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Research Article

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Antihemolytic and antioxidant activities of aerial parts extracts of *Zygophyllum album* L. and *Globularia alypum* L. from Algeria

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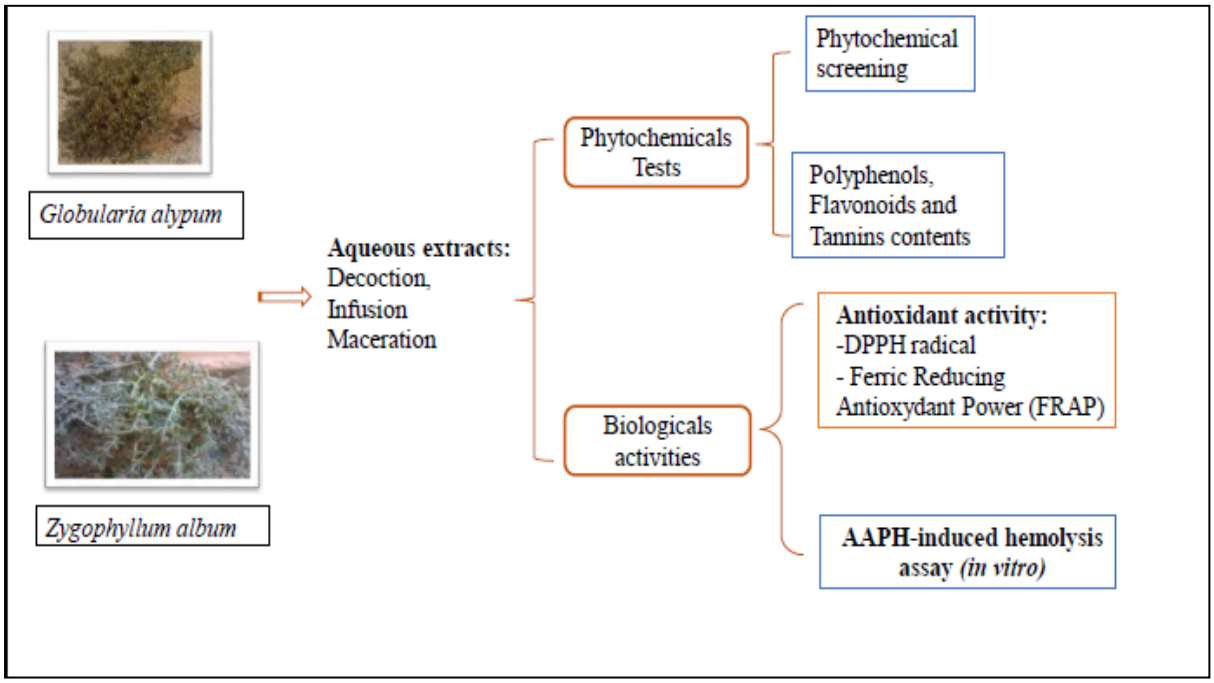
Highlights

- *G. alypum* and *Z. album* as a source of natural antioxidant ;
- The aqueous extract of *G. alypum* showed the highest level of total phenolic contents ;
- *G. alypum* extracts showed delayed hemolysis compared to control ;
- *G. alypum* extracts exhibited better antioxidant and antihemolytic activities.

Graphical Abstract



Abstract

The main intrest of this study was to evaluate the antihemolytic and the antioxidant activities of the aqueous extracts of *Globularia alypum* and *Zygophyllum album* prepared by three different methods: decoction, infusion and maceration.

Phytochemical tests, total phenolic, flavonoid and tannins contents of the different aqueous extracts were determined. DPPH radical scavenging assay and Ferric Reducing Antioxidant Power (FRAP) were employed for the evaluation of the antioxidant activity. Whereas, the antihemolytic activity was carried out by the evaluation of the protective effect of the various prepared extracts on red blood cells against the radical attack induced by 2,2′-azobis(2-amidinopropane) di-hydrochloride (AAPH). The aqueous extract of *Globularia alypum* prepared by decoction showed the highest level of total phenolic contents (196.52± 10.76 µg GAE/mg Extract). These findings confirmed the remarkably antioxidant properties of this plant with DPPH scavenging activity and FRAP test. A concentration of 160 µg/ml of this extract showed a protective effect against hemolysis induced by AAPH radical. Hence, *Zygophyllum album* extracts demonstrated lower level of total phenolic contents (30.41 ± 2.01 µg GAE/mg Extract) and lower antioxidant and antihemolytic activities.

