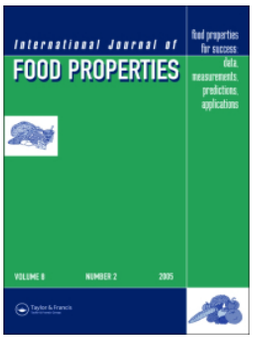

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Phytochemical composition and biological activities of extracts from *Astragalus membranaceus* arieal parts

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ABSTRACT

The application of biomolecules extracted from plants in food and pharmaceutical industry is of great interest. In the light of that fact, our interest has been aroused to study the efficiency of chlorophormic and ethyl acetat solvents for the extraction of the main molecules from *Astragalus membranaceus* aerial parts (AMA) in order to evaluate the antioxidant, inhibition of α-amylase and Angiotensin-Converting Enzyme (ACE), antibacterial, cytotoxic activies and in vitro DNA-protective capacity of these extracts. Gas chromatography-mass spectrometry (GC-MS) analysis showed the presence of methyl petroselinate acid as the main compound (45, 87%) in the ethyl acetate extract (EA-AMA) and the main compound in the chloroform extract (C-AMA) was linolenic acid (16.95%). The ethyl acetate extract exhibited strong antioxidant activity with interesting IC_{50} values not exceeding 40 µg/mL in almost all activities. The highest in vitro activity against α-amylase and ACE activity was obtained from chloroform extract with an IC_{50} of 20.36 and 20.77 µg/ml, respectively. The resazurin microtiter assay was carried out to analyze the extract potential against multi drug resistant strains of clinical bacteria origin of which the extracts had powerful antibacterial activity with minimum inhibitory concentration against *Pseudomonas aeruginosa* (0.039 ± 0 mg/ml) and *Escherichia coli* (0.039 ± 0 mg/ml). The extracts had a strong protective effect of Fenton-induced DNA damage. The XTT test revealed that the extracts had weaker cytotoxicity profile against EMT6 cell line. These findings suggest that this plant has a wide range of potential applications as a food supplement, a preventative and medicinal agent.

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Introduction

The use of plants as a source of treatment for various illnesses around the world dates back to the beginning of humanity.[1] The study of phytochemical profiles and medicinal plants biological activities is a key step to identify bioactive molecules that will be used in the pharmaceutical industry as effective substances to fight Multi-drug Resistant Pathogenic Bacteria[2] and to also develop molecules inhibit the action of the α-amylase enzyme are considered a therapeutic approach for carbohydrate absorption problems. These include conditions such as diabetes, obesity, dental caries and periodontal diseases.[3] In addition, plants may contain molecules that have an inhibitory power of ACE activity. These molecules are considered as among the main therapeutic agents used to treat hypertension and other cardiovascular diseases.[4]

Astragalus L. is a genus of flowering plants belonging to the Fabaceae family. The genus contains approximately 2500 species and *Astragalus L* plants are often found in temperate and arid regions.[5] Traditional medicine has used the plant's roots of *Astragalus membranaceus* for ages to cure a variety of illnesses as in the treatment of wounds, diabetes, leukemia, hypertension, eye disorders, nephritis, cirrhosis, uterine cancer, and immune system deterioration.[6] In terms of chemical composition, the roots of the *Astraglus* species are very rich in flavonoids, saponins and polysaccharide molecules. Currently, around 200 molecules have been purified with pharmacological and antioxidant effects such as isoflavones: calycosin (CAL), calycosin-7-glucoside (CG), formononetin (FMN) and ononin (ON).[7]

Moreover, three protein fractions purified from *A. membranaceus* waste material (AMWPDG2, AMWPDG4 and AMWPDG6) have an immunomodulatory activity potential;[8] thus, the new polysaccharide (AERP) was isolated from the residue of *A. membranaceus* was helpful in treating metabolic disturbance-related cognitive impairment by addressing the gut processes.[9]

However, the aerial parts (i.e., the stems and leaves) of *A. membranaceus* are discarded after harvest, despite various biological effects, including immune stimulation and regulation. The aerial parts comprised valuable chemical components, such as triterpene saponins, isoflavonoids, and polysaccharides.[10] The isoflavans isolated from the aerial parts of *Astragalus membranaceus* were able to inhibit tyrosinase[11] and the safety of the crude polysaccharide fraction from aerial parts was tested through a 90-day oral toxicity experiment on rats.[12] According to literature data, only a few studies have been conducted to explore the therapeutic properties of the aerial parts of *A. membranaceus*.

The purpose of our study was to assess the medicinal potentials of extracts from the aerial portions of *Astragalus membranaceus* (AMA) grown in eastern Algeria. Therefore, the present work has been carried out to investigate the ethyl acetate extract of *A. membranaceus* aerial parts (EA-AMA) and the chloroform extract of *A. membranaceus* aerial parts (C-AMA) for their TPC, TFC content, GC – MS profile, antioxidant, antibacterial, cytotoxic, DNA-protective, and alpha amylase and ACE inhibitory activities.

Materials and methods

Sampling and preparation of plant extracts

During the flowering season, the aerial parts of *A. membranaceus* were collected from the Guelma region in the northeast of Algeria, the *A. membranaceus* samples was identified at the Laboratory of Sciences and Technology, University of Mohamed Cherif Messaadia, Souk-Ahras, Algeria and a voucher specimen (MP-AM-0001), was deposited in the Laboratory of Applied Sciences, Souk-Ahras, Algeria. In addition, the plant was verified in accordance with the phenotypic characteristics reported by Fu.[6] The aerial parts of *A. membranaceus* samples were carried out after obtaining permission from local suppliers. The authors confirm that this study complies with relevant legislation and international, national, and institutional guidelines.

