identification by gas-chromatography/mass-spectronomy (GC/MS) of their alkaloidal constituents.

Materials and methods

Chemical materials

All chemicals were purchased from Sigma (represented by GETALAB Society, Tlemcen, Algeria).

Plant material

A. articulata plant stems were collected from Bechar region (Southwestern Algeria) in May 2011. Botanists of the Laboratory of "Plant Ecology", Tlemcen University, performed botanical identification using Algerian Plant Flora (Quezel et Santa 1963) and specimens that were deposited at the Herbarium of the Department of Biology of Tlemcen University, Algeria, under the code "L. 754".

Preparation of alkaloid extracts

Stems samples were air-dried at room temperature (27–32 °C) under shaded and well-ventilated conditions (65–70% relative humidity) for several days.

Dried stems were then coarsely powdered (not less than 0.5 mm) in a grinder, and approximately 65 g of the sample was extracted with 133 mL of petroleum ether for 24 h at room temperature. After filtration, the powder stems were dried out in an oven at 37 °C and subjected to two different extraction methods using organic solvents.

Extraction by polar solvents

Two extraction methods were used to purify the total alkaloid content (E1) (Harborne 1998), and basic (E2.1) and tetravalent alkaloids (E2.2), using the Stas−Otto method (Kalla 2012) (Fig. 1a, b).

Extraction by apolar solvents

To extract basic alkaloids by apolar solvents, the conventional method of Stas−Otto (Kalla 2012) was used, as shown in Fig. 1.

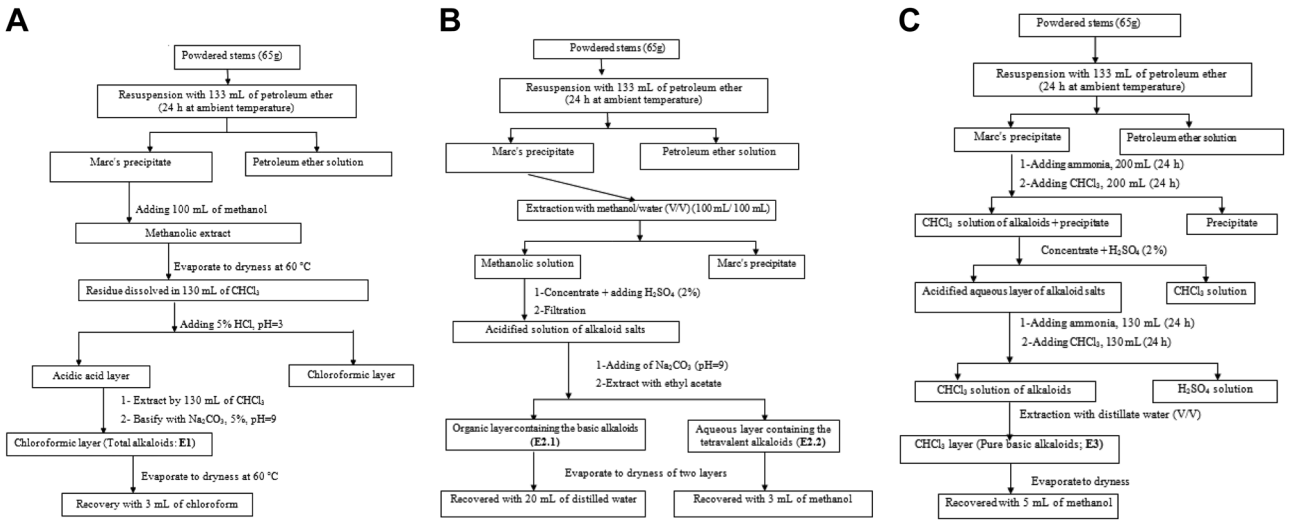

Fig. 1 Extraction protocols of different types of alkaloids. **a** Extraction of total alkaloids by polar solvent (Harborne 1998); **b** extraction of tetravalent and basic alkaloids by polar solvents according to the Stas−Otto method (Kalla 2012); **c** extraction of pure basic alkaloids by apolar solvent according to the Stas−Otto method (Kalla 2012)

Antioxidant activity

Total antioxidant capacity

Total antioxidant capacity of alkaloid extracts was evaluated by the Prieto et al. (1999) method and expressed as milligrams of ascorbic acid equivalent (mg AAE/g DM) per gram of dry matter. As a result, 0.3 mL of sample was mixed with 3 mL of standard reagent (0.6 M sulphuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate) and reaction mixture was incubated at 95 °C for 90 min. Absorbance was measured at 695 nm. The sample was analysed in triplicate.

Diphenyl picrylhydrazyl radical scavenging assay

Evaluation of the scavenging activity against the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) was measured according to the method described in Sanchez-Moreno and Larrauri (1998) and Impei et al. (2015). A volume of 50 µL of various concentrations of the extracts was added to 1950 µL of DPPH methanol solution (0.025 g/L). After 30 min of incubation at room temperature, the absorbance was read, against a blank, at 515 nm. DPPH-free radical scavenging activity was expressed in percentage (%) and calculated using the following formula:

$$\%\,\text{DPPH scavenging activity} = \left(A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}\right) \times 100,$$

where A_{blank} is the absorbance of the control, A_{sample} is the absorbance of the test compound.

EC_{50} concentration was calculated based on the graph reporting both inhibition percentages and extract concentrations. Ascorbic acid methanol solution was used as positive control.

β-carotene bleaching assay

Antioxidant activity of alkaloid extracts was determined by measuring the inhibition of linoleic acid oxidation according to the Moure et al. (2000) method. β-carotene (2 mg) was dissolved in 10 mL of chloroform. One millilitre of β-carotene solution was then mixed with 20 mg of purified linoleic acid and 200 mg of Tween 40 emulsifier. Chloroform was evaporated. The resulting mixture was immediately diluted with 100 mL of distilled water. Four millilitres of this emulsion was enriched with 0.2 mL of different concentrations of alkaloid extracts. The absorbance at 470 nm was immediately measured (t_0), against a blank.

Samples were placed at 50 °C for 120 min and then measured again at 470 nm. For positive control, the sample was replaced with gallic acid or butylated hydroxyanisole (BHA). As negative control, 0.2 mL of distilled water or solvent was used. Antioxidant activity (AA) was measured in terms of successful bleaching of β-carotene by using the following equation:

$$\text{AA} = \left(A_{\text{A}(120)} - C_{\text{C}(120)}/\left(C_{\text{C}(0)} - C_{\text{C}(120)}\right)\right) \times 100,$$

where $A_{\text{A}(120)}$ is the absorbance for the extract presence at 120 min; $C_{\text{C}(120)}$ is the control absorbance at 120 min; $C_{\text{C}(0)}$ is the control absorbance at 0 min.

Antimicrobial screening

Microbial strains

Antimicrobial activity of alkaloids extracts was evaluated using eight different microorganisms. Four Gram-positive strains (*Staphylococcus aureus* ATCC 6538, *Listeria monocytogenes* ATCC 19111, *Bacillus subtilis* ATCC 6633 and *Micrococcus luteus* ATCC 9341) and three Gram-negative bacteria (*Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 8739 and *Klebsiella pneumoniae* IBMC Strasbourg) were employed.