Globularia alypum extracts presented a powerful antioxidant and antihemolytic activities especially for the decoction extract whereas *Zygophyllum album* revealed a weaker protective effect except its maceration extract.

Keywords: *Zygophyllum album*; *Globularia alypum*; phytochemical screening; phenolics compounds; antioxidant; antihemolytic.

1. Introduction

Human mortality has been significantly increased in last few decades because of the increased prevalence of diseases such as atherosclerosis, coronary heart disease and obesity. These deseases are associated with the oxidative stress (Mopuri & Islam, 2017; Forman & Zhang, 2021; Guan et al., 2021; Hajam et al., 2022). Antioxidants are naturally substances present in fruits, vegetables and medicinal plants which have the ability to trappe or neutralise free radicals (Schramm et al., 2003; Santos-Sánchez et al., 2019; Phuyal et al., 2020). Algeria possesses a rich and a little valued flora that must be studied and charachterised from its biological and chemical activities (Benyagoub et al., 2020; Hadjadj et al., 2020). *Globularia alypum L.*, locally named «Tasselgha», is a wild plant belonging to the Globulariaceae family. It is commonly used in Algerian popular medicine as antiseptic, antifongic and antidiabetic plant. Other studies indicate that *G. alypum* is used in the treatment of diabete, as hypoglycemic plant, and allergies (Fakchich and Elachouri, 2014; Asraoui et al., 2021). *Zygophyllum album L.* belongs to a large cosmopolitan family of Zygophyllaceae (Beier et al., 2003; Bellstedt et al., 2008). Its commun name is «Al-routrat». It is known and charachterised by its antidiabetic activity (Ghoul et al., 2011; Bahlil et al., 2019; Mohammedi, 2020). In the current study, we evaluated the antioxidant and antihemolytic

activities of the aqueous extracts of the aerial parts of *Z. album* and *G. alypum* prepared by decoction, infusion and maceration.

2. Material And Methodes

2.1 Plant material and extraction

The *Zygophyllum album* L. and *Globularia alypum* L. were identified in the Botany Laboratory of the Department of Biology, University of Tlemcen (Algeria). The *Z. album* was collected from the commun of elhajeb (Biskra) wherase *G. alypum* was obtained from Ain Zaatot in Batna (Algeria), in Januray 2016. The aerial parts (leaves, stems and flowers) of the two plants were washed, dried in shade, powdered and extracted using three methods of extraction: decoction (D), infusion (I) and maceration (M). The obtained extracts were filtered, concentrated and stored in refrigerator for further studies.

2.2 Phytochemical tests

Each extract was screened for the presence of precipitation (alkaloids), color (tannins, flavonoids, free quinones, terpenoids, anthraquinones), fluorescence (coumarins) or foam saponins (Bruneton, 1999).

2.3 Polyphenols, Flavonoids ant Tannins contents

2.3.1 Total Polyphenols contnent

The total phenolic contents of the various extract were estimated using the method of Folin-Ciocalteau (Li et al., 2007). Firstly, 1 mL of Folin-Ciocalteau reagent was added to 200 µL of each extract dilution. Then, 800 µL of sodium carbonate solution Na_2CO_3 was added. After incubation, the absorbance was measured at 765 nm. The phenolic contents were determined by using the standard calibration curve of gallic acid (y= 0.0113x, R^2 0.99) and results were expressed as microgram gallic acid equivalents per milligram of extract (µg GAE/mg E).

2.3.2 Flavonoids contents

Total flavonoid content was determined by the method of Ardestani and Yazdanparast (2007). Based on this method, 250 µL of the sample was mixed with 1 mL of distilled water and 75 µL of $NaNO_2$ (15%). Then, 75 µL of $AlCl_3$ (10%) and 1 mL of NaOH (4%) were added. After 6 min., the final volume was made up to 2.5 ml with distilled water. After incubation, the absorbance of the mixture was determined at 510 nm. Total flavonoid content was determined using standard calibration curve of catechin (y=0.0028x, R^2 0.99) and results were expressed as microgram of catechin equivalents per mg of extract (µg CE/mg E).

2.3.3 Total condensed tannins contents

The concentration of condensed tannins in plant extracts was determined using vanillic acid method according to Julkunen-Titto (1985). In brief, 50 µL of the sample was mixed with vanillin at 4% and concentrated hydrochlorid acid. The reading was carried out at 550 nm.