The plant's aerial portions (40 g) were separated into two equal-weight sections (20 g each), and each portion was macerated with 200 mL of solvents (Ethyl acetate or Chloroform). Three times, the

extraction was carried out with fresh solvent, and the contents were passed through Whatman filter paper number 1. Using a rotary evaporator, the filtrate was evaporated at 40 °C and stored at 4 °C until it was used.

Qualitative phytochemical analysis

A screening for steroids/terpenoids, flavonoids, saponins, phenols and Fehling test was performed for the extracts according to Rao.[13] The tests based on color intensity or precipitate formation are considered as analytical responses to these tests.

Quantitative phytochemical analysis

Determination of total phenolic content (TPC)

The determination of total phenol content in the extracts was determined using Folin – Ciocalteu reagent according to the method described by.[14,15] As follows: 20 µL of the sample was mixed with 100 µL of Folin – Ciocalteu reagent (10%), and 75 µL of aqueous sodium carbonate solution (7.5%, w/v); then, the mixture was incubated at room temperature and in the dark for 2 h. The absorbance was measured at 765 nm, and the outcomes data were expressed as µg of gallic acid equivalent per mg of dry extract.

Determination of total flavonoid content (TFC)

The determination of total flavonoid content in the extracts was established according to the Topçu.[16] A total of 50 µL of the sample was mixed 130 µL (MeOH). This was followed by the addition of 10 µL of 1 M potassium acetate and 10 µL of 10% aluminum nitrate. The mixture was incubated at room temperature for 40 min. The absorbance of the sample was measured at 415 nm using quercetin as a positive control. The outcomes data were expressed as µg of quercetin equivalent per mg of dry extract.

GC-MS analysis

Agilent GC-MS was utilized, with an HP-5 MS column, and helium gas at a flow rate of 0.5 mL/min. The MS parameters were as follows: temperature, 280 °C; ionization current, 2 A; resolution, 1000 scan time, 5 s; electron energy, 70 eV. The temperature program that was used was as follows: the oven temperature was set 40 °C (1 min), 10 °C/min up to 260 °C (15 min). One microliter of the prepared 1% of the extracts diluted with respective solvents was injected. Relative quantity of the chemical compounds present in each of the extracts of *A. membranaceus* was expressed as percentage based on peak area produced in the chromatogram. Mass spectra were compared with the spectral libraries from Wiley and NIST to identify compounds.

Antioxidant activity

In this study, the antioxidant capacity of the extracts was carried out using seven spectrophotometric techniques which are DPPH $^{\bullet}$, reducing power, phenanthroline, $ABTS^{\bullet+}$, CUPRAC, β carotene/linoleic Acid bleaching and superoxide alkaline DMSO test. The results were compared to the following standards: BHA, BHT, ascorbic acid, quercetin, and α-tocopherol.

DPPH radical scavenging activity

The DPPH scavenging ability was produced according to the Blois,[17] and the results were presented as IC_{50} which were compared to the following standards: BHA, ascorbic acid, quercetin, α-tocopherol, and BHT.

ABTS assay radical scavenging activity

The ABTS scavenging capacity was performed according to Re,[18] and the results were presented as IC_{50} which were compared to the following standards: BHA, ascorbic acid, quercetin, α-tocopherol, and BHT.

Phenanthroline assay

The activity was measured in accordance with Szydlowska Czerniaka.[19] The results were expressed as $A_{0.5}$ values and were compared to BHA, ascorbic acid, quercetin, α-tocopherol, and BHT as standards.

Ferric reducing antioxidant power assay

Plant extracts' antioxidant capacity to reduce ferric iron was measured using a procedure described by Benzie.[20] The outcomes were provided as $A_{0.5}$ values, and they were contrasted with reference values of BHA, ascorbic acid, quercetin, α-tocopherol, and BH.

Cupric reducing antioxidant capacity assay

As proceeded by Apak,[21] the cupric reducing antioxidant capacity (CUPRAC) of the extracts was assessed, and the results were given as $A_{0.5}$ value. BHA, ascorbic acid, quercetin, α-tocopherol, and BHT were used as standards for the comparison of the obtained outcomes.

Superoxide alkaline DMSO test

The superoxide radical test was performed as developed by Kunchandy.[22] The findings were provided as IC_{50} values. BHA, ascorbic acid, quercetin, α-tocopherol, and BH were used as standards.

β-Carotene/Linoleic acid bleaching test

It was carried out according to the method of Marco[23] with few modifications, and the results were given as IC_{50} values. As standards, we used BH, α-tocopherol, quercetin, BHA, and ascorbic acid.

Inhibitory α-amylase activity

The potential of the extracts to inhibit the α-amylase enzyme was established using the iodine/potassium iodide method, with minor changes according to the method of Zengin,[24] and the results were given IC_{50} values. Acarbose was used as a standard for the comparison of the obtained results.

Inhibitory angiotensin converting enzyme activity

ACE inhibitory activity was assayed according to the method described by Martínez-Alvarez.[25] This method was based on the liberation of hippuric acid (HA) from hippuryl L-histidyl L-leucine (Hip-His -Leu, HHL) catalyzed by the ACE. The hippuric Acid (HA) released was quantified by HPLC on an analytical C18 column (Agilent1260 Infinity II Prime LC System), and the results were given IC50 values. Captopril was used as reference compound.

