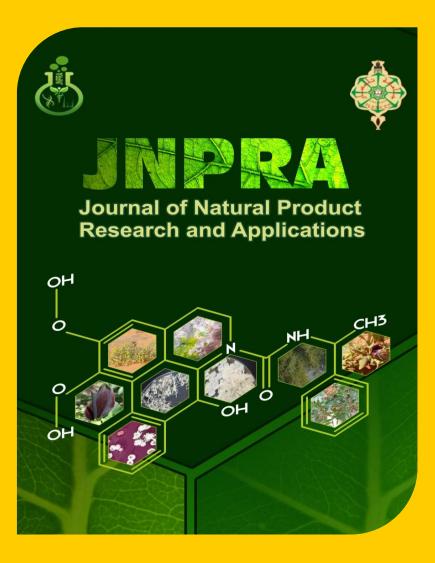
Polyphenolic Profile and Comparative Study on Phytochemicals and Antioxidant Activity of Extracts from All Parts of *Gymnocarpos decander* Forsk.

> Amel ZITOUNI Nabila BELYAGOUBI-BENHAMMOU Asma EL ZEREY-BELASKRI Fethi TOUL Nassira GHEMBAZA Fawzia ATIK-BEKKARA







## Journal of Natural Product Research and Applications (JNPRA)



Home page : https://journals.univ-tlemcen.dz/JNPRA/index.php/JNPRA

**Research Article** 

# Polyphenolic Profile and Comparative Study on Phytochemicals and Antioxidant Activity of Extracts From all Parts of *Gymnocarpos decander* Forsk.

## Amel ZITOUNI<sup>1,2</sup>\*, Nabila BELYAGOUBI-BENHAMMOU<sup>1</sup>, Asma El ZEREY-BELASKRI<sup>3,4</sup>, Fethi TOUL<sup>1</sup>, Nassira GHEMBAZA<sup>1</sup>, Fawzia ATIK-BEKKARA<sup>1</sup>

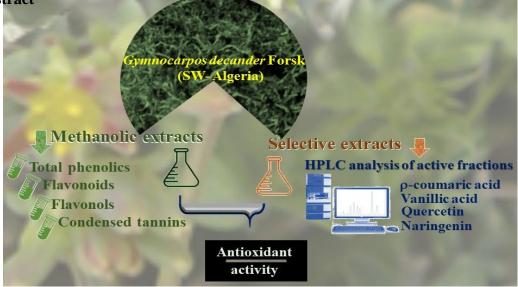
<sup>1</sup>Laboratory of Natural Products, Department of Biology, Faculty of Natural and Life Sciences, Earth and Universe, University Abou- Bekr Belkaïd, 13000 Tlemcen, Algeria. <sup>2</sup>Department of Agri-food, faculty of Science and Technology, University of Ain Temouchent, ALGERIA.<sup>3</sup>Department of Biology, Faculty of Natural and Life Sciences, University Ahmed Ben Bella, Oran1, Algeria. <sup>4</sup>Laboratoire de recherche ' Biodiversité Végétale : Conservation et Valorisation'. Université Djillali LIABES, Sidi Bel Abbes , Algérie

\*Corresponding author: biolamel@yahoo.fr.

## Highlights

- Total phenolic, flavonoid, flavonol and condensed tannin contents were evaluated.
- The antioxidant activity was assessed in different parts.
- Detection of quercetin, ρ-coumaric acid, naringenin and vanillic acid in flowers.

## **Graphical abstract**



#### Abstract

In the current study, methanolic and selective extracts from different parts of *Gymnocarpos decander* were screened for total phenolic, flavonoid, flavonol and condensed tannin contents. The antioxidant activity of extracts was also determined. The highest values of total contents of phenolics (156.097  $\pm$  2.312 mg GAE/g DM), flavonoids (14.878  $\pm$  0.275 mg CE/g DM), condensed tannins (39.388  $\pm$  1.599 mg CE/g DM) and flavonols (6.506  $\pm$  1.021 mg QE/g DM) were found in flowers. The most powerful antioxidant was found in the methanolic extract of flowers (32.27  $\pm$  2.400 mg AAE/g DM). Tannins extracted from flowers showed an interesting antioxidant activity to trap the 1,1-Diphenyl- 2-picrylhydrazyl (DPPH) free radical (0.063  $\pm$  0.000 mg/mL) and to reduce iron absorption (0.083  $\pm$  0.004 mg/mL). The highest activity in the  $\beta$ -carotene test was found in the butanolic fraction of flowers (0.314  $\pm$  0.008 mg/mL). A high performance liquid chromatography (HPLC) analysis allowed the detection of quercetin,  $\rho$ -coumaric acid and naringenin in the ethyl acetate and butanolic fractions of flowers, and also vanillic acid in the ethyl acetate fraction of flowers.

*Keywords: Gymnocarpos decander*; Polyphenolic compounds; Antioxidant activity; Identification; HPLC.

### 1. Introduction

Plants with medicinal properties play an increasingly important role in food and pharmaceutical industries for their functions on disease prevention and treatment (Manyou et al., 2021). Recently, there has been worldwide interest in finding new and safe antioxidants from natural sources, to prevent oxidative stress and to minimize oxidative injury of living cells, for the well-being and life prolongation, as well as for the prevention of many diseases (Hasbal et al., 2014). Several studies have shown that phenolic compounds, from natural antioxidants, are biologically- active compounds able to protect against free radical reactions (Toul et al., 2016; Belyagoubi- Benhammou et al., 2018; Ghembaza et al., 2021; Manyou et al., 2021).