Moreover, one species of fungus, *Candida albicans* CIP 444, was used as indicator microorganism to detect *A. articulata* antifungal activity. All bacterial strains were maintained on slants with nutrient agar (Sigma-Aldrich). *C. albicans* was grown in Sabouraud Dextrose Agar Oxoid.

Agar disc diffusion method

Inhibition zones were determined against selected microbial strains according to the disc diffusion method recommended by the Clinical Laboratory Standards Institute (CLSI) guidelines (CLSI 2012). Bacterial suspensions were prepared in Mueller Hinton Broth (MHB), while fungal suspension in Sabouraud Dextrose Broth (SDB). These two types of microorganisms were, then, incubated at 37 °C for 24 h and at 30 °C for 24–48 h, respectively. All tested microorganism suspensions were adjusted to the optical density of McFarland 0.5 (10^8 CFU/mL by MHB for bacteria and 10^6 CFU/mL for *Candida* by SDB).

Then, the suspension of each culture was spread on the solid media plates using a sterile cotton swab. Whatman No.1 sterile filter paper discs (6 mm diameter) were impregnated with 10 µL of each alkaloid extracts at different concentrations (80, 200 and 400 mg/mL). Standard antibiotic disks were used as positive controls: ampicillin (10 µg/disc; Biomaxima S.A.) and chloramphenicol (30 µg; Sigma-Aldrich-Química, S.A.) for bacteria, nystatin (100 µg; Sigma) and amphotericin B (100 µg; Sigma) for yeast.

Plates were maintained 1–2 h at 4 °C to allow alkaloid extracts diffusion. Then, Petri dishes were stored at 37 °C and incubated 24 h for bacteria and 24–48 h for fungus. After incubation, inhibition zone diameters were measured, including paper disk (in mm). Each assay was performed in triplicate and results were expressed as mean ± SD.

Determination of MIC, MBC or MFC concentration

MIC of alkaloid extracts was determined using the microbroth dilution method (NCCLS 2001), according to the Clinical and Laboratory Standards Institute (CLSI) protocols. All tests were performed in MHB (SDB for *C. albicans*) and cultures of each strain were prepared overnight.

Microorganism suspensions were adjusted by spectrophotometer to the final optical density of 10^6 CFU/mL. Then, two-fold dilutions were carried out in a 96-well microplate (100 µL per well) to obtain the dilutions of the alkaloid extracts with concentrations ranging from 0.195 to 100 mg/mL. Dimethylsulphoxide (DMSO), at a final concentration of 1% in each well, was used to dissolve the alkaloid extracts. An equal volume of the microbial inoculum of the tested strains from overnight broth culture was added to a final concentration of 5×10^5 CFU/mL in each well. After incubation for 18–24 h at 37 °C (24–48 h for *Candida*), in normal atmosphere, microorganism growth was identified by turbidity observation. MIC was defined as the lowest concentration of the alkaloid extract at which the microorganisms did not exhibit visible growth.

All tests were carried out in duplicate and MIC values were expressed in µg/mL. MBC (or MFC) value was measured by subcultivation of 10 µL of each well with no visible microbial growth on a Mueller Hinton Agar (MHA) (Sabouraud Dextrose Agar for *C. albicans*), followed by incubation at 37 °C for 24 h (24–48 h for *Candida*).

Separation of major alkaloids

In this study, the alkaloids present in the most bioactive extracts: E2.1 and E2.2 were separated. In the first step, analytical and preparative thin layer chromatography (TLC) was performed on silica gel plates (60 F 254 or aluminium plates coated with silica gel G 60) with toluene/acetone/ethanol/ammonia system (4:4:6:2) for E2.1 extract and dichloromethane/methanol system (9:1) for E2.2. extract.

In case of preparative TLC, samples were spotted in maximum quantity on TLC strip-shaped plates. After development, plates were visualised under ultraviolet light (UV) at 254 and 360 nm. Ten fractions (named C1, C2, C3, C4, C5, C6, C7, C8, and C9) were detected for E2.1 extract, while seven fractions (named B, B0, B1, B2, B3, B4, B5) were revealed after separation of E2.2 extract.

In the second step, fractioned spots were harvested and subjected to a further preparative TLC, using toluene/acetone/ethanol/ammonia system. All identified spots were recovered, dried out and subjected to GC/MS analysis for their chemical identification.

GC-MS analysis

Each sample powder was resuspended in 50 µL of acetonitrile and constantly vortexed for 15 min at room temperature. Then, 2 µL of sample was injected in a GC-MS instrument (QP2010 Shimadzu, Japan) and analysed.

Chromatographic separation was performed, in a DB-5 column (30 m × 0.25 mm × 0.25 µl; Agilent, USA), with GC oven settings as follows: 70 °C for 2 min, 200 °C (reached at 25 °C/min rate) for 5 min, 300 °C (reached at 3 °C/min rate) for 10 min.

Helium was used as carrier gas at a constant flow of 1.0 mL/min. Other instrument parameters were: ion source temperature at 230 °C, interface temperature at 250 °C, solvent cut time 2 min, acquisition scan modality starting from 100 to 1000 m/z. After the test, all the peaks detected in each sample chromatogram were analysed. In particular, molecules were identified by comparing their mass spectra with those of pure standards registered in NIST library software associated to the instrument. All samples were analysed in triplicate.

Statistical analysis

All experiments were carried out in triplicate and results were expressed as means ± SD. Microcal Origin 6 and Microsoft Excel 2003 were used for statistical and graphical evaluations. The structures of all identified molecules were drawn using ACDLabs Freeware 2016-ChemSketch software.

Results and discussion

Yields of alkaloid extracts

Three extraction methods were applied, using polar and apolar solvents, on 65 g of *A. articulata* stems, in order to obtain four different extracts. The results of Table 1 show that E2.2 extract revealed the highest yield (12.477%), followed by E3 (3.578%), while for E2.1 (1.077%) and E1 (0.377%) extracts the percentages recorded were lower.

Therefore, it is possible to conclude that the Stas-Otto method, using the methanol/water mixture, was the most adequate procedure to extract tetravalent alkaloids from the *A. articulata* stems. This type of alkaloids was the most abundant in the sample materials, justifying the high yield. Nevertheless, Benhammou et al. (2013) reported that the yield (5.95 ± 0.25%) of alkaloid extracts obtained by the Harborne (1998) method was higher than other bioactive compounds.

Antioxidant activity

Total antioxidant capacity

Total antioxidant capacity (TAC) was measured based on the reduction of Mo (VI) to Mo (V) by antioxidant compounds and formation of a green phosphate/Mo (V) complex, at acidic pH. According to the results of Table 1, all alkaloid extracts showed different antioxidant capacities. Tetravalent alkaloids (E2.2) evidenced the highest total antioxidant capacity (14.742 ± 0.224 mg AAE/g DM), followed by pure basic alkaloids (E3) (8.729 ± 0.464 mg AAE/g DM). Total and basic alkaloids had the lower TAC values. These results are in agreement with those of Benhammou et al. (2013), which revealed that the alkaloid extract of these species has a high TAC (2.86 mg AAE/g DM) in comparison with other metabolites.