Antibacterial activity

The antibacterial activity of the EA-AMA and C-AMA extract was assessed against 11 multi-drug-resistant bacterial strains, namely: *Enterococcus faecium*, *Staphylococcus aureus*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Morganella morganii*, *Escherichia coli*, *Serratia marcescens*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae*, and *Providencia rettgeri*. These strains were identified using MALDI-TOF MS (Bruker, USA) and belong to the bacterial collection of the bacteriology laboratory at Fares Yahia Hospital in Kolea, Algeria. The strains stored at −20°C were cultured in brain-heart infusion broth (BHIB, Sigma Aldrich, USA) at 37°C for 24 h, and bacterial suspensions were standardized to a cell density of 1.5 × 108 CFU/mL (McFarland no. 0,5).

Minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of extracts was determined using the 96-well microplate dilution method developed by Rios.[26] The EA-AMA and the C-AMA extract were reconstituted in 0.1% DMSO (Sigma Aldrich, USA) at a concentration of 10 mg/ml. Serial dilutions of 80 µl of plant extracts (10, 5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, and 0.039 mg/ml) and the antibiotic amikacin (5, 2.5, 1.25, 0.625, 0.312, 0.156, 0.078, and 0.039 µg/ml) as a positive control were prepared in Müller-Hinton broth (Sigma Aldrich, USA). The wells were then inoculated with 40 µl of bacterial suspensions at a cell concentration of 1.5×108 CFU/mL. DMSO was used as a negative control. Subsequently, the plates were incubated at 37°C for 24 h. After incubation, 10 µl of resazurin (Sigma Aldrich, USA) at a concentration of 6.75 mg/ml was added to the wells and incubated at 37°C for 30–45 min.[27] A pink/red color indicated bacterial growth, while a purple color indicated bacterial growth inhibition.

Minimum bactericidal concentration (MBC)

To determine the minimum bactericidal concentration (MBC), 100 µl of dilutions of extracts that showed no visible bacterial growth in the MIC test were streaked onto Müller-Hinton agar (Sigma Aldrich, USA) and then incubated at 37°C for 24 h. After counting bacterial colonies, the lowest concentration of extract indicating less than 0.01% survivors was recorded as the MBC.

Cytotoxic activity

Murine mammary carcinoma cell line (EMT6) was obtained from American Type Culture Collection (ATCC, CRL-2755). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) medium supplemented with 10% fetal bovine serum (FBS) and 1% antibiotics in a humidified atmosphere of 5% CO_2 at 37°C. To evaluate the cytotoxic effect of the EA-AMA extract and the C-AMA extract, cells were plated in 96-well plates at a density of 4,000 cells per well and treated with different concentrations of extracts ranging: 50; 100; 150; 200 µg/mL for 72 h. The cell viability was determined by XTT kit (Roche Diagnostics GmbH, Germany) according to the manufacturer's instructions. A 100 µL of mixed XTT reagents was added to each well and incubated for 4 h, and then absorbance was measured at 490 nm and 650 nm. The result was expressed as a percentage (%), relative to control cells grown in culture medium with DMSO.

In vitro *DNA-protective capacity*

The protective effect of the extracts against oxidative DNA damage was determined as described by Wang[28] using DNA extracted from human blood. For each extract, two concentrations were tested (250 µg/mL and 1 mg/mL). The reaction mixtures contained 2.5 µL of DNA (150 ng/µL), 10 µL of a Fenton reagent 30 mm hydrogen peroxide, 100 µM ferric chloride and 100 µM ascorbic acid) and 5 µL of extract or quercetin (250 µg/mL), finally add distilled water to a total volume of 20 µL. Reaction mixtures were incubated at 37 °C for 30 min and DNA. The samples were electrophoresed on a 1% agarose gel with SYBE Gold Nuclei Acid Gel (Invitrogen, USA). The DNA was visualized and photographed using a Gel Doc XR^+ (Bio Rad, USA).

Statistical analysis

The outcomes are presented as the mean value ± SD from three measurements. The IC_{50} and $A_{0.50}$ values were determined through a linear regression analysis, and one-way analysis of variance (ANOVA) was employed to identify significant differences ($p < 0.05$), utilizing XLSTAT-10.

Results and discussion

GC/MS of chloroform and ethyl acetate extract

The GC-MS analysis revealed the presence of several components which are reported in the supplementary materials (Tables S1–S2; Figures 1 and 2). The ethyl acetate extract from aerial parts of *A. membranaceus* had 47 identified molecules, while the chloroform extract from aerial parts of *A. membranaceus* had 58 molecules. In the EA-AMA extract major phytochemical identified compounds were methyl petroselinate (45.87%), palmitic acid, methyl ester (18.37%), linoleic acid, methyl ester (4,94%), oleic acid, methyl ester (4.24%) and methyl stearate (2.87%). Phenolic compounds were also identified as thymol (0.18%) and 4-vinyl-phenol (0.27%). For the C-AMA extract, the linolenic acid was present in the largest amount (16.95%), and its ester (5.61%). Other fatty acids were also identified as palmitic acid (9.3%) and linoleic acid, methyl ester (2.61%). In addition, molecules such as phytol (2.35%), eugenol (0.08) and vanillin (0.09) have been identified in the C-AMA extract. The palmitic acid and the pentadecanoic acid detected in our extracts were previously identified in the essential oil of *A. membranaceus* from China.[29] Myristic acid and oleic acid, both found in our extracts, were also detected in the flowers of *A. membranaceus*.[30]