Algeria has a rich medicinal plant heritage of wide diversity; medicinal plants are commonly prescribed by the traditional healers to treat several diseases (Baba aissa, 1999). Our choice fell on *Gymnocarpos decander*, a steppe-desert rocky shrub of the family of Caryophyllaceae. According to our ethnobotanical survey with the indigenous population of the Algerian Sahara (data not published), this plant, called in Arabic '*Djefna*', is used for the treatment of digestive diseases by traditional therapies. The whole plant or its aerial parts is used against helmintiasis and has some traditional uses in the treatment of kidney stones, psychosomatic diseases, and to break down an evil eye and bad spirits (Maiza et al., 2011; Ahmed et al., 2016). Moreover, the potential antimalarial efficacy of a hydroalcoholic extract was reported (Kaiser et al., 2007). According to Tahrouni et al. (2010), this steppic plant is considered as highly palatable to wild and domestic animals. It is widely known that animals consume instinctively medicinal plants to treat diseases from which they suffer.

Few studies have been conducted on this plant. Javidnia et al (2008), from Iran, was able to determine the chemical composition of most *G. decander* essential oils; they showed that their main compounds are hexadecanoic acid, dodecanoic acid and vinyl guaiacol. Another phytochemical investigation of *Gymnocarpos decander* aerial parts extract realized by Bechlem et al (2017), afforded many new phenolic compounds, such as flavonoids and saponins.

To the best of our knowledge, there is no detailed study reporting the antioxidant activity and phenolic profile of the different parts of G. decander. The current investigation aims to quantify the contents of phenolic compounds, evaluate the antioxidant properties of the extracts from the different parts (leaves, stems, flowers and roots) of G. decander, and also identify the molecules that are responsible for this activity, using HPLC analysis.

## 2. Materials and Methods

## 2.1. Standards and reagents

All standards and chemicals were purchased from Sigma Chemical Co. (St. Louis, Mo, USA).

## 2.2. Plant material

The various organs (leaves, stems, flowers and roots) of *Gymnocarpos decander* were collected in April 2014 from Tyout in Naama located in west of Algeria. Specimens were identified in the Laboratory of "Plant Ecology", and voucher specimen was deposited at the Herbarium of the Department of Biology, Tlemcen University, Algeria. The samples were air-dried in shadow at room temperature, and reduced to fine powder, just before extraction.

### 2.3. Preparation of methanolic extracts

The powder (1 g) of each part of *G. decander* was placed in 20 mL of methanol for 24 h. After filtration, the resulting solution was evaporated under vacuum at  $60^{\circ}$  C. The residue was then dissolved in 3 mL of methanol (Benhammou et al., 2009).

### 2.4. Ethyl acetate and butanolic fractions

The dry residue obtained by the same procedure for methanolic extract for each part, was treated with 10 mL of boiling water to dissolve the flavonoids. The filtrate aqueous solution was firstly extracted with 10 mL of ethyl acetate, then with 10 mL of butanol-1. The two fractions were evaporated and dissolved in 3 mL of methanol (Bekkara et al., 1998).

### 2.5. Tannins

The powder of plant material (2.5 g) was extracted with 50 mL acetone-water (35/15, (v/v) for 72 h at room temperature. Then, the mixture was filtrated and evaporated under vacuum at 40 °C to remove acetone. The remaining solution was washed with 15 mL of dichlomethane to remove lipidsoluble substances, then later extracted with ethyl acetate. The

water layer was separated and extracted twice more similarly. Then resulting water layers were evaporated to dryness, and dissolved in methanol (Zhang et al., 2008).

## 2.6. Saponins

Each part of plant was delipided during 2 h by 75 mL of n-hexane. After elimination of the organic phase, the precipitate obtained was macerated in 25 mL of absolute ethanol under magnetic agitation at the ambient temperature during 24 h. The ethanolic phase was evaporated at 40 °C. The dry residue was extracted by 50 mL from distilled water/petroleum ether mixture (V/V) heated at 50 °C in water bath during 30 min. The aqueous phases were mixed then treated by 5 mL of butanol during 30 min. The organic phase, evaporated at 40 °C, was weighed and dissolved in 3 mL of methanol (Bouchelta et al., 2005).

#### 2.7. Total phenolic content

The amounts of total phenolic compounds in methanolic extracts of leaves, stems, flowers and roots were determined by spectrometry method using Folin-Ciocalteu reagent (Singleton and Rossi., 1965). A volume of 0.2 mL of the extracts was mixed with 1 mL of Folin-Ciocalteu reagent diluted 10 times with water and 0.8 mL of a 7.5% sodium carbonate solution in a test tube. 30 min, the absorbance was measured at 765 nm. Gallic acid was used as a standard. The total phenolic content was expressed as milligrams of gallic acid equivalents per gram of dry matter (mg GAE/g DM).

#### 2.8. Total flavonoid content

A volume of 500  $\mu$ L of methanol solution at different concentrations of extract was added to 1500  $\mu$ L distilled water. 150  $\mu$ L of sodium nitrite (NaNO<sub>2</sub>) to 5% was added to the mixture. After 5 min, 150  $\mu$ L of aluminium trichloride (AlCl<sub>3</sub>) 10% (m/v) was added. After incubation for 6 min at room temperature, 500  $\mu$ L of sodium hydroxide (NaOH) (1M) was added. The absorbance of the solution was determined at 510 nm against the blank. The total flavonoid content of extracts was expressed as milligrams catechin equivalents per gram of dry matter (mg CE/g DM) (Zhishen et al., 1999).

#### 2.9. Total condensed tannins

The amounts of condensed tannins were estimated using the method of vanillin (Julkunen-Titto, 1985). A volume of 50  $\mu$ L of the methanolic extract was added to 1500  $\mu$ L of vanillin/methanol solution (4%, m/v). Then, 750  $\mu$ L of concentrated hydrochloric acid (HCl) was added and allowed to react at room temperature for 20 min. Absorbance was measured at 550 nm. The concentration of tannins was estimated as milligrams catechin equivalents per gram of dry matter (mg CE/g DM).

