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A comparative study on chemical profile and biological activities of aerial parts (stems, flowers, leaves, pods and seeds) of *Astragalus gombiformis*

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ABSTRACT

The present work aims to characterize the chemical profile of phenolic compounds and some biological activities of *Astragalus gombiformis*. The butanolic fractions of five aerial organs (stems, flowers, leaves, pods and seeds) were quantified and identified by LC–MS analysis. The results were revealed the presence of 13 phenolic compounds (quinic acid, p-coumaric acid, transfrulic acid, Hyperoside (quercetin-3-o-galactoside, Quercetrin (quercetin-3-o-rhamonoside), Apegenin-7-o-glucoside, kampherol, Naringenin, Apegenin, Luteolin, Cirsiliol, Cirsilineol and Acacetin. In terms of biological activities, the antioxidant,α-amylase inhibitory and anticholinesterase were determined. The butanolic extract from flowers showed the highest antioxidant activity in DPPH and ABTS(IC50: 16, 43 ± 0, 46 and 16, 13 ± 0, 35 μg/mL) very closer with standards tested and week activity with Galvinoxyl radical (GOR) (IC50: 583, 95 ± 2, 20 μg/mL), while other organ's extract exhibited moderate antioxidant activity. The leaves extract was found to exhibit the highest inhibitory effect against BChE (IC50: 165,54 ± 3,49 μg/mL) compared by the other parts which give a weak inhibitory effect at 200 μg/mL. A significant alpha-amylase inhibitory activity was displayed by seeds, leaves, pods and stems extracts (IC50:76.41 ± 3.72; 74.61 ± 3.68; 88.13 ± 1.81 and 83.81 ± 1.74 μg/mL) more the standard used. Based on these results, it is right to conclude that *A. gombiformis* is important source of the natural antidiabetic, antioxidants and anti-Alzheimer′s disease.

1. Introduction

During the past few years, increasing interest has focused in natural products from medicinal plants that represent important source of bioactive compounds for treating various human diseases(Ak and Gulç;in, 2008; Gulç;in, 2009; Gulç;in, 2010; Gulç;in, 2012; Gulç;in, 2020). With multiple biological activities, many plants contain natural antioxidants compounds attracts the attention of several research teams for its role against numerous illness. The *Astragalus* genus is member of the *Fabaceae* family, is commonly used as forage for livestock and by wild animals as well as in food and medicines (Ríos and Waterman, 1997; Zarre-Mobarakeh, 2000). In china, the roots of *A.membranaceus* have a long history in traditional chinese medicine (Yin et al., 2006) and especially for antidiabetic treatments (Kojo et al., 2013). Moreover, leaves and roots of *A.glycyphyllos* are widely used to their purifying, refreshing, diuretic and many other properties (Ionkova, 2008; Pistelli, 2002; Gođevac et al., 2008; Guarino et al., 2008).In Anatolia, *A.trojanus* aqueous extract is traditionally used against leukemia (Bedir et al., 2001). Many researches on *Astragalus* genus have been demonstrated the presence of different secondary metabolites such as *A. trojanus, A. microcephalus, A. zahlbruckneri* (Calis et al., 2001; Bedir et al., 1998, 1999). In the framework of valorization of the Algerian natural surface and to develop new products, we are interested in this work by *Astragalus gombiformis* Pomel that belongs to the *leguminosae* plant obtained from arid zoon (El Oued-Algeria).In order to have an overall picture of distribution, identifica-

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tion and quantification of phenolic compounds present in different areal organs from *A.gombiformis* and further elucidate the similarities and differences in their chemical constituents using liquid chromatography mass spectrometryanalysis (LC-MS), and also to determine their antioxidant potential, α-amylase inhibitory assay and butyrycholinesterase inhibitory activity.

2. Material and methods

2.1. Plant material

The aerial parts of *A.gombiformis* plants were collected from the saharan region (EL Oued -Algeria) in april 2019 at the end of flowering phase and beginning of fruiting phase (pod formation).The samples were cleaned, dried and ground thane stored until it is used.