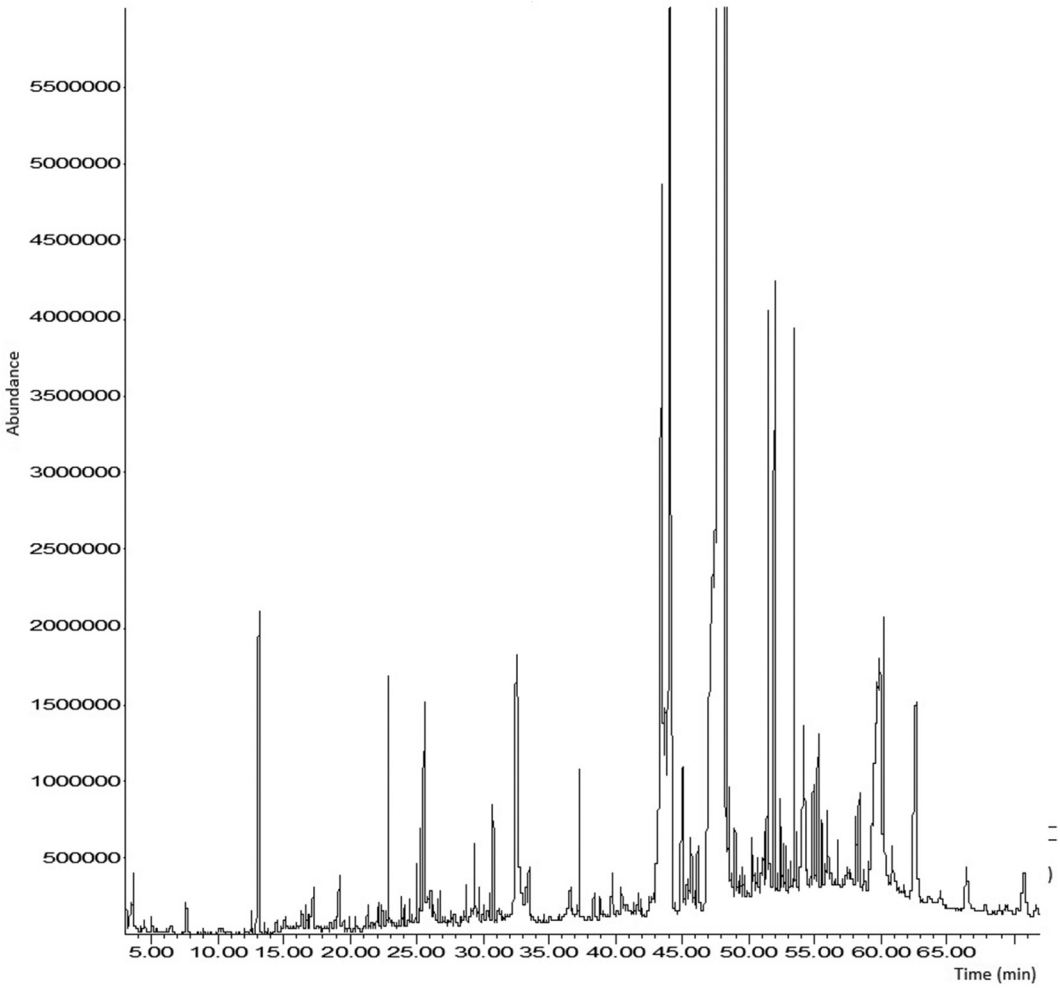

Figure 1. GC-MS chromatogram of ethyl acetate extract from *A. membranaceus* aerial parts.