#### 2.10. Total flavonol content

The flavonol content was determined as described by Kumaran et al (2007). 0.25 mL of the crude extracts were mixed with 0.25 mL of AlCl<sub>3</sub> (2 mg/mL) and 1.5 mL of sodium acetate

(50 mg/mL). The absorbance at 440 nm was recorded after 150 min. The flavonol content was expressed as milligrams of quercetin equivalents per gram of dry matter (mg QE/ g DM).

## 2.11. Reducing power assay

Various concentrations of the extracts (mg/ mL) were mixed with phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and 1% of potassium ferricyanide water solution (2.5 mL). The mixtures were incubated at 50°C for 20 min. Trichloracetic acid aqueous solution (2.5 mL, 10%) were added to the mixtures which were then centrifuged for 10 min. The supernatants (2.5 mL) were mixed with distilled water (2.5 mL) and a FeCl<sub>3</sub> solution (0.5 mL, 0.1%). The absorbance was measured at 700 nm. Gallic acid was used as a positive control (Oyaizu, 1986).

### 2.12. DPPH scavenging assay

A volume of 50  $\mu$ L of various concentrations of the extracts was added to 1950  $\mu$ L of DPPH methanol solution (0.025 g/L). After 30 min incubation period at room temperature, the absorbance was read against a blank at 515 nm. DPPH free radical scavenging activity in percentage (%) was calculated using the following formula:

DPPH scavenging activity (%) =  $(A_{blank} - A_{sample} / A_{blank}) \times 100$ Where  $A_{blank}$  is the absorbance of the control,  $A_{sample}$  is the absorbance of the test compound. Extract concentration providing 50% inhibition (EC<sub>50</sub>) was calculated from the graph plotted of inhibition percentages against extract concentrations. The ascorbic acid methanol solution was used as positive control (Sanchez-Moreno et al., 1998).

## 2.13. β-carotene bleaching assay

β-carotene (2 mg) was dissolved in 10 mL of chloroform and 1 mL of β-carotene solution was mixed with 20 mg of purified linoleic acid and 200 mg of Tween 40 emulsifier. Chloroform was then evaporated; the resulting mixture was immediately diluted with 100 mL of distilled water. 4 mL of this emulsion, 0.2 mL of different concentrations of *G. decander* extracts was added. The absorbance at 470 nm, which was regarded as t<sub>0</sub>, was measured, immediately, against a blank. The capped tubes were placed at 50 °C for 120 min. For the positive control, sample was replaced with gallic acid or BHA. A negative control consisted of 0.2 mL distilled water or solvent instead of extract or reference antioxidants. The antioxidant activity (AA) was measured in terms of successful bleaching of β-carotene by using the following equation:

AA= ((A<sub>A(120)</sub> - C<sub>C(120)</sub>/(C<sub>C(0)</sub> - C<sub>C(120)</sub>) × 100

 $A_{A (120):}$  is the absorbance in the presence of the extract at 120 min;  $C_{C (120):}$  is the absorbance of control at 120 min;  $C_{C(0)}$ : is the absorbance of control at 0 min (Moure et al., 2000).

### 2.14. Total antioxidant capacity

0.3 mL of sample was mixed with 3 mL of standard reagent (0.6 M sulfuric acid, 28 mM sodium phosphate, 4 mM ammonium molybdate). Reaction mixture was incubated at 95°C

for 90 min. The absorbance was measured at 695 nm. Antioxidant activity of the samples was expressed as milligrams of ascorbic acid equivalent (mg AAE/ g DM) and gallic acid equivalent per gram of dry matter. The sample was analyzed in triplicate (Prieto et al., 1999).

## 2.15. HPLC analyses (high performance liquid chromatography)

## 2.15. 1. Hydrolysis for HPLC

The selected extracts were hydrolysed with 1.2M HCl by refluxing in a water bath for 1 h. All samples were filtered before injection (Hertog et al., 1992).

## 2.15. 2. Chromatographic separation of phenolic compounds by HPLC

A 20  $\mu$ L of sample solution was separated using HPLC. Mobile phase consisted of solvent A (water/formic acid 0.4%) and solvent B (acetonitrile). Solvent gradient was used as followed: 0–2 min, 1% B; 2–15 min, 7% B; 15–25 min, 20% B; 25-35 min, 40% B; 35-46 min, 100% B; 46-47 min, 100% B; 47-48 min, 1% B; 48-55 min, 1% B. The flow rate was 1.2 mL /min. UV-detection was performed at 280 nm and identified phenolic compounds were quantified by comparison with curve constructed with solutions of each pure commercial compound. Standards including tannic acid, rutin, gallic acid, ascorbic acid, vanillic acid, p-coumaric acid, catechin, syringic acid, ferulic acid, quercetin and naringenin. Standards are prepared freshly and immediately injected to HPLC column.

## 2.16. Statistical analysis

Data were expressed as means  $\pm$  standard derivation (SD) using Excel programme and Origin 6. For the quantification of phenolic compounds and total antioxidant capacity, all experiences were repeated in triplicates.