DPPH test

Scavenging effect of different *A. articulata* alkaloid extracts on DPPH radical showed a dose-dependent activity, which could be probably associated to their chemical composition. EC_{50} value of each sample was determined to compare the antiradical activity of these extracts. As an indirect test, low EC_{50} values designated high antioxidant activity. Table 1 indicates that pure basic alkaloids (E3) exhibited a higher antiradical activity ($EC_{50} = 1.242 \pm 0.168$ mg/mL), followed by basic alkaloids (E2.1) ($EC_{50} = 1.380 \pm 0.037$ mg/mL). DPPH radical reduction capacity was low and equal to 5.350 ± 0.022 mg/mL for total alkaloid (E1) extract, while tetravalent alkaloid extract (E2.2) had no EC_{50} value under tested operating conditions. These EC_{50} values were higher than those of ascorbic acid ($EC_{50} = 0.090 \pm 0.002$ mg/mL) and BHA ($EC_{50} = 0.054 \pm 0.003$ mg/mL) and they were used as positive controls.

According to Benhammou et al. (2013), total alkaloids of *A. articulata* stems present an important activity in scavenging DPPH radical ($EC_{50} = 1.30 \pm 0.02$ mg/mL). Similarly, it was reported that methanolic extract of stems of this species had an important antiradical capacity against DPPH radical ($EC_{50} = 94.7$ µg/mL) and a strong anti-angiogenic effect by inhibiting the activity of nitric oxide (NO) formation, decreasing the proliferation of vascular endothelial cells and reducing expression of various proangiogenic factors (Abdulsahib et al. 2016). In addition, the ethanolic, ethyl acetate and chloroform extracts showed highest activity against DPPH test with IC_{50} inhibitory concentration of 0.14, 0.24 and 0.41 mg/mL, respectively (Mohammed et al. 2013).

β-carotene bleaching method

Table 1 presents the β-carotene bleaching activity of *A. articulata* alkaloid extracts. All alkaloid samples showed an inhibition effect on bleaching of β-carotene at different concentrations by free linoleic acid radical scavenging. E1 and E2.1 extracts showed a good antioxidant activity with values of 0.353 ± 0.175 and 0.372 ± 0.086 mg/mL, respectively,

Table 1 Yields (%), TAC, EC_{50} values of DPPH and β-carotene tests of *A. articulata* stem alkaloidal extracts, ascorbic acid, gallic acid and BHA

Alkaloids extracts	Yields (%)	TAC (mg AAE/g DM)	DPPH test EC_{50} (mg/mL)	β-carotene test EC_{50} (mg/mL)
Total alkaloids (E1)	0.377	0.684 ± 0.169	5.350 ± 0.022	0.353 ± 0.175
Basic alkaloids (E2.1)	1.077	1.03 ± 0.478	1.380 ± 0.037	0.372 ± 0.086
Tetravalent alkaloids (E2.2)	12.477	14.742 ± 0.224	—	2.313 ± 0.557
Pure basic alkaloids (E3)	3.578	8.729 ± 0.464	1.242 ± 0.168	0.943 ± 0.027
Ascorbic acid	—	—	0.090 ± 0.002	—
Gallic acid	—	—	—	3.220 ± 0.020
BHA	—	—	0.054 ± 0.003	0.028 ± 0.005

TAC total antioxidant capacity, *DPPH* 2,2-diphenyl-1-picrylhydrazyl, *BHA* butylated hydroxyanisole

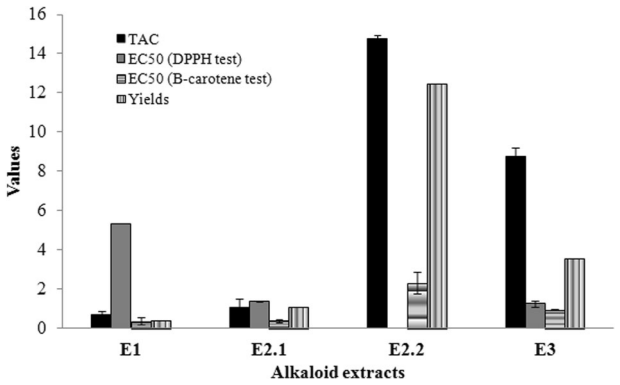

Fig. 2 Relationship between yields and antioxidant tests. E1: total alkaloids; E2.1: basic alkaloids; E2.2: tetravalent alkaloids; E3: pure basic alkaloids

while E3 measurement was 0.943 ± 0.027 mg/mL. However, E2.2 showed a high value of 2.313 ± 0.557 mg/mL.

These results were lower than gallic acid (EC_{50} = 3.220 ± 0.020 mg/mL), but higher than BHA (EC_{50} = 0.028 ± 0.005 mg/mL). In comparison with literature, total alkaloids reduced the extent of β-carotene destruction by reacting with linoleate free radical at an EC_{50} concentration equal to 1.67 ± 0.22 mg/mL (Benhammou et al. 2013).

Correlation between alkaloids classes and antioxidant activities

Figure 2 presents the relationship between yields and antioxidant tests (TAC, DPPH and β-carotene) of each extract. This relationship showed the efficacy of using different complementary techniques to measure the general antioxidant activity. In addition, it also demonstrated that the bioactivity of the extracts depended on composition and chemical structures of the alkaloids which they contain but not to their yields.

Tetravalent alkaloids (E2.2) extracts and pure basic alkaloids (E3) presented the highest yields and TAC values, while they showed the lowest DPPH and β-carotene results. On the other hand, the yields of the total (E1) and basic alkaloid (E2.1) extracts had an intermediate relationship with DPPH, β-carotene and TAC activity. These results indicated that the alkaloids might be considered responsible for the biological effect of each sample.

However, some alkaloids are more potent as radical scavengers than others, showing more reducing power (Koduru et al. 2007). These authors revealed the synergistic effect of alkaloids in crude extracts than in the isolated form.

It is important to mention that *A. aphylla* alkaloids, such as anabasine, luponin, apilupinin, anabasamine and methylanaphyllinate, have anticholinesterase activity, but the structural differences and the presence of reactive groups may play an important role in the manifestation of their biological effect (Tilyabaev and Abduvakhabov 1998). It was reported that polyhydroxyl alkaloids have a significant in vitro cytotoxic activity against some tumour cell lines (El-Desouky et al. 2007).

Some of them present a strong antioxidant activity against $^{\bullet}OH$, which is able to inhibit certain enzymes in tumour cells, inducing apoptosis and blocking growth. For example, pyrrole alkaloids revealed a great antioxidant activity (El-Desouky et al. 2007), compared to quinoline alkaloids that exhibited moderate antioxidative characteristics (Kaur and Arora 2015). In *A. articulata*, extracts showed the presence of tertiary and quaternary alkaloids with antimicrobial agents (Maatalah et al. 2012) and antidiabetic effects (Kambouche et al. 2009).