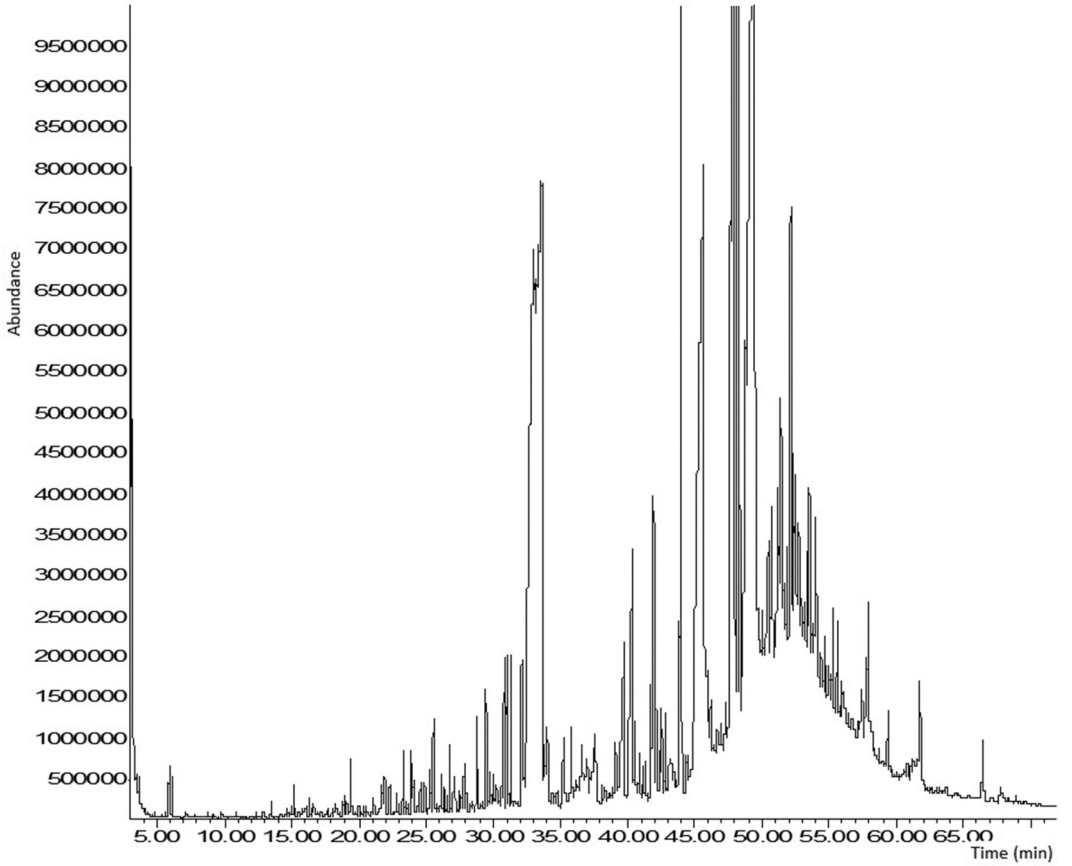

Figure 2. GC-MS chromatogram of chlorophormic extract from *A. membranaceus* aerial parts.

Qualitative phytochemical analysis

Table 1 summarizes the secondary metabolites present in *A. membranaceus* extracts for two solvents. These results demonstrated that AMA is a great source of phytochemicals that may reduce oxidative stress.[31]

Quantitative phytochemical analysis

The Total quantity of Phenolics Content (TPC) estimation of extracts was expressed as micrograms of gallic acid equivalents per milligrams of extract (μg GAE/mg) and the Total quantity of Flavonoids Content (TFC) of the extracts was expressed as micrograms quercetin equivalents per milligram of extract (μg QE/mg). Assay results are summarized in Table 2.

The EA-AMA extract has an estimated phenol content of 197.49 μg GAE/mg that is higher than the TPC of the C-AMA extract, with a content of 62.05 μg GAE/mg. A previous study showed that the TPC of the methanolic extracts of *A. membranaceus* roots originates from four regions of China varied between 135.23 and 197.40 μg GAE/mg which is close to the TPC of EA-AMA and higher than the TPC of the C-AMA.[32] In addition, EA-AMA TPC is high compared to *A. membranaceus* aqueous extract fermented by *lactiplantibacillus plantarum* which is 53.30 ± 0.72 μg GAE/mg.[33] We suggest that quantitative differences are due to different extraction methods. The estimated quantity of flavonoids in the EA-AMA extract is 42.93 ± 0.07 μg QE/mg, and in C-AMA extract is 20.58 ±

Table 1. Phytochemical screening of the EA-AMA and the C-AMA extracts.

Extracts	Steroids/terpenoids	Flavonoids	Tannins	reducing sugars
EA-AMA extract	-	+	+	+
C-AMA extract	+	+	+	+

Key: + = Present, - = Absent.

Table 2. Total phenolics and favonoids content of the EA-AMA and the C-AMA extracts.

Extracts	Total phenolics (µg GAE/mg)*	Flavonoids (µg QE/mg)**
EA-AMA extract	197.49 ± 0.54[a]	42.93 ± 0.07[a]
C-AMA extract	62.05 ± 0.53[b]	20.58 ±0.2[b]

The findings are presented as means±standard deviation based on three measurements (Tukey test, $p \leq 0.05$). Significant differences ($p < 0.05$) within the same columns are denoted by distinct superscripts (a or b). *Total phenolics is expressed as µg Gallic acid equivalents/mg of extract. **Total flavonoids are expressed as µg Quercetin equivalents/mg of extract.

0.23 µg QE/mg. Meanwhile, the amount of estimated flavonoids in our extracts is raised to the amount found in the ethyl acetate extract of *A. membranaceus* roots from the Shijiazhuang region in China (7.048 ± 0.87 RE/mg extract).[34] The geographical position of the plant, ecological conditions and climate are factors that influence the content of phenolic and flavonoid compounds.[35]

Antioxidant activities

The antioxidant power of the extracts was evaluated based on seven tests (DPPH $^{\bullet}$, reducing power, phenanthroline, $ABTS^{\bullet+}$, CUPRAC, β-carotene/linoleic Acid bleaching and superoxide alkaline DMSO test) and the results are reported as the mean values ± SD of three measurements; the IC_{50} and $A_{0.5}$ values were calculated by linear regression analysis and are summarized in Table 3.