## 3. Results and discussion

## 3.1. Total phenolic, condensed tannin, flavonoid and flavonol contents

Total phenolic, condensed tannin, flavonoid and flavonol contents of different parts of *G. decander* are shown in Table 1. It is clearly observed that the methanolic extract of flowers contain the highest total phenolic content ( $156.097\pm2.312$  mg GAE/g DM). This extract also shows a high content of flavonoids ( $14.878\pm0.275$  mg CE/g DM), flavonols ( $6.506\pm1.021$  mg QE/g DM) and condensed tannins ( $39.388\pm1.599$  mg CE/g DM). The high amount of flavonoids in flowers may be due to the presence of the anthocyanins. These are responsible for the red-purple coloration found in the Caryophyllaceae flowers and fruit (Tanaka et al., 2008). In fact, anthocyanins characterize the Caryophyllaceae family (a synapomorphic characteristic), while the other Caryophyllales are characterized by the pigments betalaines (Bate-Smith, 1962; Mabry, 1980). The flower coloration seems to be an important strategy for *G. decander* to attract pollinators in the open area, such as the vast Algerian steps where it grows. Moreover, the leaves were found to have higher amounts of phenolic compounds as

compared to the other parts. The contents of phenolic compounds in stems and roots were not significantly different.

In another study, Bouaziz et al (2009) reported that the methanolic extract of the aerial part of *G. decander* from Tunisia is rich in phenolic compounds. The total contents of phenolics, flavonoids and flavonols in this extract were found to be  $60\pm7$  (mg PyE/100 g extract),  $21\pm3$  (mg RuE/100 g extract) and  $11\pm1$  (mg QE/100 g extract), respectively.

**Table 1.** Total phenolics, flavonoids, flavonols and condensed tannins contents in different parts of *G. decander*.

Plant organs	Total phenolic content (mg GAE/g DM)	Flavonoids (mg CE/g DM)	Condensed tannins (mg CE/g DM)	Flavonols (mg QE/g DM)
Leaves	$9.734 \pm 0.181$	$2.499 \pm 0.032$	$7.602 \pm 0.179$	$1.463\pm0.085$
Stems	$7.504\pm0.347$	$1.919\pm0.008$	$5.961 \pm 0.372$	$0.614\pm0.053$
Flowers	$156.097 \pm 2.312$	$14.878 \pm 0.275$	$39.388 \pm 1.599$	$6.506 \pm 1.021$
Roots	$6.868 \pm 0.338$	$1.692\pm0.041$	$4.712\pm0.686$	$0.373\pm0.015$

Values were the mean of tree replicates  $\pm$  SD; DM: dry matter; GAE: gallic acid equivalents; CE: catechin equivalents; QE: quercetin equivalents.

#### 3.2. Antioxidant activity

#### 3.2.1. Reducing power assay

The results reported in Table 2 reveal that all compounds showed an *iron-reducing activity*. The extract from *G. decander* flowers exhibited a higher reducing power when compared to the other parts of that plant. The tannins extract of flowers showed the highest activity with an EC<sub>50</sub> value equal to  $0.083 \pm 0.004$  mg/mL, which is close to that of gallic acid ( $0.060 \pm 0.00$  mg/mL). It has been indicated, in others studies, that the tannins extract exhibits high reducing power activity (Abu zarin et al., 2016). Moreover, the recorded data showed that the other extracts have an interesting reducing power that ranges from  $0.239 \pm 0.015$  to  $3.663 \pm 0.026$  mg/mL.

## 3.2.2. DPPH radical scavenging activity

The results of the antioxidant activity are given in Table 2. The highest DPPH scavenging activity was found in tannins from flowers ( $0.063\pm0.000 \text{ mg/mL}$ ). This value indicated better antioxidant activity, even with respect to the ascorbic acid ( $0.090\pm0.002 \text{ mg/mL}$ ). This may be due to the high content of tannins found in the methanolic extract of flowers (39.388 mg CE/g DM). On the other hand, the methanolic extract of roots ( $6.260 \pm 0.052 \text{ mg/mL}$ ) and leaves ( $5.892 \pm 0.065 \text{ mg/mL}$ ) revealed the lowest hydrogen-atom donating ability. This result remains low in comparison with the one reported in the study of Bouaziz et al (2009). The EC<sub>50</sub> concentrations found by these authors were respectively ( $1.36\pm0.22$ ) and ( $1.43\pm0.24$ ) µg/mL, for methanolic extract and ethyl acetate extract of the aerial part of *G. decander*. For the remaining extracts, investigated in the present study, the EC<sub>50</sub>

concentrations were between  $(0.322 \pm 0.001)$  and  $(4.485\pm0.062)$  mg/mL. These extracts were 3 to 50 times less active than the ascorbic acid.

#### 3.2.3. $\beta$ –*Carotene/linoleic acid assay*

Saponin (EC<sub>50</sub> = 0.082±0.013 mg/mL) and methanolic extracts (EC<sub>50</sub> = 0.090±0.016 mg/mL) of leaves revealed the highest capacity to inhibit  $\beta$ -carotene when compared to all extracts from the other parts of the plant. According to the results of Khan et al (2015), saponins possess strong antioxidant properties. The methanolic extract was found to be more active in this test; this is probably due to the synergy between the molecules found in this extract, their nature and also to the effect of the oily medium used in this test. Furthermore, flower extracts that presented the best activity in previous tests (aqueous medium) showed a lower activity; this may be attributed to the nature of phenolic compounds that were found in this extract. As for the other extracts, the EC<sub>50</sub> concentrations varied between 0.121 ± 0.020 and 1.365 ± 0.104 mg/mL. These extracts turned out to be more active than gallic acid (EC<sub>50</sub> = 3.220 ± 0.020 mg/mL) but less active than the positive control BHT (EC<sub>50</sub> = 0.010 ± 0.000 mg/mL).

#### 3.2.4. Total antioxidant capacity

The results obtained indicate that all extracts have a high antioxidant capacity. The methanolic extract of flowers ( $32.27 \pm 2.400 \text{ mg AAE/g DM}$ ,  $20.281\pm1.508 \text{ mg GAE/g DM}$ ) revealed the highest antioxidant capacity compared to other extracts. According to Razali et al (2008), plants containing high levels of phenolic compounds exhibit a high antioxidant capacity.