Antimicrobial effects of alkaloids extracts

Antimicrobial activity of *A. articulata* alkaloids extracts was qualitatively and quantitatively assessed by measuring the inhibition zone diameters (IZDs), as shown in Table 2 and MIC, MBC and MFC values, indicated in Table 3. The highest antimicrobial potential by disc diffusion method was observed for E3 extract. E1, E2.1 and E2.2 extracts did not present any antibacterial activity, except for *M. luteus*

Table 2 Antimicrobial activity of alkaloid extracts of *A. articulata* (mg/mL)

	E1	E2.1		E2.2			E3			Ampicillin	Chlamphenicol
	80	80	200	80	200	400	80	200	400		
K. pneumonia	0 ± 0[a]	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	ND	ND	ND	0 ± 0 (*R*)	0 ± 0 (*R*)
P. aeruginosa	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	9 ± 1.41	ND	ND	ND	0 ± 0 (*R*)	0 ± 0 (*R*)
E. coli	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	ND	ND	ND	10.50 ± 1.00 (*R*)	0 ± 0 (*R*)
L. monocytogens	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	16.5 ± 0.71	17 ± 1.41	20 ± 0	33.67 ± 3.51 (*S*)	0 ± 0 (*R*)
M. luteus	0 ± 0	13 ± 0	14 ± 0	0 ± 0	0 ± 0	0 ± 0	9 ± 4.24	13 ± 2.12	16 ± 0	28 ± 0 (*S*)	22 ± 0 (*I*)
S. aureus	0 ± 0	ND	ND	0 ± 0	0 ± 0	0 ± 0	ND	ND	ND	40.20 ± 0.45 (*S*)	10 ± 0 (*R*)
B. sublitis	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	11 ± 1.41	13.75 ± 1.06	14.25 ± 1.06	0 ± 0 (*R*)	0 ± 0 (*R*)
C. albicans	0 ± 0	0 ± 0	0 ± 0	0 ± 0	0 ± 0	8 ± 0	0 ± 0	0 ± 0	ND	30 ± 0[b](*S*)	26 ± 0[c] (*S*)

S: Micoorganism classified as **S**usceptible by CLSI criteria to the antimicrobial compound; ***I***: **I**ntermediate; ***R***: **R**esistant

ND not determined

[a]Expressed as the size of the inhibition zones (mm) as an average of triplicates ± SD, including diameter of paper disk (6 mm)

[b]Nystatin (100 µg/disc)

[c]Amphotericin B (100 µg/disc)

and *P. aeruginosa*. This effect was very remarkable against Gram-positive pathogens, notably *M. luteus* and *L. monocytogenes*, which presented the largest IZD at 400 mg/mL of E3 alkaloid extract (16 and 20 mm, respectively), followed by *B. subtilis* with the IZD of 14.25 mm. For *C. albicans*, E2.2 extract at 400 mg/mL produced the smallest inhibition zone (8 mm). Alkaloids generally affect more Gram-positive than Gram-negative bacteria. It is well known that Gram-negative bacteria have a complex barrier system that can regulate and sometimes prevent the passage of biocides through the cytoplasmic membrane into the cytoplasm (Denyer and Maillard 2002). It is also reported that Gram-positive bacteria should be more susceptible since they have only an outer peptidoglycan layer, which is not an effective barrier (Scherrer and Gerhardt 1971). Several results have confirmed these observations; thus, some alkaloids were found to be more active against Gram-positive bacteria than against the Gram-negative ones (Ajayeoba et al. 1995; Manosalva et al. 2016).

The results by Maatalah et al. (2012) proved that saponin extracts exhibit a higher degree of antimicrobial activity against all tested bacterial strains than the *A. articulata* alkaloid extract. The inhibitory activity of the alkaloid extracts was comparable to those of standard antibiotics (ampicillin and chloramphenicol) used as positive controls. Antibiogram results of the four Gram-negative bacteria strains showed that almost all of these microbes were resistant to pure antibiotics, while this study demonstrates the existence of a variability of antibiotic susceptibility on Gram-positive bacteria.

Table 3 indicates that MICs and MBCs (or MFCs) determined by micro-well dilution assay showed a broad-spectrum of antimicrobial activities against eight pathogenic microorganisms. As a result, MIC values ranged from 0.781 to 25 mg/mL for bacteria and from 10 to >100 mg/mL for fungus, while MBC values ranged from 1.562 to >20 mg/mL for bacteria and from 10 to >100 mg/mL for fungus.

Table 3 Minimal inhibitory (MIC) and minimal bactericide (or fungicidal) concentration (MBC or MFC) (mg/mL) of alkaloids extracts of *A. articulata* against various microbes

Microbial strains	MIC (mg/mL)			MCB (mg/mL)		
	E1	E2.2	E3	E1	E2.2	E3
S. aureus	10	20	0.781	10	>20	1.562
B. subtilis	10	>20	1.562	10	>20	3.125
M. luteus	5	>20	1.562	5	>20	3.125
L. monocytogenes	ND	ND	ND	ND	ND	ND
E. coli	10	>20	3.125	10	>20	3.125
K. pneumoniae	10	>20	1.562	10	>20	3.125
P. aeruginosa	5	>20	25	10	>20	25
C. albicans	10	20	>100	10[a]	>20	>100

ND not determined

[a]For *C. albicans* minimal fungicidal concentration (MFC) (mg/mL)

Finally, the highest MBC values (25 mg/mL) were found in E3 alkaloid extract against *P. aeruginosa*. This extract exhibited the strongest inhibitory effects against all tested bacteria and the lowest MIC and MBC values, although they showed a weak activity against *C. albicans* (MIC and MBC >100 mg/mL). Among all the extracts, E3 sample efficiency could be associated with its content in *A. articulata* pure basic alkaloids extracted by the Stas-Otto method with apolar solvents.