In the DPPH assay, the ethyl acetate extract from *A. membranaceus* (EA-AMA) exhibited the moderat antioxidant activity (IC_{50}: 195 ± 0.55 µg/mL). However, the chlorophormic extract (C-AMA) not give any activity at 800 µg/mL. In terms of the IC_{50} values of DPPH in EA-AMA was higher than Dry Roots (IC_{50}: 40.97 ± 3.63 µg/mL).[36] In the ABTS assay, the EA-AMA extract possessed the highest antioxidant capacity (IC_{50}: 7.97 ± 0.88 µg/mL) closer activity to BHT, BHA, ascorbic acid, tannic acid, α-tocopherol and Trolox (IC_{50}: 1.29 ± 0.30, 1.81 ± 0.10, 1.74 ± 0.10, 1.01 ± 0.16, 7.59 ± 0.53 and 3.21 ± 0.06 µg/mL, respectively) and followed by C-AMA extract (42.72 ± 1.35 µg/mL). These ABTS assay results are in agreement with values obtained of *A. membranaceus* flower and Dry Roots extracts (IC_{50}: 22.02 ± 2.1, 40.97 ± 3.63 µg/mL, respectively).[36,37] Moreover, reducing power assay result showed that EA-AMA extract and C-AMA extract give moderate activities ($A_{0.5}$:81.80 ± 1.75, 109.99 ± 0.68 µg/mL, respectively) by comparing them to tannic acide standart ($A_{0.5}$: 41.07 ± 2.36 µg/mL). Samuel reported that the $A_{0.5}$ of Reducing power assay of *A. membranaceus* dry leaves is 612,40 ± 28,73 which capacity is lower than ours.[36] Data analysis of CUPRAC assay indicated that EA-AMA extract give the best activity ($A_{0.5}$: 21.93 ± 1.64 µg/mL) compared with six used standards. This CUPRAC assay result is in agreement with the value previously reported by Kirkan for *Astragalus ponticus* aerial parts with ($A_{0.50}$: 51.85 ± 0.79 µg/mL).[38] The phenanthroline test showed that the EA-AMA extract ($A_{0.50:}$ 21.93 ± 1.64 µg/mL) had strong and better activity then the ethanolic fraction of aerial parts of *Astragalus armatus* ($A_{0.50}$: 66.93 ± 1.14 µg/mL).[39] Regarding β-carotene linoleic acid assay, EA-AMA extract exhibited high activity (IC_{50}: 35.66 ± 0.61 µg/mL) then the C-AMA extract (IC_{50}: 132.35 ± 1.15 µg/mL). Our data are similar to Albayrak finding, the results found in the study of the methanolic extracts of four *Astragalus* species growing wild in Turkey which the IC_{50} were varied between 54.23 and 80.60 µg/mL.[40] The EA-AMA extract had the highest superoxide alkaline DMSO test (IC_{50}: <3.125 µg/mL) which is in agreement with the results obtained for *astragalus armatus* (IC_{50}: 13 ± 1.25 µg/mL).[39] The plant extracts antioxidant activity is related to the nature of the used solvent; the plant extraction methods, the experimental protocols as well as the nature of the phytochemical molecules. A number of studies have shown that plant polyphenols can be used as antioxidants against different

Table 3. Antioxidants activity of the EA-AMA and the C-AMA extracts by DPPH $^{\bullet}$, reducing power, phenanthroline, $ABTS^{\bullet+}$, CUPRAC, βcarotene/linoleic acid bleaching and superoxide alkaline DMSO test.

Extracts	$DPPH^{\bullet}$ Assay IC_{50}(μg/mL)	$ABTS^{+}$ assay IC_{50}(μg/mL)	CUPRAC assay ($A_{0.5}$ μg/mL)	Phenanthroline ($A_{0.5}$ μg/mL)	DMSO alkaline assay IC_{50} μg/mL	β-carotene linoleic acid assay IC_{50} μg/mL	Reducing power assay $A_{0.5}$ μg/mL
Acetate extract	195 ± 0.55^{a}	7.97 ± 0.88^{a}	21.93 ± 1.64^{a}	21.93 ± 1.64^{a}	<3.125	35.66 ± 0.61^{b}	81.80 ± 1.75^{b}
Chloroform extract	NA	42.72 ± 1.35^{b}	NA	78.87 ± 0.83^{b}	41.64 ± 0.54^{a}	132.35 ± 1.15^{c}	109.99 ± 0.68^{a}
Acarbose	NA	NA	NA	NA		NA	NA
BHT	12.99 ± 0.41^{b}	1.29 ± 0.30^{d}	8.97 ± 3.94^{cd}	2.24 ± 0.17^{d}	>200	1.05 ± 0.01^{e}	>200
BHA	6.14 ± 0.41^{c}	1.81 ± 0.10^{cd}	6.62 ± 0.05^{de}	0.93 ± 0.07^{d}	>200	0.90 ± 0.02^{e}	7.99 ± 0.87^{e}
Ascorbic acide	13.94 ± 2.81^{b}	1.74 ± 0.10^{cd}	12.43 ± 0.09^{c}	NA	7.59 ± 1.16^{c}	52.59 ± 1.98^{b}	6.37 ± 0.42^{e}
Tannic acide	7.74 ± 0.19^{bc}	1.01 ± 0.16^{d}	3.76 ± 0.73^{e}	NA	0.94 ± 0.22^{d}	7.46 ± 0.26^{d}	41.07 ± 2.36^{c}
α-tocopherol	$13.02 \pm 5,17^{b}$	7.59 ± 0.53^{b}	19.92 ± 1.46^{b}	NA	31.52 ± 2.22^{b}	1.79 ± 0.03^{e}	34.93 ± 2.38^{d}
Trolox	5.12 ± 0.21^{c}	3.21 ± 0.06^{c}	8.69 ± 0.14^{cd}	5.21 ± 0.27^{c}	NA	NA	5.25 ± 0.20^{e}

The IC_{50} and $A_{0.50}$ values denote the concentrations at which 50% inhibition and 0.50 absorbance are achieved, respectively. Linear regression analysis was employed to calculate IC_{50} and $A_{0.50}$, and the results were presented as Mean±SD (n=3). Significantly different values in the same columns are indicated by distinct superscripts (a, b, c, d, or e) with a significance level of $p < 0.05$. BHA butylated hydroxyanisole, BHT butylated hydroxytoluene, NA not absorbance.

oxidative stress.[41] Based on the results of seven antioxidant tested methods, the aerial parts of *A. membranaceus* have a potential antioxidant effect like the roots part which has been proven by several studies its richness in biomolecules with high antioxidant activity.[32] The *A. membranaceus* may be used in the treatment of different diseases associated with oxidative stress.