Catechin was used as the standard (y=0.0002x, R^2 0.98) and the results were expressed as microgram of catechin equivalents per mg of extract (µg CE/mg E).

2.4 Antioxidant activity

2.4.1 DPPH radical scavenging activity

Free radical scavenging activity (DPPH, 2,2-diphenyl-1-picrylhydrazyl) was applied as described by Boumerfeg et al. (2009). A volume of 50 µL of the sample from different concentrations was added to 1250 µL of DPPH (0.004%). After 30 min in darkness, the absorbance was measured at 517 nm against a negative control. The inhibition percentage of free radical DPPH was calculated as the following:

% inhibition= [(A control– A sample)*100]/A control. The A control represented the absorbance without samples.

2.4.2 Ferric Reducing Antioxydant Power (FRAP)

The reducing power of the various extracts was assayed according to the method described by Oyaizu (1986). 200 µL of the sample was added to 500 µL of phosphate buffer (0.2 M, pH 6.6) and 500 µL of potassium ferricyanide $K_3Fe(CN)_6$ at 1%. After incubation at 50°C, 500 µL of Trichloroacetic acid (10%) was added. The tubes were then centrifuged and 500 µL of the upper layer was collected and mixed with 500 µL of distilled water then 100 µL of $FeCl_3$ at 0.1%. The absorbance was measured at 700 nm. The standards used in this test were ascorbic acid and Butylated hydroxyanisole (BHA) (from0- to500 µg/mL). Results were expressed by calculating EC_{50} values form of a linear or logarithmic regression curve.

2.5 AAPH-induced hemolysis assay in vitro

The protective effect of different aqueous extracts of *Z. album* and *G. alypum* towards the erythrocytes was investigated according to the protocol described by Niki et al. (1998) and Takebayashi et al. (2010). Blood was obtained from healthy young donors and collected into heparinized tubes. Samples were centrifuged at 2500 rpm for 5 min at 4 C°. Then, the plasma was then carefully discharged. Erythrocytes were washed three times with phosphate-buffered saline (300 mOsm; pH 7.4). Thermal decomposition of AAPH (50 mM) was applied to generate aqueous peroxyl radicals inducing free radical chain oxidation in erythrocytes. To study the protective effects of the aqueous extracts against AAPH-induced hemolysis, an erythrocyte suspension (1/40) was pre-incubated with the aqueous extracts/standards (10–160 µg/mL) at 37°C for 10 min followed by incubation with AAPH radical. Ascorbic acid (AA) was used as reference. The mixture was shaken slowly every 15 min while being incubated for 5 h at 37 °C. The kinetics of progressive disappearance of red blood cells (RBCs) was followed by the dynamic measurement of decrease in absorbance at 630 nm. The resistance of RBCs to the radical attack was expressed in the absence and presence of extracts required for 50% erythrocyte lysis (The half-time of hemolysis: HT_{50}).

2.6 Statistical analysis

Experiment tests were recorded in triplicates for all the analysis. Results were calculated as the mean (n=3) ± SD (standard deviation) for each sample. The results of phenolic compounds and the antioxidants assays were demonstrated using Microsoft Excel 2010. One-way ANOVA was performed using GraphPad Prism version 5 to analyze values of antihemolytic activity.

3. Results

3.1 Phytochemicals screening

The aqueous extracts of aerial parts of *G. alypum* demonstrated the presence of flavonoids, free quinones, tannins, terpenoids, reduced compounds and saponins. On the other hand, free quinones, coumarins, flavonoids, saponins, terpanoids and reduced compounds were observed in the aqueous extracts of the *Z. album.*The results obtained are given in Table 1.

Table 1. Phytochemical screening of the aqueous extracts of the aerial parts of *G. alypum* and *Z. album*. (+ : presence, - : absence).

