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3 **Cytotoxicity and antioxidant activities of leaf extracts of *Varthemia sericea* (Batt. et Trab.)**

4 **Diels**

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15 **ABSTRACT**

16 *Introduction:* *Varthemia sericea*, is a plant which belongs to the Asteraceae family, and grows
17 wild in the Central Algerian Sahara. It is used in traditional medicine pharmacopeia in order to
18 treat several diseases. This study aims at assessing the phytochemical profile of leaf extracts of
19 *Varthemia sericea* and to investigate *in vitro* cytotoxicity and antioxidant activities.

20 *Methods:* Total phenolic and flavonoids were analyzed through using colorimetric methods. The
21 antioxidant activity was evaluated by using different complementary tests: Free-radical
22 scavenging, β -carotene bleaching inhibition, reducing power and copper chelation ability. The
23 cytotoxicity was determined by the MTT colorimetric assay.

24 *Results:* The highest total phenolic content recorded with methanol extract with a value of
25 $283.61 \pm 0.58 \mu\text{g GAE/mg}$. The ethyl acetate and aqueous residue extracts contained the highest
26 flavonoids contents with values of $54.48 \pm 1.29 \mu\text{g QE/mg}$ and $44.56 \pm 0.14 \mu\text{g QE/mg}$,
27 respectively. Interesting antioxidant activity was exhibited by the different extracts especially the
28 ethyl acetate and aqueous residue, ethanol and methanol extracts. Furthermore, the petroleum
29 ether, ethyl acetate and aqueous residue extracts showed a significant cytotoxicity activity
30 against MCF-7, HeLa and HL60 cells with IC_{50} values ranging from 16.15 ± 0.79 to 211.96 ± 3.38
31 $\mu\text{g/ml}$, after 72 h of treatment. There is a significant correlation to high level amounts of
32 flavonoids in these extracts which can be attributed to their cytotoxicity effects.

33 *Conclusions:* The extracts from leaves of *V. sericea* showed promising antioxidant and
34 cytotoxicity effects. It suggests that it could be a potential natural source for the development of
35 a new cancer drug.

36 **Keywords:** *Varthemia sericea*, Phenolic compounds, Flavonoids, Cancer, Cytotoxicity activity,
37 Antioxidant activity.

38 **1. Introduction**

39 The oxidative environment is represented by chemical species, consisting of superoxide,
40 hydroxyl radical, nitric oxide and peroxy nitrite. Living organisms with their lipids, DNA, RNA,
41 and proteins, are unfavorably affected by oxidative effect from these free radicals, and this
42 ultimately leads to human chronic diseases [1]. Free radicals are responsible for causing a wide
43 variety of serious diseases including cancer [2], cardiovascular diseases [3], neurological
44 disorders [4,5,6,7], alcohol- induced liver disease [8], ulcerative colitis [9] and aging [10].

45 The human body display enzymatic and non-enzymatic antioxidant defenses, which respond
46 to the harmful outcomes of free radicals and different oxidants. Antioxidants play an important
47 role in the prevention of the damaging effects of free radicals by neutralizing them. They can

48 also prevent the formation of free radicals by sequestering transition metals like iron and copper
49 [11].

50 The non-natural antioxidants such as propylgallate (PG), butylated hydroxytoluene (BHT)
51 and butylated hydroxyanisole (BHA) had been the cause of carcinogenesis and liver damage in
52 animals [12,13]. Thus, there is a necessity to search for antioxidants of natural origin with higher
53 efficiency and lower side effects [14,15,16].

54 According to literature, the consumption of medicinal herbs either in the form of raw extracts
55 or chemical constituents is correlated to a lower threat of many diseases due to their content in
56 antioxidant molecules like phenols, flavonoids and carotenoids to name a few [17,18].

57 Furthermore, phenolic molecules found in plants such as phenolic acids and flavonoids are
58 common components of the human diet and involved in several biochemical mechanisms like
59 antioxidant, antimicrobial, antithrombotic, antiatherogenic, anti-inflammatory, anticarcinogenic,
60 and antimutagenic activities [19, 20, 21]. Most of these biological effects are believed to be due
61 to their antioxidant properties.

62 Polyphenol, phenolic acids and in particular flavonoids are responsible for their antioxidant
63 properties, which is manifested by quenching free radicals through donating hydrogen in the active
64 hydroxyl, canceling their effects [22]. They operate by chelating metal ions [23,24, 25] and,
65 more importantly, by scavenging free radicals [26,27,28].

66 In addition, flavonoids can be significant dietary chemoprotective agents against cancer due to
67 their antioxidant activities and UV light-absorbing ability. The flavonoids can exert a significant
68 antioxidant function through the regulation of crucial signaling pathways. Thus, the inclusion of
69 a flavonoid-rich diet could be a realistic approach to diminish the risk of oxidative stress and
70 resulting cancer [29].