#### 3.3. Identification and quantification of phenolic compounds using HPLC

High-performance liquid chromatographic (HPLC) analysis was used to select the two most active extracts (ethyl acetate and butanolic fractions of flowers) out of the twenty under studied. The phenolic compounds existing in these fractions were identified by comparing their retention times to those of eleven reference standards, namely tannic acid, rutin, gallic acid, ascorbic acid, vanillic acid,  $\rho$ -coumaric acid, catechin, syringic acid, ferulic acid, quercetin and naringenin.

The results of the quantitative analysis ( $\mu g/g$  DM) of each identified compound are presented in Table 3. The ethyl acetate fraction of flowers contains ascorbic acid, vanillic acid,  $\rho$ -coumaric acid, quercetin and naringenin. The amounts of identified compounds varied widely; they ranged from 623.14  $\mu g/g$  DM for naringenin to 18.24  $\mu g/g$  DM for vanillic acid. However, tannic acid, rutin, gallic acid, catechin, syringic acid, ferulic acid were not found. The butanolic fraction of flowers encloses  $\rho$ -coumaric acid, quercetin and naringenin.

From these results, it can easily be stated that the most abundant molecules in these two fractions are quercetin and naringenin. Quercetin, a major representative of the flavonol subclass, has both antioxidant and anti-inflammatory activities and prevents cancer (Shaik et al., 2006; Skider et al., 2014). According to Ashraful et al (2014) and Subburaman et al (2014), naringin and its aglycone naringenin were found to display strong anti-inflammatory

activity; they are potential bioflavonoids with excellent antioxidant properties and freeradical scavenging capability.

Table 2. Reducing power	DPPH radical sc	cavenging, β-caroten	e bleaching and total
antioxidant capacity of bioa	ctive compounds fi	from leaves, stems, f	lowers and roots of G.
decander.			

	Tests	Bioactive	Leaves	Stems	Flowers	Roots
		compounds				
		Methanolic extract	$2.242\pm0.044$	$1.643\pm0.023$	$0.107\pm0.007$	$3.663 \pm 0.026$
	<b>Reducing</b> power	Ethyl acetate fraction	$1.207\pm0.021$	$0.511 \pm 0.007$	$0.237\pm0.001$	$0.769\pm0.000$
	lci Vel	Butanolic fraction	$1.065\pm0.002$	$0.659 \pm 0.000$	$0.141\pm0.003$	$0.239 \pm 0.015$
	keducing power	Tannins	$1.151\pm0.016$	$1.229\pm0.007$	$0.083 \pm 0.004$	$1.246\pm0.001$
	L R	Saponins	$1.399\pm0.017$	$1.458\pm0.014$	$0.166\pm0.008$	$1.408\pm0.087$
		Gallic acid	$0.060\pm0.00$			
		Methanolic extract	$5.892 \pm 0.065$	$4.095\pm0.042$	$0.350\pm0.000$	$6.260\pm0.052$
<b>F</b>	say	Ethyl acetate fraction	$4.485\pm0.062$	$0.653 \pm 0.012$	$0.401\pm0.011$	$1.261\pm0.013$
(m	asi	Butanolic fraction	$1.687\pm0.012$	$1.385\pm0.005$	$0.322\pm0.001$	$0.701\pm0.008$
ш	DPPH assay	Tannins	$1.302\pm0.046$	$1.314\pm0.029$	$0.063 \pm 0.000$	$1.294\pm0.029$
	Id	Saponins	$2.026\pm0.011$	$2.247 \pm 0.001$	$0.126\pm0.000$	$0.649\pm0.005$
EC <sub>50</sub> (mg/mL)	A	Ascorbic acid	$0.090\pm0.002$			
-		Methanolic extract	$0.090 \pm 0.016$	$0.121 \pm 0.020$	$0.325 \pm 0.037$	$1.365 \pm 0.104$
	a	Ethyl acetate fraction	$0.432 \pm 0.014$	$0.457 \pm 0.003$	$0.359 \pm 0.018$	$0.489 \pm 0.018$
	v en	Butanolic fraction	$1.168 \pm 0.392$	$0.441 \pm 0.019$	$0.314 \pm 0.008$	$0.246 \pm 0.005$
	β-carotene assay	Tannins	$1.129\pm0.022$	$3.623 \pm 0.025$	$0.328 \pm 0.003$	$0.493 \pm 0.038$
	a, G	Saponins	$0.082\pm0.013$	$0.122\pm0.000$	$0.386 \pm 0.003$	$0.529 \pm 0.007$
	ä	Gallic acid	$3.220\pm0.020$			
		BHT	$0.010\pm0.000$			
		Methanolic extract	$8.293 \pm 1.624$	$9.319 \pm 0.401$	$32.27 \pm 2.400$	$5.275\pm0.694$
ng	0.0	Ethyl acetate fraction	$6.431 \pm 1.271$	$6.447 \pm 0.191$	$3.270 \pm 0.121$	$6.311 \pm 0.214$
TAC (mg	AAE/g DM)	Butanolic fraction	$4.350\pm0.156$	$5.766 \pm 1.059$	$9.135 \pm 0.771$	10.453±1.756
Ą	P A	Tannins	$6.477 \pm 1.396$	$6.369\pm0.075$	$3.628 \pm 0.087$	$8.184 \pm 0.771$
		Saponins	$8.819 \pm 1.175$	$6.532 \pm 0.994$	$3.311 \pm 0.451$	$3.896 \pm 0.438$
		Methanolic extract	$5.212 \pm 1.020$	$5.856 \pm 0.252$	20.281±1.508	$3.315 \pm 0.436$
ng	Z	Ethyl acetate fraction	$4.030 \pm 0.799$	$4.052 \pm 0.120$	$1.485 \pm 0.916$	$3.966 \pm 0.135$
TAC (mg	50 L	Butanolic fraction	$2.333 \pm 0.098$	$3.626 \pm 0.663$	$5.730 \pm 0.484$	$3.539 \pm 1.103$
AC	E/	Tannins	$4.070\pm0.877$	$3.997 \pm 0.047$	$2.280 \pm 0.054$	$5.143 \pm 0.485$
Ĥ	GAE/g DM)	Saponins	$5.536 \pm 0.738$	$4.155\pm0.625$	$2.093 \pm 0.283$	$2.448\pm0.275$