	G. alypum			***Z. album***		
	D	I	M	D	I	M
Saponins	+	+	-	+	+	+
Tannins	+	+	+	-	-	-
Flavonoids	+	+	+	-	+	+
Coumarins	-	-	-	+	+	+
Free quinines	+	+	+	+	+	-
Anthraquinones	-	-	-	-	-	-
Terpenoids	+	+	+	+	+	+
Alkaloids	-	-	-	-	-	-
Reduced compounds	+	+	+	+	+	+

3.2 Polyphenols, flavonoids and tannins contents

A determination of phenolics, flavonoids and tannins contents of the different extracts were realised (Table 2). It was observed that the three preparations (infusion, decoction and maceration) of the aqueous extracts of *G. alypum* contained the lowest amount of tannins. In addition, the extract prepared by maceration revealed the lowest amounts of total phenolic contents (134.82 ± 7.05µg GAE/ mg E) and total flavonoids contents (47.52 ± 1.83 µg CE/ mg E).

Table 2. Polyphenols, flavonoids and tannins contents of the aqueous extracts of the aerial parts of *G. alypum* and *Z. album*.

	Polyphenols contents (µg GAE/mg E)	Flavonoids contents (µg CE/mg E)	Tannins contents (µg CE/mg E)	IC_{50} (µg/mL)	EC_{50} (mg/mL)
GA D	196.52± 10.76	228.79± 10.45	23.36± 11.71	25.62± 0.48**	0.33± 0.01
GA I	195.50± 2.90	223.82± 9.12	2.15± 0.77	23.04± 0.73*	0.31± 0.01
GA M	134.82± 7.05	47.52± 1.83	7.1± 3.11	85.37± 1.48***	0.68± 0.19
ZA D	22.88± 6.76	5.11± 1.98	11.15± 5.44	719.6± 11.36***	20.22± 0.41***
ZA I	28.47± 1.45	9.56± 3.10	4.45± 5.02	765.4± 21.44***	8.33± 1.31***
ZA M	30.41± 2.01	5.65± 0.21	16.73± 8.45	446.1± 2.36***	15.56± 1.09***

Values were expressed as the mean ± SD in triplicate. Comparisons were made with ascorbic acid (IC_{50}= 3.74 ± 0.16µg/mL, EC_{50}= 0.13± 0.002 mg/mL) and BHA (IC_{50}= 1.97 ± 0.05µg/mL, EC_{50}= 0.09 ± 0.003 mg/mL), (*: significatif : $p \geq 0.05$ *: $p \leq 0.05$, **: $p \leq 0.01$, ***: $p \leq 0.001$). GAD: *G. alypum* decoction; GAI: *G. alypum* infusion*; GAM: G. alypum* maceration; ZAD: *Z. album* decoction; ZAI: *Z. album* infusion; ZAM: *Z. album* maceration.

3.3 Antioxidant activity

The study of the antioxidant activity was estimated using two methods: DPPH radical scavenging assay and the ferric reducing antioxidant power (FRAP).

The obtained results showed that the scavenging activity of *G. alypum* (IC_{50} 223.04± 0.73 to 85.37± 1.48 µg/mL and EC_{50} 0.31± 0.01 to 0.68± 0.19 mg/mL) was better than *Z. album* (IC_{50} 446.1± 2.36 to 765.4± 21.44 µg/mL and EC_{50} 8.33± 1.31 to 20.22± 0.41 mg/mL), but lower than the standard (ascorbic acid and BHA) (Table 2). There was no significant difference between the EC_{50} of the decoction and infusion extracts of *G. alypum*. Among the aqueous extracts of *Z. album*, the infusion extract showed the most important activity in ferric reducing antioxidant (EC_{50} 8.33± 1.31 mg/mL) which was significant compared to ascorbic acid (0.13± 0.002 mg/mL) and BHA (0.09± 0.003 mg/mL).

3.4 AAPH-induced hemolysis assay

From the kinetics of hemolysis (Figures 1 and 4), the hemolysis inhibition was noticed in all extracts (both *G. alypum* and *Z. album*). Generally, the curves of tested extracts of *G. alypum* showed a hemolysis delay compared to the control (Figure 1) which was confirmed by the values of HT_{50} (Figure 2). The powerful activity was noticed for 160µg/mL in the extracts of *G. alypum* (203.9 ± 2.85, 219.3 ± 4.51 and 221.9 ± 7.42 min, respectively to *G. alypum* (D), *G. alypum* (I) and *G. alypum* (M). In addition, these results were considered slightly less than ascorbic acid (Figure 3). These tested concentrations (10, 20, 40 µg/ml) of the aqueous extracts of *Z. album* (Figure 4) showed a shift of sigmoid to the left of the control contrary to the 80 and 160µg/ml concentrations. These results presented a low protective activity in interaction with the erythrocyte membrane compared with the *G. alypum* extracts (Figures 4

and 5) and ascorbic acid which could explain the hemolysis effect of *Z. album* depending on concentrations.