2.2. Extraction

The extraction was realized according to Bensouici et al. (2019) method. Each part of *A.gombiformis* was extracted with ethanol–water (70:30, v/v).After concentration under reduced pressure, the residue was suspended in water and extracted successively with petroleum ether and butanol respectively. The butanolic fractions were the objective of our study.

2.3. Total phenolic content (TPC)

The total phenolic content of the butanolic fractions was determined following the Folin–Ciocalteu method (Le et al., 2007) the results was expressed as micrograms of gallic acid equivalents per milligrams of extract (μg GAE/mg) (Bursal et al., 2019; Gulcin et al., 2019; Taslimi et al., 2020a, 2020b).

2.4. Total flavonoids content (TFC)

The quantification of total flavonoids content was performed by Tel et al. (2013) method and the results was expressed as micrograms quercetin equivalents per milligram of extract (μg QE/mg). (Türkan et al., 2020; Gülçin et al., 2019, Gülçin et al., 2020).

2.5. Method of phenolic compounds quantification BY LC-MS techniques

The analysis for phenolic compounds was performed on a Shimadzu UFLC XR system (Kyoto, Japan), equipped with a SIL-20AXR auto-sampler, a CTO-20 AC column oven, a LC-20ADXR binary pump and a quadripole 2020 detector system. This instrument was equipped with a Inertsil ODS-4 C18 3 μm column (L150 × 3.0 mm i.d.). The column temperature was set at 40 °C and the injection volume was 20 μl with a flow rate of 0.5 mL/min Eau95% + MeOh5% + 0.2% Acetic acid and 50%ACN + 50%H2O + 0.2%Acetic acid were used as mobile phases A and B, respectively. The analysis was performed using a linear gradient programmed as follows: 0,01–14 min, from 10% to 20% B; 14–27 min, 0 from 20% to 55% B; 27–37 min, from 55% to 100% B; 37–45 min, 100% B; 45–50 min 10% B. Dessolving line temperature was 275 °C, nebulizing gas flow 1,50 L/min, the drying gas was set at 15,00 L/min and Temperature of Heat block was 450°c. LC-ESI(−) MS mass spectra [MH]- were acquired using Lab Solutions software. Phenolics were identified by comparison with retention time of the standards of phenolic compounds. The lab standards were LGC and Sigma Aldrich.

2.6. Determination of antioxidant activity

2.6.1. DPPH free radical-scavenging assay

The antioxidant activity of different areal parts of *A.gombiformis* was evaluated following Blois (1958) method using the free radical DPPH (2,2-diphenyl-1-picrylhydrazyl). The results were given as 50% inhibition concentration (IC50) and compared with the antioxidant standards (BHA, BHT, α-Tocopherol, Ascorbic acid and Tannic acid).

2.6.2. ABTS cation radical assay

The ABTS scavenging activity was determined according to the method described earlier (Re et al., 1999). The results were given as 50% inhibition concentration (IC50).

2.6.3. GOR scavenging assay

The Galvinoxyl radical (GOR) scavenging assay activity was determined according to Shi et al. (2001) method.

2.7. Enzymatic inhibitory assay

2.7.1. The α-amylase inhibitory assay

The α-amylase inhibitor activity was investigated using the method described elsewhere (Behvar et al., 2018). Acarbose was used as a standard to compare our results (Taslimi et al., 2017; Taslimi and Gulçin, 2017; Gulçin et al., 2018; Taslimi et al., 2018).

2.7.2. Anti-Alzheimer activities

Butyrylcholinesterase inhibitory of the areal parts of *A.gombiformis* was evaluated using the spectrophotometric method developed by Ellman et al. (1961). The butyrylthiocholine chloride was used as enzyme substrates and galanthamine was used as standard compound and the results were given as 50% inhibition concentration (IC50). (Taslimi and Gulçin, 2018; Cakmak et al., 2019; Taslimi et al., 2020a, b).

2.8. Statistical analysis

All determinations were carried out in triplicate of each sample. The results reported as mean ± standard deviation (SD) were calculated from the data obtained. The analysis of variance of bioassays were the subject of one-way analysis of variance (ANOVA) using the PRISM GRAPHPAD V: 5.00 (Trial) followed by Tukey test. The level of significance was fixed at $p < 0.05$.