71 Herbal medicine and its products are the most suitable and safe to be used as alternative
72 medicine [30]. *Varthemia sericea* (*V. sericea*) is an endemic plant that belongs to the Asteraceae

73 family, growing wild in the central Algerian Sahara. The leaves of the plant are used in
74 traditional medicine against digestive diseases: stomach and headaches [31]. In our previous
75 work, the extracts of *Varthemia sericea* leaves were initially screened for phytochemical class,
76 antioxidant properties and α -amylase and α -glucosidase inhibitory activities [32].

77 The present research was conducted to investigate the cytotoxicity and antioxidant activities
78 of methanol and its extracts, ethanol and water extracts from *V. sericea* leaves, to assess the
79 possibility of their usage as a potential alternative to synthetic drugs for cancer management.

80 **2. Materials and Methods**

81 2.1. Plant material

82 In this study, the plant is identified by a botanist from the University of Bejaia, then the leaves
83 of *V. sericea* were selected and collected in Tamanrasset (South Sahara of Algeria) in November
84 2015. A voucher specimen was at the Herbarium of Université de Montpellier 2, Institut de
85 Botanique (MPU) (UM-MPU-MPU302208). The selected parts were washed with distilled water
86 to remove all the debris and were cut into small pieces.

87 2.2. Preparation of plant extracts

88 *V sericea dry leaves* plant material (90 g) was ground into small pieces of around 250 μ m of
89 diameter to get a homogeneous dry powder by using the laboratory crusher; then it is divided
90 into three equal portions.

91 Extracts were obtained using two extraction procedures. First, one portion (30 g) of plant
92 material was extracted with aqueous-methanol (75% V/V), the methanol extract was evaporated
93 then subjected to successive extractions with 300 ml of petroleum ether, chloroform, and ethyl
94 acetate according to the adjusted procedure of Markham, [33]. Second, the other two portions
95 per 30 g of leaves plant material were individually extracted with 300 ml of aqueous-ethanol
96 (50% V/V) or distilled water as it was described before [34]. In both cases, extraction was
97 performed during 72 h at room temperature (10% W/V) under agitation. This process was

98 repeated twice in the same conditions. The extracts were gathered and then filtered through a
99 filter paper (Whatman No.1), evaporated under reduced pressure and stored at 4 °C for further
100 experiments.

101 2.3. *Phytochemical content*

102 2.3.1. *Determination of Total Phenolic Content*

103 Total phenolic content of leaf extracts of *V. sericea* was determined using the Folin–Ciocalteu
104 method adopted by Singleton and Rossi, [35]. A volume of 20 µl of different extracts was mixed
105 with 100 µl of Folin–Ciocalteu reagent (10 % V/V) and 75 µl of sodium carbonate [Na₂CO₃]
106 (7.5% W/V). After the incubation at room temperature in the dark for 2 h. The absorbance of the
107 reaction mixture was measured at 765 nm with UV/VIS spectrophotometer microplate (Perkin
108 Elmer, Enspire). The phenolic content was calculated using the equation obtained with gallic
109 acid standard curve: $y = 0,0034x + 0,1044$ ($R^2 = 0,9972$). The results were expressed as
110 microgram gallic acid equivalents per milligram (µgGAE/mg).

111 2.3.2. *Determination of Total Flavonoid Content*

112 The total flavonoid content of extracts was performed using the microplate method adopted
113 by Topçu et al. [36]. A volume of 50 µl of sample solution was added to 130 µl of methanol, 10
114 µl of potassium acetate [CH₃COOK], and 10 µl of aluminum nitrate [Al(NO₃)₂·9H₂O] (10%).
115 After incubation for 40 min at room temperature, the absorbance was measured at 415 nm with
116 UV/VIS spectrophotometer using a microplate reader of 96 microwells (Perkin Elmer, Enspire).
117 The quercetin was used as a standard for the calibration curve ($y = 0.0048x$ and $R^2 = 0.997$). The
118 results were expressed as microgram quercetin equivalents per milligram (µgQE/mg).

119 2.4. *Antioxidant activities*

120 2.4.1. *Free radical scavenging activity DPPH*

121 The free radical-scavenging activity was determined spectrophotometrically by the 2,2-
122 diphenyl-1-picrylhydrazyl (DPPH) assay as described by Blois [37]. A volume of 160 µl of

123 DPPH solution (1 mM), dissolved in methanol, was mixed with 40 µl of different samples
124 concentrations. After incubation for 30 min at room temperature, the absorbance was measured
125 at 517nm. The results were expressed as in percentage (%) calculated using the following
126 formula:

$$127 \quad I \% = ((Ac-As) / Ac) * 100$$

128 Where I (%) is the percentage of inhibition, Ac and As are the absorbances of the control and
129 the test sample after 30 min respectively. The α -tocopherol, BHT and BHA were used as
130 antioxidant standards for comparison of the activity.