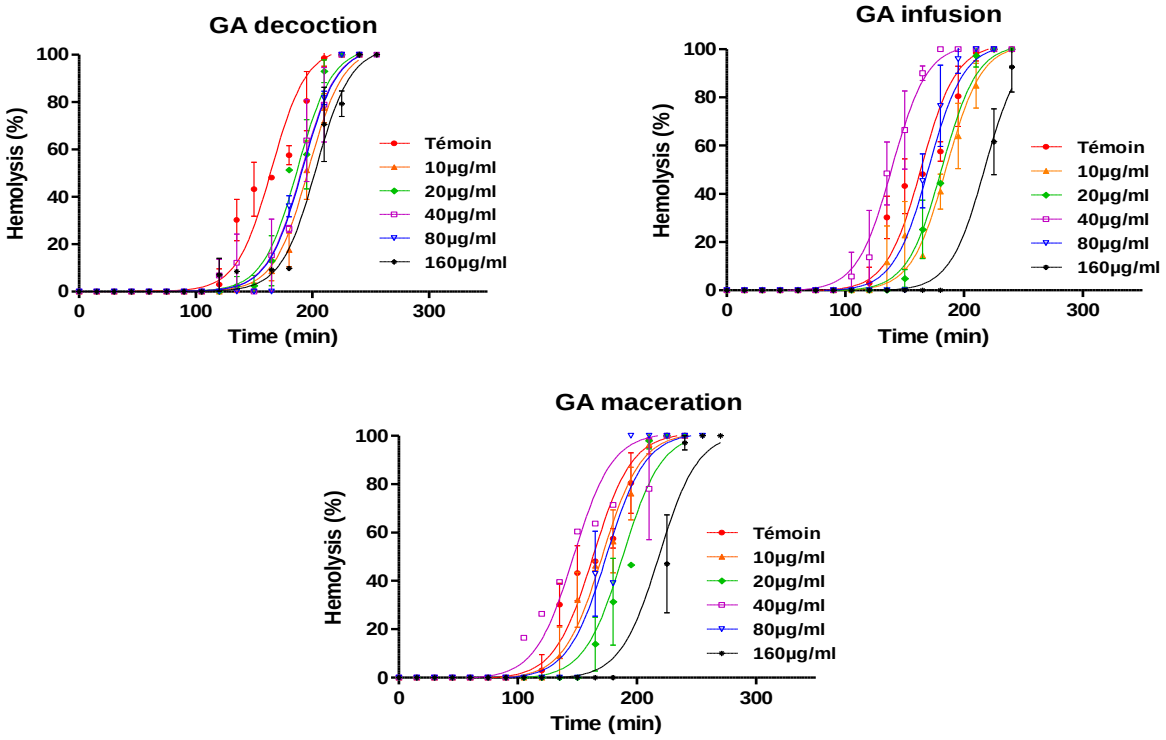


Figure 1. Hemolysis curve of fresh human blood in the presence of AAPH and different concentration of of *G. alypum* extracts. Data are mean ± SD (n=4).