3. Results

3.1. Total phenolic and flavonoids content

Based on obtained results, the total phenolic content (TPC) of the butanolic extracts was estimated according to the calibration curve prepared from gallic acid ($y = 0{,}0034x + 0{,}1044$, $R^2 = 0{,}997$), while the total flavonoids content (TFC) was calculated following the calibration curve prepared from quercetin ($y = 0{,}0048\times$, $R^2 = 0{,}997$).As shown in Figs. 1 and 2, there is a large variation in TPC content in different parts examined. The average values of all parts of *A.gombiformis* showed that flavonoids represented more than 50% of the phenolic compounds (TPC: 71, 19) (TFC:50,592). The TPC of butanolic extract from the flowers (110, 56 ± 19,62 μg GAE/mg) was found to be higher than the other part's extracts, followed by leaves, stems, seeds and pods with values of (92, 52 ± 7, 90; 83, 21 ± 15, 34; 52, 23 ± 2, 29 and 17, 43 ± 3, 38 μg GAE/mg, respectively). The same trend was observed with regard to TFC distribution, the highest TFC was registered in butanolic extract of flowers (94,65278 ± 10,43401 μg QE/mg), followed by leaves, stems, seeds

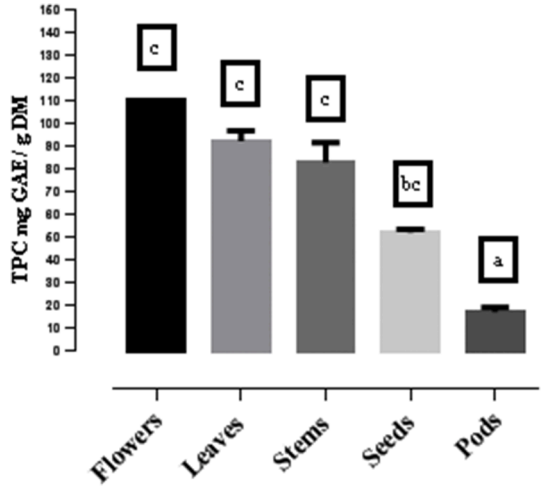

Fig. 1. Total phenolic content (TPC) of areal parts of *A.gombiformis*.

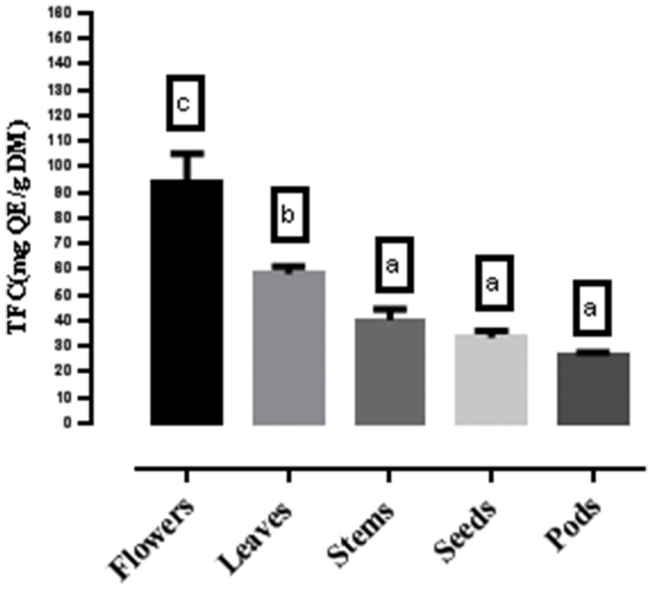

Fig. 2. Total flavonoids content (TFC) of areal parts of *A.gombiformis*.

and pods by values of 58,33 ± 2,80; 40,20 ± 4,30; 33,47 ± 2,65 and 26,31 ± 1,27 μg QE per mg).

3.2. Identification and quantification of phenolic and flavonoids contents

The quantitative analysis results of major phenolic compounds identified in different plant's parts of *A.gombiformis* are summarized in Table 1. Following the total phenolic and flavonoids content results, the LC-MS analysis confirms that flavonoids contents in this plant represent more than 50% of the polyphenolic fraction. The Cirsiliol or Dimethoxyflavone (DMF) is represented the major flavonoids in this plant. The highest concentration of this metabolite is fawned in flowers by a value of 64.768 ppm, followed by leaves stems seeds and pods (62, 337; 38,774; 24,915 and 11,538. As shown in Fig. 3, dimethoxyflavone is flavone substituted by methoxy groups at positions 6 and 7 and hydroxy groups at positions 5, 3′ and 4′ respectively.