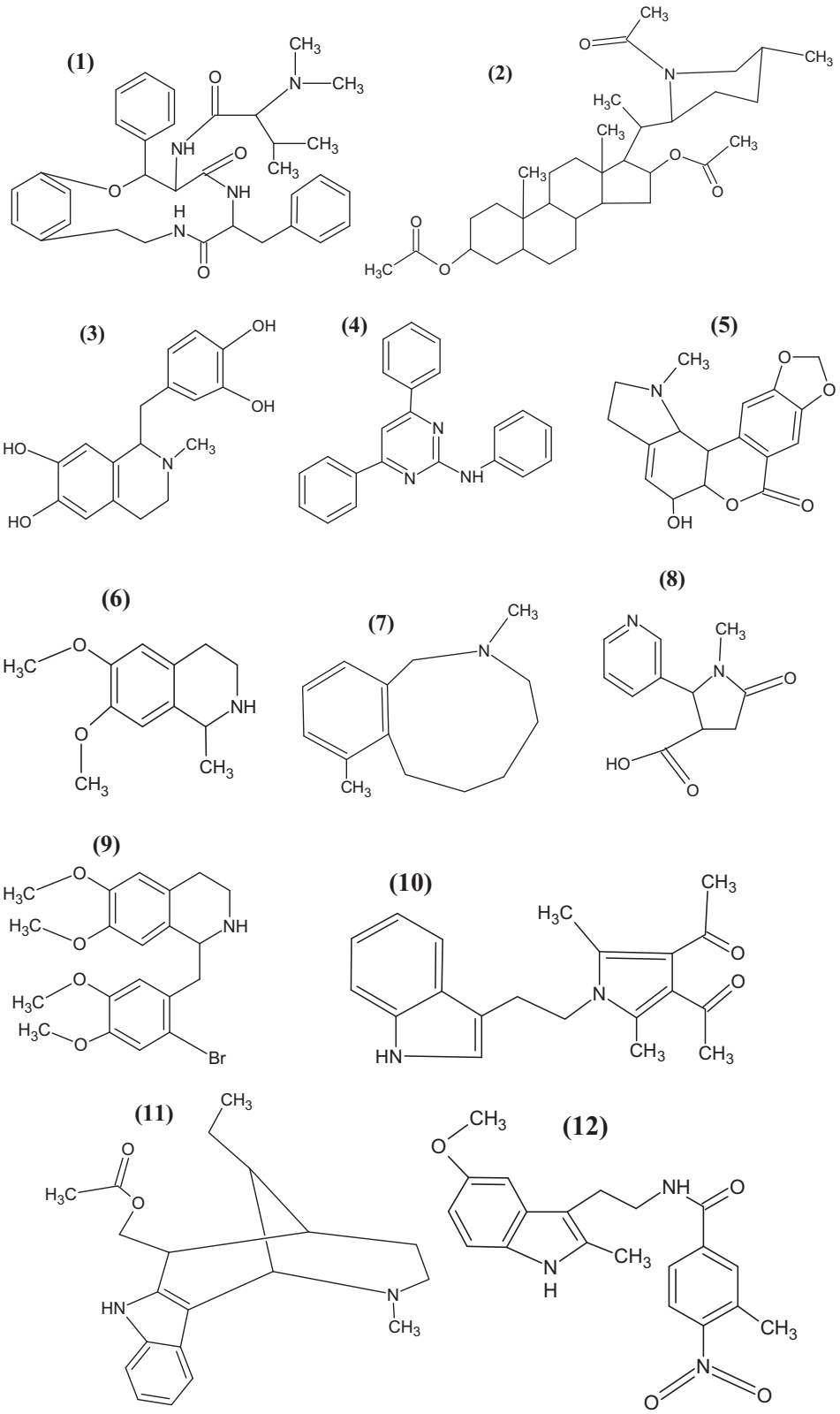

Fig. 3 Structures of alkaloids identified in E2.1 and E2.2 alkaloid extract of *A. articulata* stems. C1, C4, C6, C7, C8 and C9 were identified in the E2.1 extract; B, B0, B1, B3, B4 and B5 were identified in the E2.2 extract

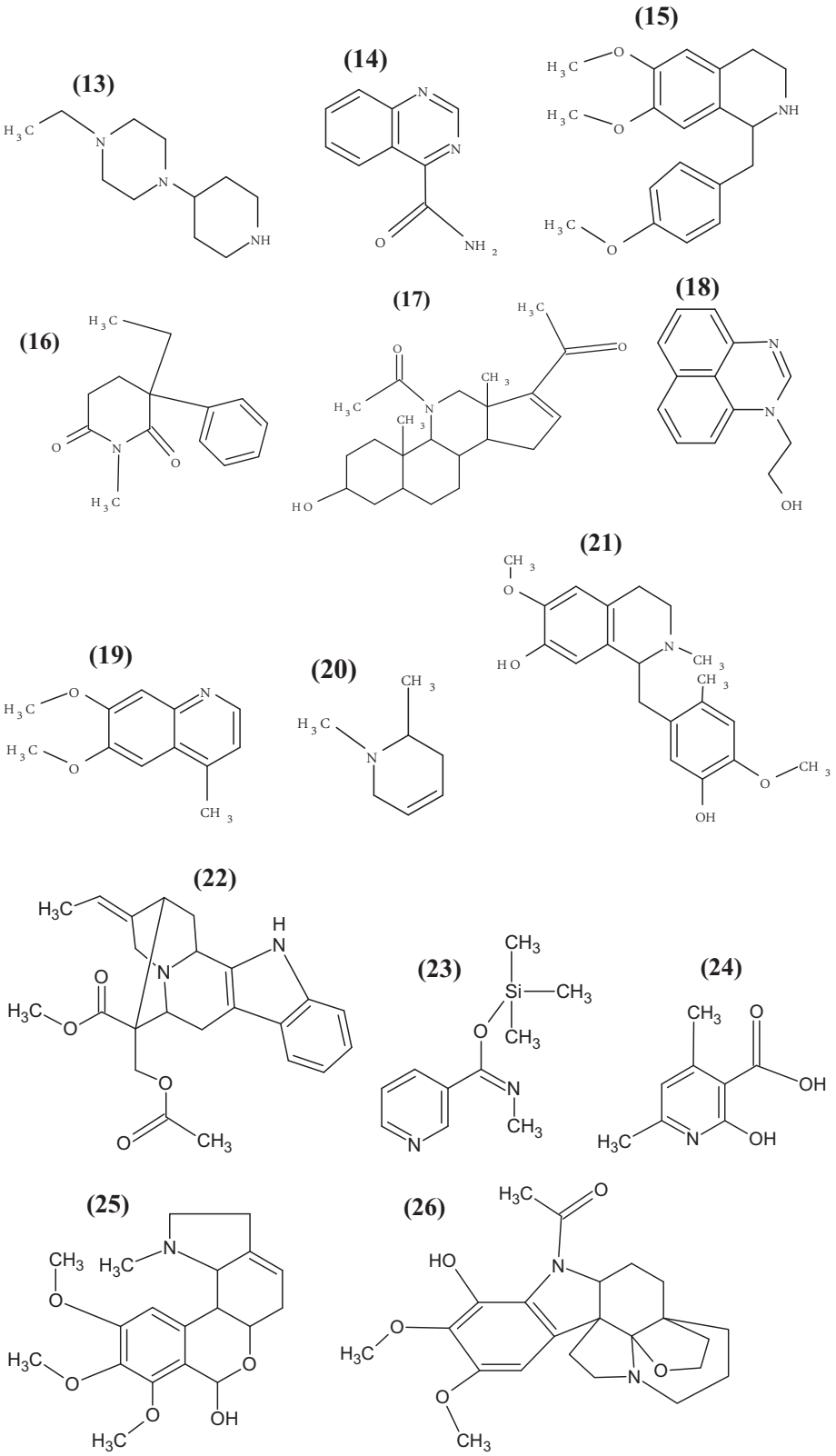

Fig. 3 (Continued)