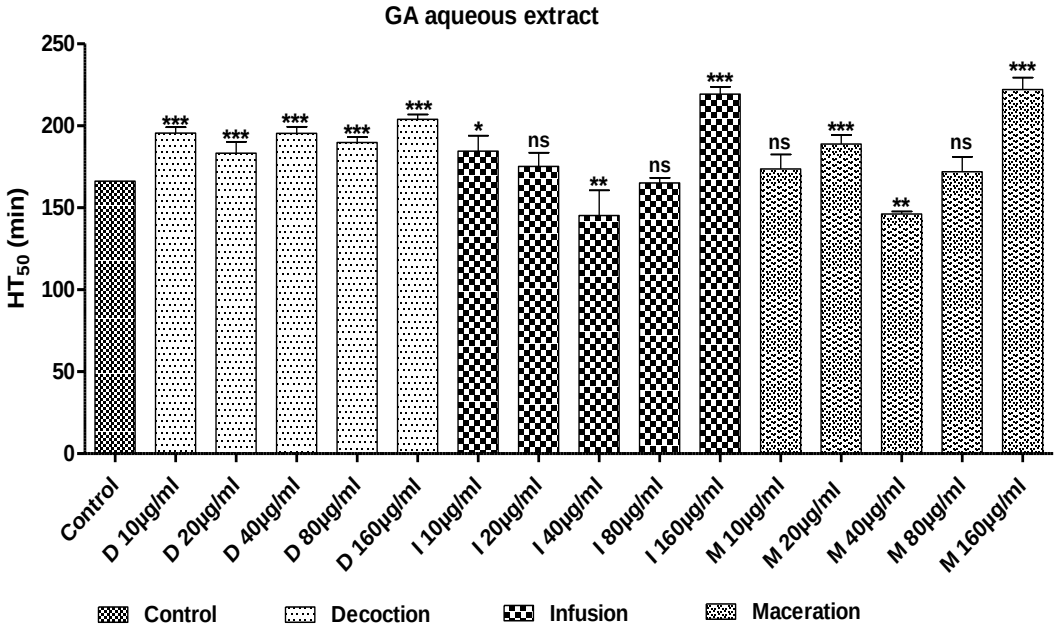


Figure 2. HT_{50} of the different concentrations of of *G. alypum* extracts. Comparison was realised against Control (ns: $p \geq 0.05$, ***: $p \leq 0.001$).

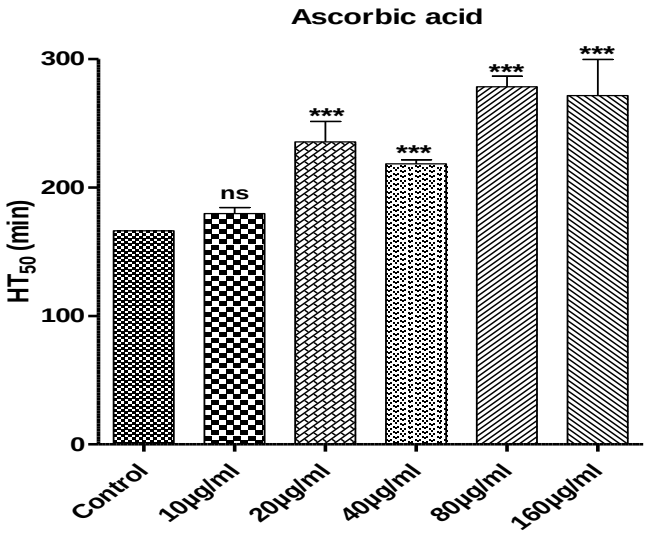


Figure 3. HT_{50} of the different concentrations of ascorbic acid. Comparison was realised against Control (ns: $p \geq 0.05$, ***: $p \leq 0$.