By comparing the results of LC-MS analysis with the reference compounds referring to their retention times, the characterization of phenolic compounds of *A.gombiformis* revealed 13 compounds. Five common phenolic compounds [**quercetin-3-o-rhamonoside(18)**, **Kampherol(22)**, **Cirsiliol(29)**, **p-coumaric acid(9)**, **Hyperoside (12)**] were presented by varied concentration in different organs. The highest content of quercetin-**3-o-rhamonoside(18)**, **Kampherol(22)** and **Cirsiliol(29)** was detected in flowers (8.352; 1.53 and 64.768 ppm,respectively). Whereas **p-coumaricacid(9)** was detected in seeds and **Hyperoside(12)** in leaves (4.744; 7.341 ppm).

Moreover, this analysis showed the presence of **quinic acid(1)** in stems and pod (2.268 and 3.859 ppm), **Apegenin-7-o-glucoside(19)** in leaves, flowers, seeds and pod (0.873; 0.977; 0.376 and 1.281 ppm), **Apegenin(27)** in stems, flowers, seeds and pod (0.357; 0.335; 0.101 and 0.463 ppm) and **Luteolin(28)** in flowers, seeds and pod (0.872; 1.326; 0.776 ppm). All this results are significant difference in concentrations ($p < 0.05$). Moreover, **Transfrulic acid(11), Naringenin(27)**, **Cirsilineol(30)**, **Acacetin(31)** were found as traces with lowest concentration compared with the other compounds detected.

3.3. Antioxidant properties

In our study, the butanolic fractions of areal part of *A.gombiformis* was tested for its antioxydante activity with more than one method (DPPH scavenging, ABTS radicals scavenging activities and Galvinoxyl radical (GOR) scavenging assay) and compared the results with several reference standards with the aim to achieve more informative and arguably necessary results. The results are shown in Table 2.

The results of DPPH scavenging show that butanolic extract of the flowers was exhibited the highest antioxidant activity (IC50: 16, 43 ± 0, 46 μg/mL) closer activity to that of BHT, α-tocopherol and ascorbic acid (IC50: 12.99 ± 0.41, 13.02 ± 5.17 and 13.94 ± 2.81 μg/mL) respectively, and less than BHA and Tannic acid (IC50: 6.14 ± 0.41 and7.74 ± 0.19 μg/mL), followed by the leavs (IC50: 147.82 ± 0.28 μg/mL). But the stems, seeds and pods extracts demonstrated a week activity at 200 μg/mL (IC50: 219.12 ± 4.14; 244, 63 ± 1, 94 and 306.43 ± 2.48 μg/mL). Similarly, The analysis data of the ABTS assay showed that the same fraction of flowers of *A.gombiformis* give the best activity (IC50: 16, 13 ± 0, 35) compared with standards, very closer with α-tocopherol (IC50: 7.59 ± 0.53 μg/mL) and moderate activity compared with BHA, BHT, ascorbic acid and tannic acid (IC50:1.81 ± 0.10, 1.29 ± 0.30; 1.74 ± 0.10 and 1.01 ± 0.16 μg/mL, respectively) followed by leaves seeds stems and pods (IC50 : 143,45 ± 4,06; 241,87 ± 1,33; 257,55 ± 3,96; 266,95 ± 2,26 μg/mL). Furthermore, a weak activity of all parts tested of *A.gombiformis* was observed with galvinoxyl radical (GOR) by values ranging from 489,23 ± 1,09 to 655,51 ± 1,92 μg/mL.

3.4. α-amylase inhibitory assay

The evaluation of *A.gombiformis* effect against α-amylase inhibitory assay was not investigated before in worldwide. For this objective, the anti-diabetic activity of this plant will be of major importance. As shown in Fig. 4, butanolic extracts of the five parts of *A.gombiformis* were tested with 4 mg/mL, the high half maximal inhibitory concentration were registered in flowers and Leaves (76.41 ± 3.72 and 74.61 ± 3.68), no significant difference between the both organs ($p < 0.005$). Followed by pods and stems by value of 88.13 ± 1.81 and 83.81 ± 1.74, these results are founded to be higher than Ascarbose (IC50: 275.43 ± 1.00) used as a standard.