Values were the mean of two replicates  $\pm$  SD. DM: dry matter; GAE: gallic acid equivalents; AAE: ascorbic acid equivalents.

All the phenolic compounds identified in the present study were characterized for the first time in ethyl acetate and butanolic fractions of *G. decander* flowers. The results obtained suggested that the antioxidant activity of the extracts of flowers is closely related to their composition in polyphenols. These findings may be useful in establishing the relationship between the chemical composition and the highest antioxidant activity of *G. decander* flowers' extracts, hence the interest of studying the flower part of this plant.

Standards r.t (min)	1 1.58	2 1.85	3 3.50	4 4.60	5 15.20	6 15.48	7 18.48	8 21.50	9 24.80	10 31.68	11 34.00
Flower ethyl acetate fraction	N.D.	N.D.	N.D.	71.65	18.24	54.37	N.D.	N.D.	N.D.	72.95	623.14
Flower butanolic fraction	N.D.	N.D.	N.D.	N.D.	N.D.	33.12	N.D.	N.D.	N.D.	124.84	454.20

**Table 3.** Quantitative analysis of phenolic content ( $\mu g/g$  DM) of ethyl acetate and butanolic fractions of the flowers, and ethyl acetate fraction of roots of *G. decander*.

(1) tannic acid; (2) rutin; (3) gallic acid; (4) ascorbic acid; (5) vanillic acid; (6) p-coumaric acid; (7) catechin; (8) syringic acid; (9) ferulic acid; (10) Quercetin; (11) naringenin. N.D.: not determined. r.t: retention time.

#### 3.4. Relationship between contents of phenolic compounds and antioxidant activities

The correlation, using the Pearson coefficient ( $\rho$ ) between the contents of phenolic compounds and the three antioxidant power tests used in the present study, is presented in Table 4. A negative correlation was found between the reducing power capacity and the total contents of phenolics, flavonoids, flavonols and condensed tannins ( $\rho = -0.821$ , -0.831, -0.847 and -0.841). Also, a significant negative correlation ( $\rho \ge -0.915$ ) appeared between DPPH assay and all contents of phenolic compounds. However, a weak correlation was found between the  $\beta$ -carotene test and all contents of phenolic compounds, suggesting the influence of the oily medium used in this test on the nature of the compounds. The overall relationship between the total antioxidant capacity and total phenolic, flavonoid, flavonol and condensed tannin contents were found positive; a significant linear correlation was observed. This finding suggests that the phenolic compounds contribute significantly to the antioxidant capacity. The data obtained are found to be in good accordance with previously reported studies which showed that high total phenolic content increases the antioxidant activity (Kumaran and Karunakaran, 2007; Beddou et al., 2014; Sadeghi et al., 2015).

potentiai:					
Р	Reducing	DPPH	β-carotène	TAC (mg	TAC (mg
	power	assay	assay	AAE/g DM)	GAE/g DM)
Total phenolics	-0.821	-0.935	-0.177	0.991	0.991
Flavonoids	-0.831	-0.933	-0.204	0.992	0.992
<b>Condensed tannins</b>	-0.841	-0.936	-0.224	0.994	0.994
Flavonols	-0.847	-0.915	-0.273	0.987	0.987

**Table 4.** Pearson's correlation coefficients ( $\rho$ ) of phenolic compounds and antioxidant potential.

#### 4. Conclusion

According to the data obtained from the current study, *G. decander* appears to be rich in phenolic constituents and demonstrates a important antioxidant activity measured by different methods. The results of the present investigation suggest that flower extracts of *G. decander* 

exhibited highest antioxidant activity and higher phenolic content. The results indicate notably that the antioxidant activity of this plant could be attributed to the presence of ascorbic acid, vanillic acid,  $\rho$ -coumaric acid, quercetin and naringenin. This study would provide bases for future studies. It will be interesting to continue the investigation of this plant in order to identify other active components, which would be responsible for the studied and/or other biological activities.

## **Author Contribution Statement**

Amel ZITOUNI: Carried out the experiment, wrote and corrected the manuscript; Nabila Belyagoubi-Benhammou and Asma El Zerey-Belaskri: Planned the experiment protocol, analysed the data, corrected the manuscript and supervised the findings; Fethi TOUL and Nassira GHEMBAZA: Contributed in experiment and discussion of results; Fawzia ATIK-BEKKARA: Designed the study and planned the experiment protocol. All authors contributed to the final manuscript.

### **Conflict of interest**

The authors declare that they have no conflict of interest

## ORCID

D Amel ZITOUNI : 0000-0002-7145-8591.