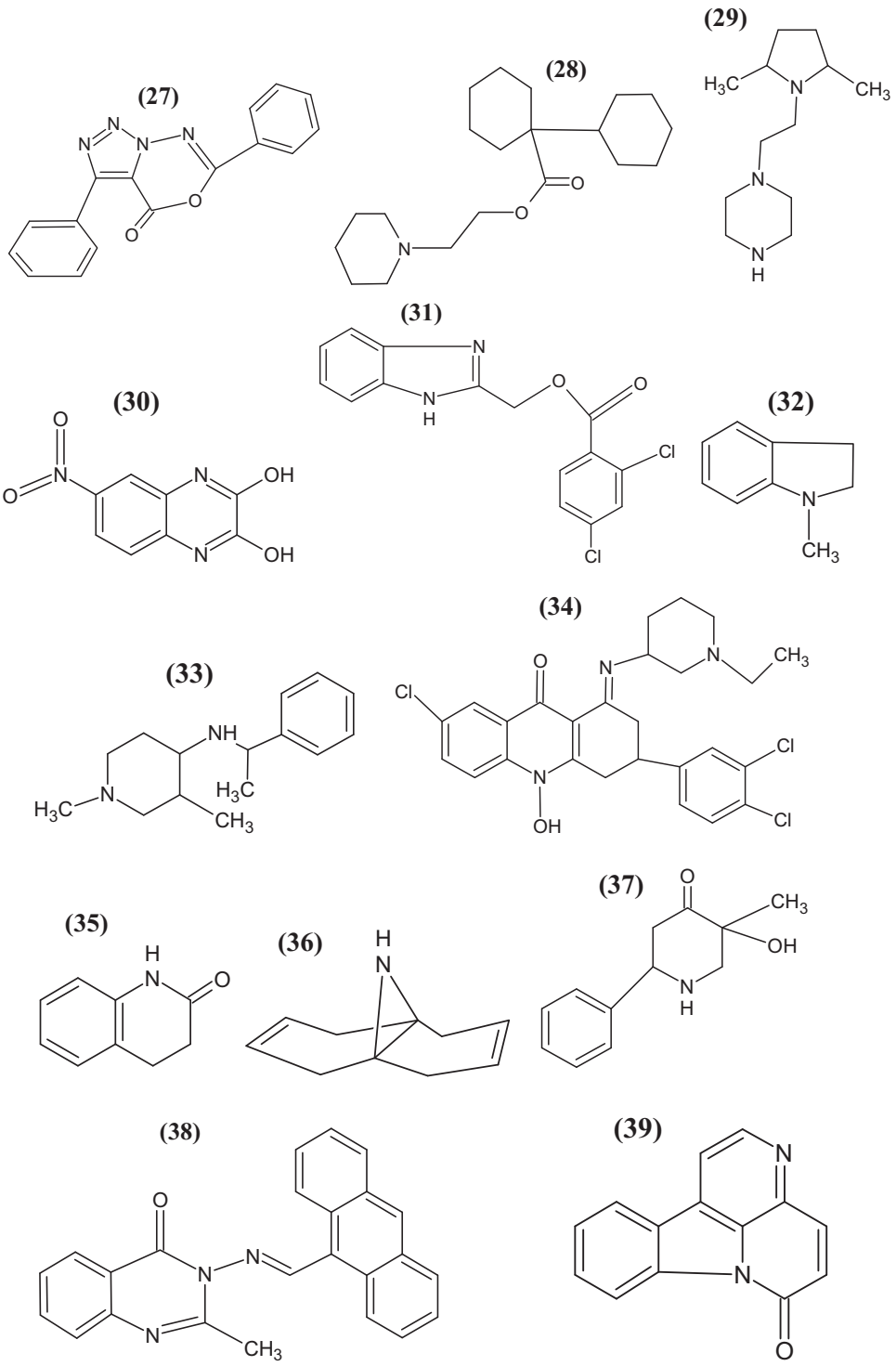

Fig. 3 (Continued)

GC/MS analysis

GC-MS analysis aimed to characterise the chemical compounds present in our extracts. The results obtained are shown in Fig. 3 and Table 4. Therefore, 49 compounds were recognised and documented for the first time in literature in *A. articulata*. Indeed, only few species of *Anabasis* genus, in particular *A. aphylla*, were chemically investigated. In addition to pyridine and piperidine alkaloids like anabasine (neonicotine = 2-(3-pyridyl)-piperidine)),

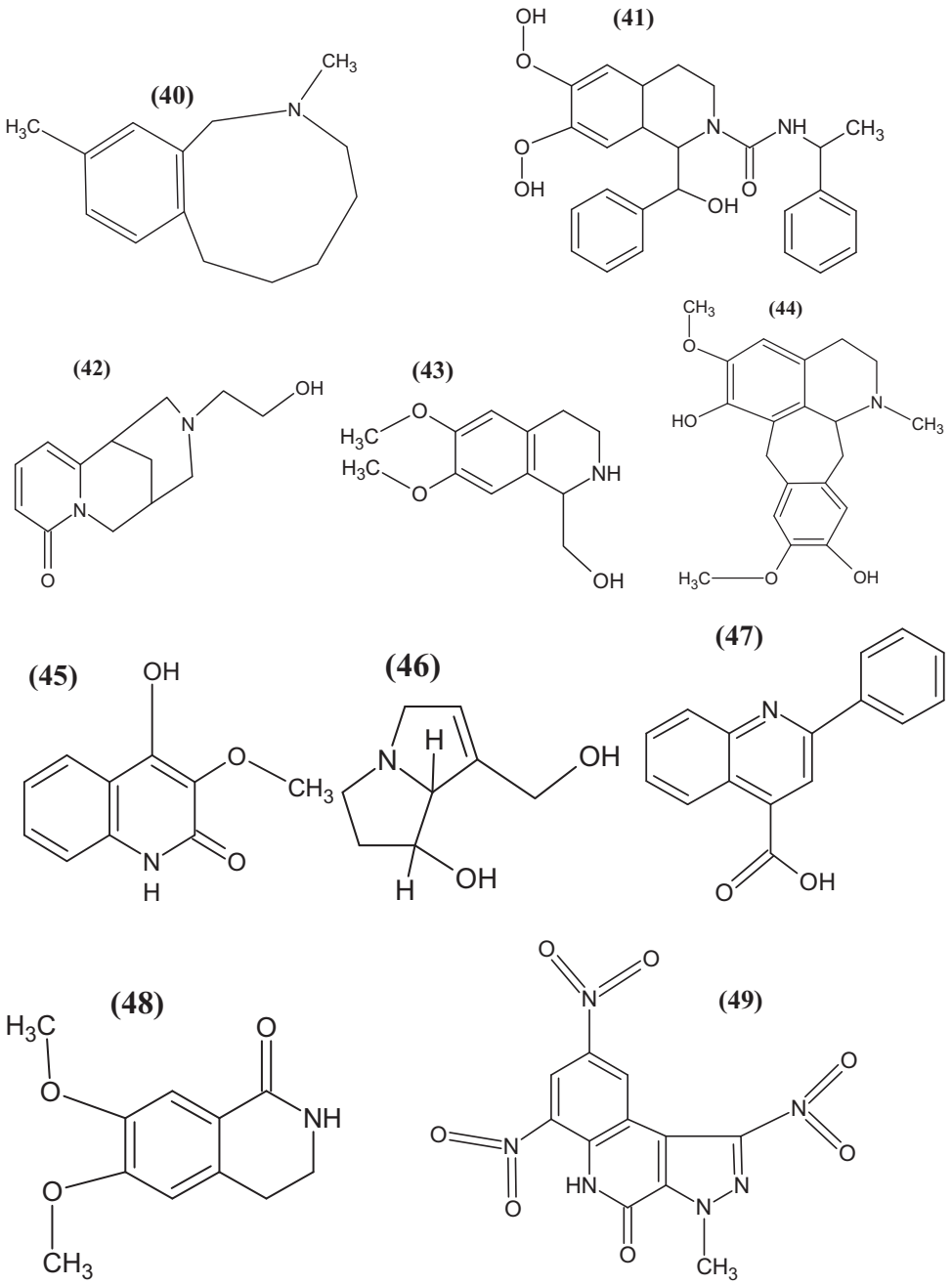

Fig. 3 (Continued)