3.5. Butyrycholinesterase inhibitory activity

This study evaluated the capacity of *A.gombiformis* as inhibitors enzyme of cholinesterase family that used as a control and treatment to fight against Alzheimer's disease. (Hebert et al., 1995). As provided in Table 3, the results of fractions tested was compared by the positive control (galanthamine).The butanolic extract obtained from the leaves exhibited the highest inhibitory effect against BChE (IC50:

Table 1
LC-MS analysis of different organs of *A.gombiformis*.

	Analyte	Retention time	([M-H]-)	Butanolic fractions Concentration (ppm)				
				TPC: 38.5 Seeds	TPC:46.176 Stems	TPC: 99.304 Flowers	TPC:28.419 Pods	TPC:78.355 Leavs
1	quinic acid	2.017	191.00	2.268[a]	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	3.859[b]
2	Gallicacid	–	169.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
3	protocatchuic acid	–	153.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
4	Catechin (+)	–	289.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
5	caffeic acid	–	179.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
6	syringic acid	–	197.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
7	1,3-di-O-caffeoyquinic acid	–	515.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
8	Epicatechin	–	289.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
9	p-coumaric acid	22.283	163.00	4.370[b]	2.039[a]	5.082[db]	7.341[c]	6.069 [db]
10	Rutin	–	609.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
11	trans frulic acid	24.550	193.00	N.D.(Peak)	0.578[a]	N.D.(Peak)	N.D.(Peak)	0.217[a]
12	Hyperoside (quercetin-3-o-galactoside	25.829	463.00	4.744[c]	0.698[a]	2.622[b]	1.002[a]	1.221[a]
13	Luteolin-7-o-glucoside	–	447.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
14	3,4-di-O-caffeoyquinic acid	–	515.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
15	Naringin	–	579.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
16	Rosmarinic acid	–	359.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
17	4,5-di-O-caffeoyquinic acid	–	515.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
18	Quercetrin (quercetin-3-o-rhamonoside)	28.050	447.00	0.684[a]	0.285[a]	8.352[c]	5.454[b]	0.629[a]
19	Apegenin-7-o-glucoside	28.028	431.00	N.D.(Peak)	0.873[a]	0.977[a]	0.376[a]	1.281[b]
20	o-coumaric acid	–	163.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
21	Salviolinic acid	–	717.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
22	kampherol	33.350	285.00	0.006[a]	2.159[d]	1.531[c]	0.291[ab]	0.781[ab]
23	quercetin	–	301.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
24	trans cinnamic	–	147.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
25	Silymarin	35.398	481.00	N.D.(Peak)	N.D.(Peak)	14.765	N.D.(Peak)	N.D.(Peak)
26	Naringenin	35.083	271.00	0.269[a]	0.277[a]	N.D.(Peak)	0.268[a]	N.D.(Peak)
27	Apegenin	35.717	269.00	0.357[a]	N.D.(Peak)	0.335[a]	0.101[a]	0.463[b]
28	Luteolin	36.283	285.00	N.D.(Peak)	N.D.(Peak)	0.872[a]	1.326[b]	0.776[a]
29	Cirsiliol	36.975	329.00	24.915[b]	38.774[c]	64.768[c]	11.538[a]	62.337[c]
30	Cirsilineol	40.139	343.00	1.187	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)
31	Acacetin	42.117	283.00	N.D.(Peak)	N.D.(Peak)	N.D.(Peak)	0.722[a]	0.722[a]

Different capital letters represent significant variations ($P < 0.05$) between the concentrations.

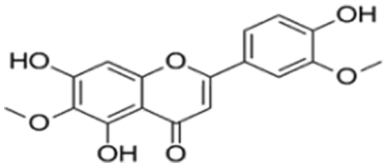

Fig. 3. Chemical structure of major flavonoids identified in *A. gombiformis* (Cirsiliol: dimethoxyflavone).