### References

- Abu Zarin, M.H., Isha A., & Armania, N. (2016). Antioxidant, antimicrobial and cytotoxic potential of condensed tannins from *Leucaena leucocephala* hybrid-Rendang. *Food science and humain wellness*, 5, 65–75. https://doi.org/10.1016/j.fshw.2016.02.001
- Ahmed, S., Hasan, M.M., & Mahmood, Z.A. (2016). Antiurolithiatic plants in different countries and cultures. *Journal of Pharmacognosy and Phytochemistry*, 5,102–115. https://www.phytojournal.com/archives/2016/vol5issue1/PartB/4-4-45.pdf.
- Ashraful, A., Subhan, N., Mahbubur, R, Reza, H., & Sarker, S. (2014). Effect of Citrus Flavonoids, Naringin and Naringenin, on Metabolic Syndrome and Their Mechanisms of Action. *Advanced Nutrients*, 5, 404–417. doi: 10.3945/an.113.005603.
- Baba Aïssa, F. (1999). Encyclopédie des plantes utiles. Ed : Librairie Moderne Rouïba. Alger, pp 368.
- Bate-Smith, E. (1962). The phenolic constituents of plants and their taxonomic significance. Botanical Journal of the Linnean Society, 58: 95–173. https://doi.org/10.1111/j.1095-8339.1962.tb00890.x
- Bechlem, H., Mencherini, T., Bouheroum, M., Benayache, S, Cotugno, R., Braca, A., & De Tommasi, N. (2017). New Constituents from *Gymnocarpos decander .Planta Medica*, 83,1200–1206. DOI: 10.1055/s-0043-111599

- Beddou, F., Bekhechi, C., Ksouri, R., Chabane Sari, D., & Atik Bekkara, F. (2014). Potential assessment of Rumex vesicarius L. as a source of natural antioxidants and bioactive compounds. *Journal of Food Science and Technology*, 52, 3549–3560. doi: 10.1007/s13197-014-1420-9
- Bekkara, F., Jay, M., Viricel, M.R., & Rome, S. (1998). Distribution of phenolic compounds within seed and seedlings of two *Vicia faba* cvs differing in their seed tannin content, and study of their seed and root phenolic exudations. *Plant Soil*, 203, 27–36. https://doi.org/10.1023/A:1004365913726
- Belyagoubi-Benhammou, N., Belyagoubi, L., El Zerey-Belaskri, A., Zitouni, A., Ghembaza, N., Benhassaini, H., & Atik-Bekkara, F., Piras, A., Falconieri, D., Rosa, A. (2018). Fatty acid composition and antioxidant activity of *Pistacia lentiscus* L. fruit fatty oil from Algeria. *Journal of Food Measurement and Characterisation*, 12 ,1408– 1412. https://doi.org/10.1007/s11694-018-9755-y
- Benhammou, N., Atik-Bekkara, F., & Kadifkova-Panovska, T. (2009). Antioxidant activity of methanolic extracts and some bioactive compounds of *Atriplex halimus*. *Comptes Rendus Chimie*, 12, 1259–1266. Doi : 10.1016/j.crci.2009.02.004
- Bouaziz, M., Dhouib, A., Loukil, S., Boukhris, M., & Sayadi, S. (2009). Polyphenols content, antioxidant and antimicrobial activities of extracts of some wild plants collected from the south of Tunisia. *African Journal of Biotechnology*, 8, 7017–7027. doi:10.4314/ajb.v8i24.68789
- Bouchelta, A., Boughdad, A., & Blenzar, A. (2005). Effets biocides des alcaloïdes, des saponines et des flavonoïdes extraits de *Capsicum frutescens L*. (Solanaceae) sur *Bemisia tabaci* (Gennadius) (*Homoptera :Aleyrodidae*). *Biotechnologie, Agronomie Société et Environnnement*, 9, 259–269. https://popups.uliege.be/1780-4507/text/v9n4/259.pdf
- Ghembaza, N., Belyagoubi-Benhammou, N., Zitouni, A., Toul, F., Michalet, S., & Atik-Bekkara, F. (2021). Rapid identification analysis of chemical constituents of Sedum villosum L. (Orpin.) by UHPLC-DAD-HRSM. *Journal of Natural Products Research* and Applications, 1, 15–23.

https://ojs.univ-tlemcen.dz/index.php/JNPRA/article/view/967/852

- Hasbal, G., Yilmaz-Ozden, T., & Can, A. (2014). Antioxidant and antiacetylcholinesterase activities of *Sorbus torminalis (L.) Crantz* (wild service tree) fruits. *Journal of food and drug analysis*, 23, 57–62. doi: 10.1016/j.jfda.2014.06.006.
- Hertog, M.G., Hollman, P.C.H., & Venema, D.P. (1992). Optimization of a quantitative HPLC determination of potentially anticarcinogenic flavonoids in vegetables and fruits. *Journal of Agricultural and Food Chemistry*, 40, 1591–1598. https://doi.org/10.1021/jf00021a023
- Javidnia, K., Miri, R., Jamalian A., Sarkarzadeh, H., & Nasiri, A. (2008). Composition of Essential Oil of Gymnocarpos decander Forrssk. from Iran. Journal of Essential Oil Research, 20, 477–478. https://doi.org/10.1080/10412905.2008.9700062