Inhibition of α-amylase activitity

Extracts inhibitory effects on α-amylase enzyme were established using the Acarbose as a standard. The results were given as IC_{50} values and are summarized in Table 4.

The α-glucosidase and the α-amylase are key enzymes in the digestion of dietary carbohydrates. Inhibition of these enzymes slows carbohydrate digestion and glucose absorption, which may help prevent postprandial hyperglycemia. In this study, we explored the ability of our extracts to inhibit the activity of α-amylase. As shown in Table 3, only the C-AMA extract exhibited highest α-Amylase inhibitory activity (IC_{50}:20.36 ± 0.78 µg/mL) more than acarbose standard (IC_{50}: 275.43 ± 1.36 µg/mL). The ability of C-AMA extract to inhibit α-amylase activity is consistent with the results of previous studies which showed that the methanolic extract from *A. membranaceus* roots had IC_{50}: 10.1 ± 0.9 µg/mL[42] as well as the ethanolic extract (IC_{50}: 13.11 ± 0.55 µg/mL).[43] The strongest α-Amylase inhibition of C-AMA extract is probably due to the synergistic effect of fatty acids including linolenic acid, palmetic acid and linoleic acid, which were disclosed their involvement in anti-diabetes activity[44, 45] The activity of α-glucosidase is strongly inhibited by these fatty acids,[46] which suggests that the aerial parts may be possess a significant inhibitory effect on α-glucosidase. Moreover, treatment with polysaccharides extracted from *A. membranaceus* roots significantly decreased blood glucose levels, insulin resistance, and oxidative stress in type 2 diabetes mellitus (T2DM) mice.[47] In order to confirm the advantages of the aerial parts of *A. membranaceus* against type 2 diabetes, it is necessary to conduct *in vivo* approaches and clinical investigations.

Inhibitory angiotensin converting enzyme activity

The EA-AMA and the C-AMA extracts were tested for their in vitro activities to inhibit ACE. The captopril was used as standard and the results were given as IC_{50} values as shown in Table 5.

Table 4. α-Amylase inhibitory activity of the EA-AMA extract, the C-AMA extract and acarbose.

Extracts	α-amylase inhibitory assay IC50 (µg/ml)
EA-AMA extract	NA
C-AMA extract	20.36±0.78[a]
Acarbose	275.43±1.36[b]

The IC_{50} values denote the concentrations at which 50% inhibition is achieved. The findings are presented as means±standard deviation based on three measurements (Tukey test, $p \leq 0.05$). Significant differences ($p < 0.05$) within the same columns are denoted by distinct superscripts (a or b). NA not absorbance.

Table 5. Angiotensin converting enzyme inhibitory of the EA-AMA extract, the C-AMA extract and Captopril.

Extracts	Angiote,sin converting enzyme inhibitory assay IC50 (µg/ml)
EA-AMA extract	NA
C-AMA extract	20.77±0.84[a]
Captopril	0.58±0.02[b]

The IC_{50} values denote the concentrations at which 50% inhibition is achieved. The findings are presented as means±standard deviation based on three measurements (Tukey test, $p \leq 0.05$). Significant differences ($p < 0.05$) within the same columns are denoted by distinct superscripts (a or b). NA not absorbance.

One remedial strategy for the treatment of hypertension is the inhibition of the Angiotensin converting enzyme. In this work, we investigated our extracts' capacity to impede ACE activity. The C-AMA extract showed the high level of Angiotensin converting enzyme inhibiting action (IC50: 20.77 ± 0.84 µg/ml). This capacity is due to the presence of unsaturated fatty acids in the extract, such as linolenic acid, which were disclosed for their involvement in the prevention of primary hypertension in humans.[46] A recent study showed that the aqueous extract of the root part has potential power to inhibit ACE with an IC50 of 1.85 ± 0.01 µg/ml.[48] The antihypertensive potential of *A. membranaceus* can be revealed through further clinical studies.

Antibacterial activity

MIC values of chlorophormic extract, ethyl acetate extract and amikacin against multi drug resistant (MDR) strains of clinical bacteria origin are presented in Table 6. Resazurin as an indicator showed an antimicrobial effect of the extracts.

The C-AMA and the EA-AMA extracts exhibited a comparable antibacterial property against pathogenic strains, with a powerful effect mainly against two bacteria namely *Pseudomonas aeruginosa* and *Escherichia coli* (MIC value of 0.039 ± 0 mg/ml). However, no significant antibacterial activity of our extracts was observed against the other multi-drug-resistant strains, unlike amikacin, which demonstrated variable antibacterial activity against these strains. Regarding the minimum bactericidal concentrations (MBC), both extracts showed bactericidal effects with a value of 0.3125 ± 0 mg/ml against the two tested bacteria, *Pseudomonas aeruginosa* and *Escherichia coli*. Our obtained results reinforce the results prior research which demonstrated that the ethyl acetate fraction of *A. membranaceus* arieal parts showed good antibacterial activity against several test bacteria, particularly against *Bacillus subtilis* with MIC value of 12.5 mg/ml.[49] The fatty acids produced by plants are known for their antibacterial activity.[50] The antibacterial properties of *A. membranaceus* may be attributed to several fatty acids present in the plant, such as palmitic acid which possesses a biological activity against *Pseudomonas aeruginosa*.[51] The *A. membranaceus* aerial parts, with its richness in fatty acids, could potentially be a source for the development of new antibacterial agents.