anabasamine and isonicoteine, several quinolizidine alkaloids were identified as aphylline, aphylline N-oxide, aphyllidine, oxaphylline and lupinine (Sadykov and Tumur 1960; Zakharov et al. 1974), and a new pyridine alkaloid, 2-(pyridin-3-yl)-6-(2-(pyridin-3-yl) pyridin-5-yl)piperidine, was also reported (Du et al. 2008).

This study presents a GC-MS procedure which was applied to identify alkaloids in *A. articulata* stems (Algerian origin). According to the literature, 49 compounds divided into 16 classes are new for this species, cyclopeptide alkaloids (Integerressine (**1**)), steroid alkaloids (Tetrahydro-solasodine triacetate (**2**); 11-Azapregn-16-en-20-one, 11-acetyl-3-hydroxy-, (3.beta.)- (**17**)), quinoline alkaloids (6,7-dimethoxy-4-methylquinoline (**19**); 3,4-dihydroquinolin-2(1*H*)-one (**35**); Quinolin-2(1*H*)-one, 4-hydroxy-3-methoxy- (**45**); Cinchophen (**47**); 3-methyl-1,6,8-trinitro-3,5-dihydro-4*H*-pyrazolo[3,4-c] quinolin-4-one (**49**); 6-Nitro-2,3-quinoxalinediol (**30**)), camptothecin alkaloids (3,11-Diazatricyclo[7.3.1.0(3,8)]trideca-5,7-dien-4-one, 11-(2-hydroxyethyl)- (**42**)), quinazoline alkaloids (Quinazoline-4-carboxamide (**14**); Quinazolin-4(3*H*)-one, 2-

Table 4 Alkaloids of *A. articulata* stems characterised through GC-MS with their retention time (min)

Fractions	Sub-fractions	Name and number of molecules	Retention time (min)
E2.1	C1	Integerressine (**1**)	3.86
		Tetrahydro-solasodine triacetate (**2**)	5.33
		l-Laudanosoline hydrobromide (**3**)	18.9
		2-Phenylamino-4,6-diphenylpyrimidine (=*N*,4,6-Triphenyl-2-pyrimidinamine) (**4**)	31.6
	C4	Hippeastrine (=Trispherine) (**5**)	6.14
	C6	(+)-Salsolidine (**6**)	9.75
		2,8-Dimethyl-2,3,4,5,6,7-hexahydro-1*H*-2-benzazonine (**7**)	11.48
		Cotinine,4-carboxy (**8**)	15.36
		Papaveroline, 2′-bromo-, tetramethyl ether) (**9**)	16.23
		(4-Acetyl-1-[2-(1*H*-indol-3-yl)-ethyl]-2,5-dimethyl-1*H*-pyrrol-3-yl)-ethanone (**10**)	31.87
		Dasycarpidan-1-methanol, acetate (ester) (**11**)	25.72
	C7	Benzamide, *N*-[2-(5-methoxy-2-methyl-1*H*-indol-3-yl)ethyl]-3-methyl-4-nitro- (**12**)	40.026
		Piperazine, 1-ethyl-4-(4-piperidyl)- (=1-Ethyl-4-(4-piperidinyl)piperazine) (**13**)	30.803
		Quinazoline-4-carbonamide (=4-Quinazolinecarboxamide) (**14**)	28.46
		Norrecticuline, 7-*O*-methyl-3′-desoxy- (=6,7-Dimethoxy-1-(4-methoxybenzyl)-1,2,3,4-tetrahydroisoquinoline) (**15**)	16.56
		2,6-Piperidinedione, 3-ethyl-1-methyl-3-phenyl- (=Glutethimide + tmah = Gluthethimide methyl derivative = 3-Ethyl-1-methyl-3-phenyl-2,6-piperidinedione) (**16**)	11.35
		11-Azapregn-16-en-20-one, 11-acetyl-3-hydroxy-, (3.beta.)- (**17**)	26.45
		1H-Perimidine-1-ethanol (=2-(1*H*-Perimidin-1-yl)ethanol) (**18**)	17.97
		Quinoline, 6,7-dimethoxy-4-methyl- (=6,7-dimethoxy-4-methylquinoline) (**19**)	10.7
	C8	Pyridine, 1,2,3,6-tetrahydro-1,2-dimethyl- (=1,6-Dimethyl-3-piperideine = 1,2-Dimethyl-1,2,3,6-tetrahydropyridine) (**20**)	20.1
	C9	Reticuline, 6′-methyl (=1-(5-Hydroxy-4-methoxy-2-methylbenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydro-7-isoquinolinol) (**21**)	9.55
		Sarpagan-16-carboxylic acid, 17-(acetyloxy)-, methyl ester, (16R)- (=Acetylakuammidine) (**22**)	22
E2.2	B	*N*-Methyl nicotinimidate, *O*-trimethylsilyl (**23**)	39.09
		Nicotinic acid, 1,2-dihydro-4,6-dimethyl-2-oxo- (**24**)	29.46
		Nerinine (**25**)	7.07
		Aspidospermidin-17-ol, 1-acetyl-19,21-epoxy-15,16-dimethoxy- (**26**), 6-Diphenyl-4*H*-(1,2,3)-triazolo(1,5-d)(1,3,4)oxadiazin-4-one (=3,6-Diphenyl-4H-(1,2,3)triazolo(1,5-d)(1,3,4)oxadiazin-4-one) (**27**)	11.15 23.98
		Dihexyverine (**28**)	24.38
		Piperazine, 1-[2-(2,5-dimethyl-1*H*-pyrrol-1-yl)ethyl]- (**29**)	32.0
		6-Nitro-2,3-quinoxalinediol (**30**)	33.2
		Benzoic acid, 2,4-dichloro-, (benzimidazol-2-yl)methyl ester (**31**)	20.84
		1*H*-Indole, 2,3-dihydro-1-methyl- (=1-Methylindoline) (**32**)	22.11
	B0	1,3-Dimethyl-4-(1-phenylethylamino)piperidine **(33)**	24.5
		7-Chloro-3-[3,4-dichlorophenyl]-1-[[1-ethyl-3-piperidinyl]imino]-10-hydroxy-1,3,4,10-tetrahydro-9(2*H*)-acridinone (=(1*E*)-7-Chloro-3-(3,4-dichlorophenyl)-1-[(1-ethyl-3-pi) (**34**)	12.42
		2(1*H*)-Quinolinone, 3,4-dihydro- (=3,4-Dihydroquinolin-2(1*H*)-one) (**35**)	13.22
		Naphthalen-4a,8a-imine, 1,4,5,8-tetrahydro- (**36**)	17.2
		E-Hydroxy-3-methyl-6-phenyl-4-piperidone (=3*E*-Hydroxy-3-methyl-6-phenyl-4-piperidone) (**37**)	10.15
		Quinazolin-4(3*H*)-one, 2-methyl-3-(9-anthracenylmethylenamino)- (**38**)	11.087
		6H-Indolo[3,2,1-de][1,5]naphthyridin-6-one (=Canthin-6-one) (**39**)	15.36
	B1	2,9-Dimethyl-2,3,4,5,6,7-hexahydro-1*H*-2-benzazonine (**40**)	10.82 16.16