- Julkunen-Titto, R. (1985). Phenolic constituents in the leaves of northern Willows methods for the analysis of certain phenolics. *Journal of Agricultural and Food Chemistry*, 33, 213–217. https://doi.org/10.1021/jf00062a013
- Kaiser, J., Yassin, M., Prakash, S., Safi, N., Agami, M., Lauw, S., Ostrozhenkova , E., Bacher, A., Rohdich, F., Eisenreich, W., Safi, J., & Galan-Goldhirsh, A.(2007). Anti-malarial drug targets: screening for inhibitors of 2C-methyl-D-erythritol 4- phosphate synthase (IspC protein) in Mediterranean plants. *Phytomedicine*, 14, 242–249. doi: 10.1016/j.phymed.2006.12.018.
- Khan, N., Akhtar, M.S., Khan, B.A., Braga, V., & Reich, A . (2015). Antiobesity, hypolipidemic, antioxidant and hepatoprotective effects of *Achyranthes aspera* seed saponins in high cholesterol fed albino rats. *Archives of medicale science*, 11, 1261–1271. doi: 10.5114/aoms.2015.56353
- Kumaran, A., & Karunakaran, R.J. (2007). In vitro antioxidant activities of methanol extracts of five Phyllanthus species from India. *LWT Lebensmittel-Wissenschaft & Technologie*, 40, 344–352. https://doi.org/10.1016/j.lwt.2005.09.011
- Kumaran, S.P., Kutty, B.C., Chatterji, A., Subrayan, P.P., & Mishra, K.P. (2007).
  Radioprotection against DNA damage by an extract of Indian green mussel. *Perna* viridis (L). Journal of environmental pathology, toxicology and oncology, 26, 263–272. doi: 10.1615/jenvironpatholtoxicoloncol.v26.i4.30.
- Mabry, T. (1980). Betalains. In: Bell E, Charwood B, eds. Encyclopedia of plant physiology, secondary plant products. Berlin, Germany: Springer Verlag, pp 513–533.
- Maiza, K., Hammiche, V., & Maiza-benabdesselam, F. (2011). Traditional medicine in north Sahara: the "Deffi". *Life Sciences Leaflets*, 16, 551–560.
- Manyou, Y., Gouvinhas, I., Rocha, J., Ana I. R. N., & Barros, A. (2021). Phytochemical and antioxidant analysis of medicinal and food plants towards bioactive food and pharmaceutical resources. *Scientific reports*, 11, 10041. https://doi.org/10.1038/s41598-021-89437-4
- Moure, A., Franco, D., Sineiro, J., Dominguez, H., Nunez, M.J., & Lem, J.M. (2000). Evaluation of extracts from *Gevuina avellana* hulls as antioxidants. *Journal of Agricultural and Food Chemistry*, 48, 3890–3897. doi: 10.1021/jf000048w.
- Oyaizu, M. (1986). Studies on products of browning reaction prepared from glucose amine. *Japan Journal of Nutrition*, 44, 307–315. http://dx.doi.org/10.5264/eiyogakuzashi.44.307
- Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Analytical Biochem*istry, 269, 337–341. doi: 10.1006/abio.1999.4019.
- Razali, N., Razab, R., Mat Junit, S., & Abdul Aziz, A. (2008). Radical scavenging and reducing properties of extracts of cashew shoots (*Anacardium occidentale*). Food Chemistry, 111, 38–44. DOI:10.1016/j.foodchem.2008.03.024

- Sadeghi, Z., Valizadeh, J., Shermeh, O., & Akaberi, M. (2015). Antioxidant activity and total phenolic content of *Boerhavia elegans*(choisy) grown in Baluchestan, Iran. American journal of psychiatry, 5, 1–9.
- Sanchez-Moreno, C., Larrauri, J.A., & Saura-Calixto, F. (1998). A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of food and Agriculture*, 76, 270–276.

https://doi.org/10.1002/(SICI)1097-0010(199802)76:2<270::AID-JSFA945>3.0.CO;2-9

- Shaik, Y.B., Castellani, M.L., Perrella, A., Conti, F., Salini, V., Tete, S., Madhappan, B., Vecchiet, J., De Lutiis, M.A., Caraffa, A., & Cerulli, G. (2006). Role of quercetin (a natural herbal compound) in allergy and inflammation. *Journal of Biological Regulators and Homeostatic Agents*. 20, 47–52.
- Singleton, V.L., & Rossi, J.A. (1965). Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16,144– 158.
- Sikder, K., Das, N., Kesh, SB., & Dey, S. (2014). Quercetin and B-sitosterol prevents high fat diet induced dyslipidemia and hepatotoxicity in swiss albino mice. *Indian Journal of Experimental Biology*, 52, 60–66.
- Subburaman, S., Ganesan K., & Ramachandran M. (2014). Protective role of naringenin against doxorubicin-induced cardiotoxicity in a rat model: histopathology and mRNA expression profile studies. *Journal of Environnemental Patholology, Toxicology and Oncology*, 33, 363–376. doi: 10.1615/jenvironpatholtoxicoloncol.2014010625.
- Tanaka, Y., Sasaki, N., & Ohmiya, A. (2008). Biosynthesis of plant pigments: anthocyanins, betalains and carotenoids. The *Plant Journal*, 54, 733–749. doi: 10.1111/j.1365-313X.2008.03447.x.
- Tarhouni, M., Ben Salem, F., Ouled Belgacem, A., & Neffati, M. (2010). Acceptability of plant species along grazing gradients around watering points in Tunisian arid zone. *Flora*, 205, 454-461. DOI:10.1016/j.flora.2009.12.020
- Toul, F., Belyagoubi-Benhammou, N., Zitouni, A., & Atik-Bekkara, F. (2016). Antioxidant activity and phenolic profile of different organs of *Pistacia atlantica* Desf. subsp. *atlantica* from Algeria. *Natural Products Research*, 30, 1–6. https://doi.org/10.1080/14786419.2016.1217205
- Zhang, S.Y., Zheng, C.G., Yan, X.Y., & Tian, W.X. (2008). Low concentration of condensed tannins from catechu significantly inhibits fatty acid synthase and growth of MCF-7 cells. *Biochemical Biophysical Research Communications*, 371, 654–658. doi: 10.1016/j.bbrc.2008.04.062
- Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry*, 64, 555–559. https://doi.org/10.1016/S0308-8146(98)00102-2