Table 2
Antioxydants activity of butanolic extracts of *A.gombiformis* by DPPH, ABTS Reducing, Galvinoxyl radical (GOR).

	DPPH IC50µg/mL	ABTS IC50µg/mL	GOR IC50µg/mL
Pods	306 ± 2.48[a]	266 ± 2.26[a]	578 ± 4.01[b]
Leavs	147 ± 0.28[b]	143 ± 4.06[b]	655 ± 1.92[c]
Seeds	244 ± 1.94[c]	241 ± 1.33[c]	603 ± 0.09[d]
Stems	219 ± 4.14[d]	257 ± 3.96[d]	489 ± 1.09[a]
Flowers	16.43 ± 0.46[e]	16.13 ± 0.35[e]	583 ± 2.20[b]
BHA	6.14 ± 0.41	1.29 ± 0.30	5.38 ± 0.06
BHT	12.99 ± 0.41	1.81 ± 0.10	3.32 ± 0.18
α-Tocopherol	13.02 ± 5.17	7.59 ± 0.53	NT
Ascrobic acid	13.94 ± 2.81	1.74 ± 0.10	NT
Tannicacid	NT	1.01 ± 0.16	NT

IC50 values are expressed as means ± SD of three parallel measurements ($P < 0.05$) Different capital letters represent significant variations ($P < 0.05$) between varieties. Reference compound: BHA butylated hydoxylanisole, BHT butylated hydroxyltoluene, α-Tocopherol Ascrobic acid Tannic acid NT: not tested.

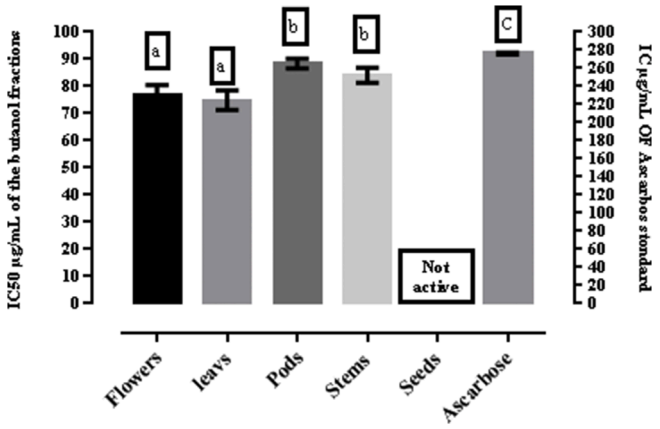

Fig. 4. Alpha-amylase inhibitory assay of different organs of *A.gombiformis*.

165,54 ± 3,49) compared by the other parts. Moreover, a weak inhibitory effect was detected by the stems, flowers, pod and grains (IC50: <200µg/mL).

4. Discussion

In the present work, we investigated the chemical constitution of phenolic fraction from five organs of *A.gombiformis.* The inhibition of alpha-amylase and butyrylcholinesterase as well as the antioxidant activity were reported with a view to prospect for possible bioactive compounds with antidiabetic, anti-alzheimer and antioxidant properties. Phenolic compounds are one of the widest groups of secondary

Table 3
Anticholinesterase activity (BchE) of butanolic extracts obtained from stems, flowers, leaves, Pods and seed of *A.gombiformis*.

	BchE Inhibitory activity							
	3.125 µg	6.25 µg	12.5 µg	25 µg	50 µg	100 µg	200 µg	IC50 µg/mL
seeds	NA	NA	NA	NA	NA	5.69 ± 1,45	40.80 ± 3,53	>200
Leavs	NA	NA	NA	NA	1.96 ± 2.91	39.72 ± 1.45	57.10 ± 2,08	165.54 ± 3.49
pod	NA	NA	NA	NA	NA	19.47 ± 0.95	44.16 ± 3.94	>200
Stems	NA	NA	NA	NA	NA	4.97 ± 4.15	38.41 ± 1.45	>200
Flowers	NA	NA	NA	NA	NA	4.83 ± 1.05	34.78 ± 0.96	>200
Galantamine[1]	35.9 ± 2.28	43.8 ± 0.0	68.5 ± 0.31	80.7 ± 0.41	85.8 ± 1.63	91.8 ± 0.20	94.8 ± 0.34	6.27 ± 1.15