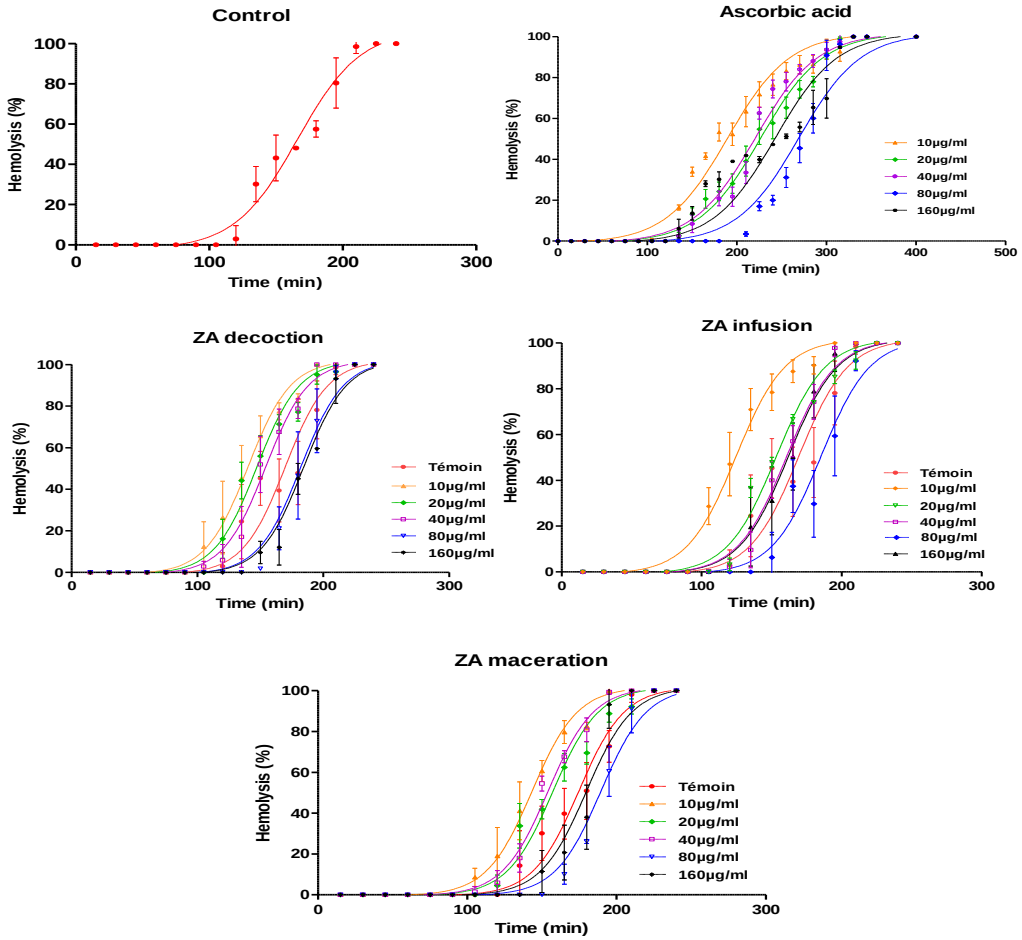


Figure 4. Hemolysis curve of fresh human blood in the presence of AAPH and different concentration of AA and *Z. album* extracts. Data are mean ± SD (n=3).

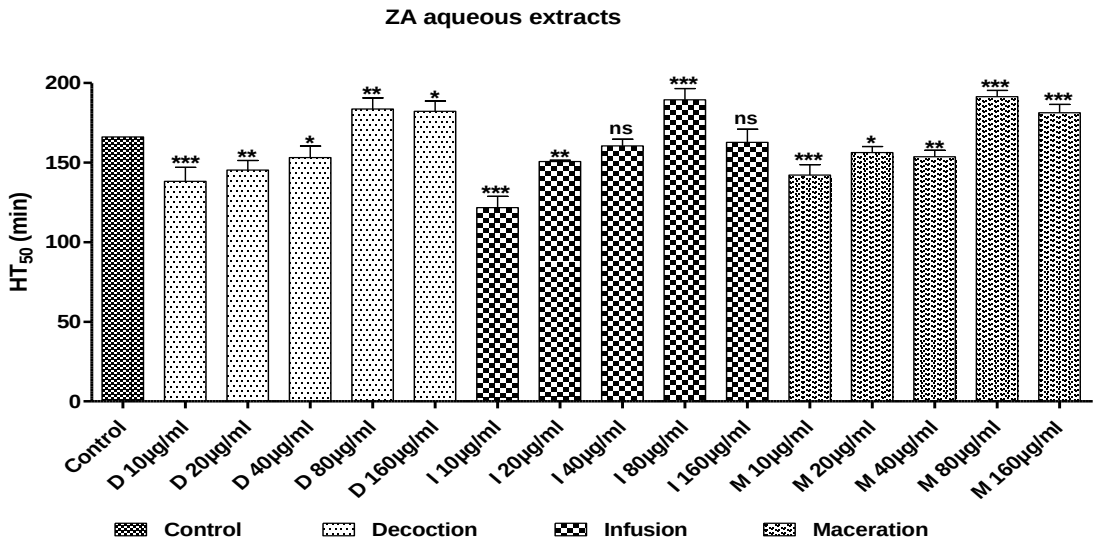


Figure 5. HT_{50} of the different concentrations of *Z. album* extracts. Comparison was realised against Control (ns: $p \geq 0.05$, ***: $p \leq 0.001$).

4. Discussion

Aerial parts of *G. alypum* and *Z. album* are recognized by their biological activities, especially antidiabetic, anti-inflammatory and antioxidant activities (Djeridane et al., 2006; Belmimoun et al., 2016). Azzi et al. (2012), Ouelbani et al. (2016) and Telli et al. (2016) reported that these medicinal plants are used in the Algerian tradition for the treatment of many diseases. In the present study, the phytochemical screening of the various extracts of both plants showed the presence of a large range of secondary metabolites. Amessis-Ouchemoukh et al. (2014) reported that several studies affirmed the presence of polyphenols, terpenoids and coumarins contrary to saponins in *G. alypum*. Ksouri et al. (2013), were confirmed the presence of polyphenols, saponins and terpanoids in *Z. album.* Elgamal et al. (1995) and Hassanean et al. (1993) identified and isolated a saponin triterpenoid and a quinovic acid from the aerial parts of *Z. album* which are probably responsible for many biological activities.