Cytotoxic activity

The cytotoxic effect of C-AMA and EA-AMA extracts was identified on murine mammary cancer cells (EMT6) using the XTT assay. The results of cytotoxicity assay are presented in Table 7.

Generally, the C-AMA and the EA-AMA extracts exhibited weaker cytotoxicity profile toward EMT6 cell line. The extracts showed an inhibitory effect less than 25% for concentrations ranging from 100 to 200 µg/ml. According to the American National Cancer Institute, crude extracts must have an IC_{50} below 30 µg in the preliminary assay to be considered cytotoxic.[52] Contrary to our findings, Zhou showed an inhibitory effect of AM roots extract on breast cancer cell proliferation.[53]

Table 6. Average Minimum Inhibitory Concentrations (MIC) and standard deviation of plant extracts and amikacin.

Bacteria	C-AMA extract (mg/ml)	EA-AMA extract (mg/ml)	Amikacin (µg/ml)
Enterococcus faecium	10 ± 0	5 ± 0	5 ± 0
Staphylococcus aureus	10 ± 0	10 ± 0	0.83 ± 0.23
Acinetobacter baumannii	8.33 ± 2.35	10 ± 0	2,5 ± 0
Pseudomonas aeruginosa	0.039 ± 0	0.039 ± 0	0.625 ± 0
Morganella morganii	5 ± 0	5 ± 0	0.312 ± 0
Escherichia coli	0.039 ± 0	0.039 ± 0	0,312 ± 0
Serratia marcescens	10 ± 0	5 ± 0	0.625 ± 0
Klebsiella pneumoniae	10 ± 0	10 ± 0	2.5 ± 0
Proteus mirabilis	5 ± 0	5 ± 0	0.83 ± 0.23
Enterobacter cloacae	5 ± 0	5 ± 0	1.25 ± 0
Providencia rettgeri	10 ± 0	5 ± 0	0.625 ± 0

Table 7. The EA-AMA extract and the C-AMA extract percentage inhibition of EMT6 cell line.

	EMT6 % Inhibition	
Concentration (µg/mL)	C-AMA extract	EA-AMA extract
50 µg/mL	5% ± 0.063[a]	7% ±0.01[a]
100 µg/mL	22% ±0.014[b]	19% ±0.014[b]
150 µg/mL	23%±0.007[b]	20% ±0.014[b]
200 µg/mL	24%±0.021[b]	23% ±0.045[b]

The findings are presented as means±standard deviation based on three measurements (Tukey test, $p \leq 0.05$). Significant differences ($p < 0.05$) within the same columns are denoted by distinct superscripts (a or b).

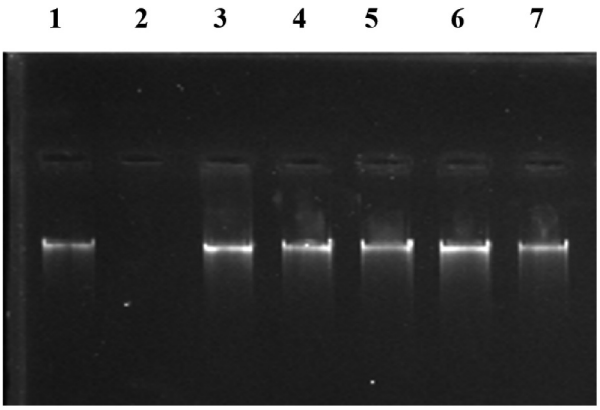

Figure 3. NA damage protection assay. 1- DNA (human blood), 2- DNA + Fenton's reagent, 3- DNA + Fenton's reagent + 250 µg quercetin, 4- DNA + Fenton's reagent + 250 µg EA-AMA extract, 5- DNA + Fenton's reagent + 1 mg EA-AMA extract, 6- DNA + Fenton's reagent + 250 µg C-AMA extract, 7- DNA + Fenton's reagent +1 mg C-AMA extract.

In vitro DNA-protective capacity

Astragalus membranaceus aerial parts extracts have been evaluated for their ability to protect human DNA from free radicals generated by H_2O_2 (Figure 3).

The free radicals resulting from the Fenton reaction caused a complete disappearance of the DNA (line 2) comparing to the control (line 1). The electrophoretic profile of positive control showed DNA preservation which underwent a Fenton reaction in the presence of 250 µg Quercetin (line 3). The appearance of visible DNA bands with the same intensity as the DNA control band for tests involving 250 µg or 1 mg of plant extracts (lines 4, 5, 6 and 7). This finding indicates that the C-AMA and the EA-AMA extracts protect the DNA. In the line of our results, Curnow[54] found that the aqueous extract of *A. membranaceus* roots significantly reduced the destructive effect of ultraviolet radiation on cellular DNA. Therefore, the *Amembranaceus* could have a considerable capacity to prevent DNA damage from the oxidative stress.

Conclusion

As far as we are concerned, our research is regarded as the first of its kind in Algeria to examine the potential uses and medicinal benefits of *Astragalus membranaceus* aerial parts. The results revealed that our AMA have significant antioxidant, DNA damage protecting, antidiabetic, antihypertensive and antibacterial *in vitro* effects. In addition to their promising biological activities, our extracts do not induce cytotoxicity. Therefore, the AMA could be an excellent source for bioactive compounds which might be used as a therapeutic agent for a range of human diseases (diabetes, hypertension, and infection disease) and/or a dietary supplement.

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Author contributions

All authors have read and agreed to the published version of the manuscript.

Consent for publication

All authors agree to the publication.

Data availability statement

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All authors approved.

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