Table 4 (continued)

Fractions	Sub-fractions	Name and number of molecules	Retention time (min)
		-)-1,2,3,4-Tetrahydroisoquinoline, 2-[(1-phenethyl)aminocarbonyl]-1- (=1-[Hydroxy(phenyl) methyl]-6,7-dimethoxy-*N*-(1-phenylethyl)-3,4-dihydro-2(1*H*)-isoquinolinecarboxamide) (**41**)	
	B3	3,11-Diazatricyclo[7.3.1.0(3,8)]trideca-5,7-dien-4-one, 11-(2-hydroxyethyl)- (**42**)	11.087
		(+)-Calycotomine (**43**)	9.75
		Benzocycloheptano[2,3,4-I,j]isoquinoline, 4,5,6,6a-tetrahydro-1,9-dihydroxy-2,10-dimethoxy-5-methyl- (**44**)	15.56
	B4	Quinolin-2(1*H*)-one, 4-hydroxy-3-methoxy- (**45**)	7.67
		Heliotridine 1*H*-Pyrrolizine-7-methanol, 2,3,5,7a-tetrahydro-1-hydroxy-, (1*S*-cis)- (**46**)	19.56
		Cinchophen (**47**)	26.32
	B5	Corydaldine (=6,7-Dimethoxy-3,4-dihydro-1(2*H*)-isoquinolinone) (**48**)	15.76
		3-Methyl-1,6,8-trinitro-3*H*-pyrazolo[3,4-c]quinolin-4(5*H*)-one (=3-Methyl-1,6,8-trinitro-3,5-dihydro-4*H*-pyrazolo[3,4-c]quinolin-4-one) (**49**)	16.16

methyl-3-(9-anthracenylmethylenamino)- (**38**)), isoquinoline alkaloids (R/S Laudanosoline (**3**); Salsolidine (**6**), Papaveroline, 2′-bromo-, tetramethyl(ether) (**9**); Norrecticuline,7-*O*-methyl-3′-desoxy-(**15**); Reticuline, 6′-methyl (**21**); 1-[Hydroxy(phenyl)methyl]-6,7-dimethoxy-*N*-(1-phenylethyl)-3,4-dihydro-2(1*H*)-isoquinolinecarboxamide (**41**); (+)-Calycotomine (**43**); Benzocycloheptano[2,3,4-I,j]isoquinoline, 4,5,6,6a-tetrahydro-1,9-dihydroxy-2,10-dimethoxy-5-methyl- (**44**); 1Naphthalen-4a,8a-imine, 1,4,5,8-tetrahydro- (**36**)), isoquinolone alkaloids (Corydaldine (**48**)), indole alkaloids (Hippeastrine (**5**); 1-Methylindoline (**32**), (4-Acetyl-1-[2-(1*H*-indol-3-yl)-ethyl]-2,5-dimethyl-1*H*-pyrrol-3-yl)-ethanone (**10**); Benzamide, *N*-[2-(5-methoxy-2-methyl-1*H*-indol-3-yl)ethyl]-3-methyl-4-nitro- (**12**); Canthin-6-one (**39**), terpene indole alkaloids (Acetylakuammidine (**22**); Dasycarpidan-1-methanol, acetate (**11**); Aspidospermidin-17-ol, 1-Acetyl-19,21-epoxy-15,16-dimethoxy-) (**26**), pyridine/Pyrrolidine alkaloids (2-phenylamino-4,6-diphenylpyrimidine (**4**); Cotinine,4-carboxy (**8**); Piperazine, 1-[2-(2,5-dimethyl-1*H*-pyrrol-1-yl)ethyl]- (**29**); *N*-Methyl nicotinimidate, *O*-trimethylsilyl (**23**); Nicotinic acid, 1,2-dihydro-4,6-dimethyl-2-oxo-) (**24**), piperidine alkaloids (2,6-Piperidinedione, 3-ethyl-1-methyl-3-phenyl- (**16**); Piperazine, 1-ethyl-4-(4-piperidyl)- (**13**); Dihexyverine (**28**); 1,3-Dimethyl-4-(1-phenylethylamino)piperidine (**33**); 3E-hydroxy-3-methyl-6-phenyl-4-piperidone (**37**); 1,6-dimethyl-3-piperideine (**20**)), pyrrolizidine alkaloids (Heliotridine 1*H*-Pyrrolizine-7-methanol, 2,3,5,7a-tetrahydro-1-hydroxy-, (1S-cis)- (**46**)), purine alkaloids (3,6-Diphenyl-4*H*-[1,2,3]triazolo[1,5-d][1,3,4]oxadiazin-4-one) (**27**), acridone alkaloids (7-Chloro-3-[3,4-dichlorophenyl]-1-[[1-ethyl-3-piperidinyl] imino]-10-hydroxy-1,3,4,10-tetrahydro-9(2*H*)-acridinone (**34**)), benzazonine alkaloids (2,8-Dimethyl-2,3,4,5,6,7-hexahydro-1*H*-2-benzazonine (**7**); 2,9-Dimethyl-2,3,4,5,6,7-hexahydro-1*H*-2-benzazonine (**40**)), homolycorine-type Amaryllidaceae alkaloids (Nerinine (**25**)).

Conclusion

Based on the results described in this study, it can be concluded that *A. articulata* alkaloids, purified by polar or apolar extraction procedure, possess antimicrobial and antioxidant activities, due to their chemical structures. A GC/MS analysis was carried out on the alkaloid extracts obtained to identify their composition in order to improve knowledge of this plant species.

Therefore, 49 alkaloid structures were characterised and documented for the first time in literature on *A. articulata*. Moreover, the detection of all these different alkaloids in this study's extracts supported and explained the use of this plant in traditional medicine. However, based on the promising findings presented in this paper, additional studies can be suggested to evaluate other biological properties in vivo of all identified groups of alkaloids, which were isolated during tests, to better understand the scientific mechanism of each of them.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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