Regarding the data collected in the study of different aqueous extracts of *Z. album*, lower contents in phenolics, flavonoids and tannins were observed. Our results were different from those obtained by Belguidoum et al. (2015) and Belmimoun et al. (2016) on the hydroethanolic extract prepared by maceration and the methanolic extracts, respectively. This variation in phenolics contents is also dependent on the season of collection, geographic site and the temperature. The maceration method is considered as the most suitable because it prevents the degradation of molecules (Csupor, 2015).

The study of the antioxidant activity was estimated using two methods: DPPH radical scavenging assay and the ferric reducing antioxidant power.

Polyphenols and flavonoids are known for their power to scavenge free radicals and inhibit lipid peroxidation (Punitha et al., 2005; Djeridane et al., 2006). For its active components, *G. alypum* is considered interesting for the treatment of diseases related to oxidative stress.

We have chosen the oxidative hemolysis of human erythrocytes as a model to study the free radical-induced damage of biological membranes and the protective effect of the different studied extracts as a barrier against the free radicals formed by the peroxidation process (Girard et al., 2006). The erythrocyte is rich in polyunsaturated fatty acids susceptible to be oxidized that one initiating radical could lead up to twenty propagation reactions (Niki et al., 1988). In this process, the red blood cell was treated by a water-soluble azo compound AAPH that generates free radicals by its unimolecular thermal decomposition (Sato et al., 1995), which could attack the RBC membrane to induce lipid peroxidation and cause hemolysis. This kind of hemolysis provided a good approach for the study of the peroxidation of biomembranes and the action of native bioantioxidants (vitamin E and ubiquinol-10) (May et al., 1998; Takebayashi et al., 2010). Several studies confirmed the antihemolytic effect of hydromethanolic extracts prepared from medicinal plants (Ramchoun et al., 2015; Chansiw et al., 2018). In this study, the hemolysis inhnibition was noticed in all extracts, both *G. alypum* and *Z. album*. This activity could be attributed to many factors, including the nature of flavonoids to be effective scavengers against free radical-induced damages, the degree of their ability to penetrate lipid bilayers in erythrocyte (Blasa et al., 2007), the contact between the endogeneous and exogenous antioxidant, the capacity of the erythrocyte to increase the defense against free radicals and the sturcture-activity relationship properties (Es-Safi et al., 2007). Moreover, the hemolysis is lagged because of endogenous antioxidants presented by a number of mechanisms such as enzymes and vitamins which can protect erythrocyte from damage (Yousif et al., 2012). It was demonstrated that the binding of flavonoids with the RBC membrans inhibites significantly the lipid peroxidation and enhances their integrity against lysis (Yousif et al., 2012).

From the results of phytochemichal screening, *Z. album* was charachterised by the presence of saponins which are the principal cause of the blood hemolysis. Baumann et al. (2000) reported that the hemolytic properties are generally attributed to the interaction between the saponins and the sterols of the erythrocyte membrane. As a result, the membrane bursts causing an increase in permeability and a loss of haemoglobin. In addition, *Z. album* contained lower amount of phenolic compounds.

5. Conclusion

The three modes of preparation of the extracts (infusion, decoction and maceration) revealed different results. Thus, the compounds presented in *G. alypum* (D) exhibited an antioxidant and antihemolytic activities. The present study highlights the use of this plant as a source of natural antioxidant. The *Z. album* extracts showed the lowest antioxidant and antihemolytic activities. It contained saponins which are considered as responsible of the hemolysis effects. It would be interesting to complete this study with separation, identification or purification of bioactive components in the extracts especially for *G. alypum* and to test the activity of the

fractions. Moreover, further studies concerning the *in vivo* methods are needed. Toxicological study is required.

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Author Contribution Statement

Azzi Rachid supervised the findings of this work; Khaoula OUFFAI and Fayza ABBOOU: Carried out the survey; Farid Boucif LAHFA: discussed the results and contributed to the final manuscript.

Conflict of interest

No conflict of interest was reported by the authors.

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