IC50 values is defined as the concentration of 50% inhibition percentages and calculated by linerar regression analysis and expessed es Mean ± SD (n = 3) NT: not absorbance. b: reference compounds.

metabolites described in the literature they are ubiquitous in plants with broad diversity in structure (Havsteen, 2002).The *Astragalus* genus is well known by its richness in phenolic compounds and flavonoids (Jing et al., 2011).The phenolic compounds and flavonoids were quantified in the butanolic extracts of the different organs. Regarding the content of total phenol of leaves and flowers extract, our results are higher than that obtained by Bronislava et al. (2018) from leaves and flowers extract of *A.glycyphyllos* (25.99 and 23.71 mg GAE/g). Moreover, our findings of TFC are relative higher when compared with flavonoids content from leaves and flowers extract of *A.glycyphyllos* (21.00 and 16.71 mg RE/g)(Bronislava et al., 2018). It is not necessarily to compare our data directly with the results of literature due to the differences of species, maturity, plant part and the heterogeneity of the plant tested. Overall, we can conclude that distribution of TPC and TFC are organ-dependent, and the variation of results due to several factors, such as ecological condition and climate, genotypic and environmental stress within geographical positions of herbal tested (Lobanova, 2011).

On the other hand, the identification and quantification of phenolic compound and flavonoids were well documented in previous studies (Yasinov and Khaitov, 1988; Yasinov et al., 1983) as well as their isolation was carried out by chemical and spectroscopic chromatographic methods in order to elucidate their structure. Previous experiments on *Astragalus* have shown the presence of major similar phenolic compounds obtained from our study such as hyperoside, naringenin, quercetin, kaempferol, apigenin, p-coumaric acid, rosmarinic acids from *A. schizopterus* methanol extract (Haşimi et al., 2017). Many papers reported a similar phenolic and flavonoid compounds such as kaempferol, quercetin, rutin, rosmarinic acid, calycosin,ononin, formononetin which detected in *Astragalus* species by using HPLC-DAD and HPLC-MS/MS.(Qi et al., 2008; Yunfei et al. (2008); Montoro et al. (2012); Zhang et al.,. 2013. Our findings are in agreement with Sevil and Onur (2019) who found almost same compounds from *Astragalus taxa* such as rutin, chlorogenicacid, kaempferol, ferulic acid and syringic acid. Anyway, Jun et al. (2012) also has documented the presence of thirty phenolic compounds in *A.membranaceus*by using the high performance liquid chromatography (HPLC). So according to literature and also based on our results, we may consider *A.gombiformis* a potential source of bioactive molecules with dual action in the pharmacological development. However, the variation in chemical composition depended to plant's part, plants species of the various *Astragalus* spp. within the maturityand vegetation period (Lobanova, 2011). Platikanov et al. (2005).

The antioxidant potential of five phenolic fractions of *A.gombiformis* has been assessed by using three different methods based on different principles in order to take in consideration the nature of bioactive molecules through their various mechanisms actions and the capacity of radical scavenging (Li et al., 2008).Butanolic extract obtained from flowers was found to exhibit the best antioxidant activity by scavenging free radicals, which appears to be strongly correlated with total phenolic content in almost all tests. Based in our results, the highest activity in flower and leaves using DPPH method are in agreement with study previously reported by Bronislava et al. (2018),*Astragalus. cicer* leaves exhibited a particularly high antioxidant activity (128.6 µmol/g) close to our results of leaves extract (IC50: 147.82 ± 0.28 µg/mL).Moreover, our half maximal inhibitory concentration registered in ABTS assay of flowers was founded to be lowest compared by methanolic extract from flowers of *A.membranaceus* var.*mongholicus* (22.02 µg/mL) (Yuan et al., 2019).In contrary, *A.cicer* seeds revealed a strong antioxidant capacity with 67.2% inhibition compared with our results (306.43 ± 2.48; 266,95 ± 2, 26), due to the action and the different concentration of antioxidant metabolites present in the corresponding medicinal plants (Bronislava et al., 2016).Indeed, leaves and flowers extracts which are richer with these compounds were generally more actives. Similarly, Tepavcević al. (2010) have documented that the DPPH scavenging activity correlated well with total polyphenolic content, According to literature, Many researchers have found that the medicinal plants with high amounts of flavonoids and phenols have potent antioxidant actions (da Silva et al., 2006; Ksouri et al., 2009; Falleh et al., 2011; Dehshiri et al., 2013).

Diabetes mellitus is one of the chronic diseases caused by metabolic disorders (Pallavi et al., 2015) which are characterized by high levels of glucose in the blood resulting from the poor production and action of insulin (kojo et al., 2013). This disease is treated either by injecting insulin or by oral anti-diabetic drugs. (Cheribet et al., 2019). The alpha amylase inhibitor compounds are considered as actives principle in oral antidiabetic drugs, they cause delay in the release of D-glucose from complex carbohydrates as well as glucose absorption which causing reduced of plasma glucose level. In current research on anti-diabetic drugs, plant medicine has played an important role to discover many plants with a high potential against alpha-amylase. Anyway, good activity were founded by *A.gombiformis* extract against alpha-amylase inhibitor assay. Similarly to our results,*A.ponticus.pall* reported a high α-amylase and α-glucosidase inhibitory potential in leaves and roots extract (Arumugam et al. (2019).From literature, Kojo et al. (2013) and Zanget al. (2011) have been well noted that *A.membranaceus* have a long history for the treatment of diabetic, the decoction of this herb reduce fasting blood glucose and homeostatic model assessment (HOMA) levels in type 2 diabetes mellitus patients. On the other hand, only the seeds extract didn't exhibited any α-amylase inhibitory activity at 400 µg/mL, this finding may be explained by the presence of other bioactive compounds in flowers, leaves, stems and pods that distinguished by strong α-amylase inhibitory and were not identified in our study. According to our literature survey, several researches documented that polysaccharides, saponins in A*stragalus* are the major chemical constituents demonstrating biological activity to diabetes mellitus (WHO, 1999; Thorone, 2003),which reinforces and confirms the hypothesis of presence of other compounds which have not been detected by LC-MS.

Alzheimer's disease (AD) or dementia disease, that affects around 10% of the population over the age of 65-year old, is caused by alteration of neurons which cause in decreasing of the neurotransmitter levels and lead to block the cholinergic transmission and decline in

cognitive function in patients (Choi et al., 2012). The inhibitors of cholinesterase family are the major advances in the treatment of Alzheimer's disease (Ezio, 2004; Melkinova, 2007). Through our study which also reported the effect of butanolic fractions from five different organs of *A.gombiformis* against butyrylcholinesterase (BChE), only the leaves fraction was showed a considerable activity, this observation suggest the implication of other bioactive molecules than phenolic compounds in the inhibition of these enzymes, and their interaction with secondary metabolites in extract as well as their concentrations. According to our literature survey, *A.schizopterus* did not exhibit any enzyme inhibitory activity against AChE and BChE (Haşimi et al., 2017), while *A.leporinus Boiss. var. hirsutus* exctact showed strong inhibitory by a values of IC50: 46.96 ± 4.06 and 66.15 ± 4.08 against acetyl- and butyryl-cholinesterase. Furthermore, Hassen et al. (2013) documented that *A.gombiformis* growing in Tunisia exhibited a high anticholinesterase activity with an IC50 of 110 μg/mL.

5. Conclusion

For the first time, our study described the comparative chemical analysis of phenolic compounds as well as the antioxidant, alpha amylase and anticholinesterase activities of five organs from *A.gombiformis* plant growing in Algeria. A total of 13 phenolic compounds were identified of which the major constituents with high concentration were flavonoids. The flower part of this herbal was found to have the highest concentration of the phenolic compounds witch assure the strongest antioxidant activity obviously trough scavenging several radical. There is a growing interest to incorporate the *A.gombiformis* extracts into food products to replace food additives such as BHA and BHT in order to avoid their side effects. Therefore, the anti-diabetic potential of *A.gombiformis* suggest this species as anattractive substitute for diabetic's management as recommended in the literature. The isolated bioactive molecules from *Astragalus gombiformis* extracts may provide more information necessary for desirable pharmacological.

Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2020.101668.

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