131 2.4.2. Assay of superoxide radical scavenging capacity (Pyrogallol)

132 The superoxide radical scavenging (Pyrogallol) capacity was determined according to the
133 method of Liang et al. [38]. Solutions containing Tris-HCl buffer (50 mM, pH 8.2, 140 µl), 25
134 mM pyrogallol solution (20 µl) and sample (20 µl) were incubated at 25 °C for five min. Then, 8
135 mM HCl solution (20µl) was added into the mixture promptly to terminate the reaction. The
136 absorbance was measured at 420 nm. Ascorbic acid was used as a positive standard. The
137 superoxide radical scavenging capacity was calculated using the following formula:

$$138 \quad \text{Scavenging capacity (\%)} = [1 - (A1 - A2)/A0] \times 100\%$$

139 Where A0 is the absorbance of the control, A1 is the absorbance of the test sample and A2 is
140 the absorbance of product control (Tris-HCl buffer rather than pyrogallol solution).

141 2.4.3. β -carotene-linoleic acid bleaching assay

142 The antioxidant activity was evaluated by using β -carotene-linoleic acid emulsion and was
143 determined using the method of Marco [39]. The emulsion linoleic acid- β -carotene was prepared
144 by dissolving 0.5 mg of β -carotene in 1 ml of chloroform, 20 µl of linoleic acid, 200 µl of Tween
145 40 were added, and the solvent was evaporated under reduced pressure. The residue was then
146 taken with 50 ml of H₂O₂ and the absorbance was adjusted to 0.8–0.9 at 470 nm. 160 µl of the
147 emulsion were mixed with 40 µl of each extract or BHA and BHT (as antioxidant standards for

148 evaluation of the activity) at different concentrations in micro-plate and the zero-time absorbance
149 reading at 470 nm was recorded. The micro-plate was stored at 45 °C and the absorbance values
150 were recorded over 120 min. The percentage of inhibition I (%) of the tested extracts was
151 determined using the following formula:

$$152 \quad I (\%) = 1 - \left(\frac{A(t = 0) - A(t = 120)}{A0(t = 0) - A0(t = 120)} \right) * 100$$

153 Where I (%) is the percentage of inhibition, A (t = 0) and A (t = 120) are the absorbencies
154 (470 nm) of the tested samples at 0 min and 120 min, respectively. A0 (t = 0) and A120 (t = 120)
155 are the absorbencies (470 nm) of the positive control (Methanol without extract) at 0 min and
156 120 min, respectively.

157 2.4.4. Reducing power

158 Reducing power was measured by the reduction of $[\text{Fe}^{+3} (\text{CN}^-)_6]$ to $[\text{Fe}^{2+} (\text{CN}^-)_6]$ using the
159 method of Oyaizu [40], with some modifications. 20 μl of each extract or standard (BHA and
160 quercetin), dissolved in methanol, at different concentrations were mixed with 40 μl of sodium
161 phosphate buffer (0, 2 M, pH 6,6) and 40 μl of potassium ferricyanide $[\text{K}_3\text{Fe}(\text{CN})_6]$ (1%). The
162 mixture was incubated at 50 °C for 20 min. After incubation, the reaction mixture was acidified
163 with 40 μl of tri-chloroacetic acid (10%). 40 μl of distilled water and 20 μl of FeCl_3 (0.1%) were
164 then added to the mixture, and the absorbance was measured at 700 nm. The increased
165 absorbance of the reaction mixture designates greater reduction capability. The concentration
166 giving an $A_{0.5}$ was determined from the absorbance curves.

167 2.4.4. Copper Chelating assay (CCA)

168 Copper chelating activity (CCA) of leaf extracts of *V. sericea* was determined to chelate Cu^{2+}
169 using the method that employs pyrocatechol violet (PV) as the chromogen agent as described by
170 Sánchez Vioque [41], with a slight modification.

171 In aqueous medium buffered at slightly acidic medium (pH 6.0), phenolic compounds may
172 bind Cu^{2+} , but depending on the chemical structure, the reactivity of formation of the complex
173 phenol- Cu^{2+} is increased, but there is generally a leftover of Cu^{2+} in the reactional medium
174 because not all the copper is bound by chemical compounds present in a test sample. Then, the
175 residual Cu^{2+} reacts with PV violet at a proportion of (2:1), forming a dark-colored complex that
176 can be observed at 632 nm [42]. However, the dark color goes into yellow in the presence of
177 chelating agents that dissociate the complex, and the chelating activity can thus be assessed by
178 the measurement of the rate of color reduction.

179 Briefly, 40 μl of each extract was added to 140 μl sodium acetate buffer (50 mM, pH 6.0).
180 Then, 10 μl (5 mM) ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$) and incubation for 30 minutes. After, we added 10 μl of
181 PV (4 mM) and was incubated for 30 minutes. The absorbance was measured using a micro-well
182 plate reader at 632 nm. The EDTA was considered as a positive control.

183 2.5. Cytotoxicity activity

184 The MTT colorimetric assay, Mosmann [43], which is based totally on the reduction of MTT
185 [3-(4,5-dimethyl thiazolyl)-2,5-diphenyl-tetrazolium bromide] by mitochondrial dehydrogenase
186 to a purple formazan product, used to assess the cytotoxicity action of leaf extracts of *V. sericea*
187 against three cancer cells in human Leukemia (HL-60), Breast Cancer cells (MCF-7) and
188 Cervical cells (HeLa).

189 • Cells, Cell cultures and Treatment

190 The HL60 cells were grown and maintained in a humidified incubator at 37 °C and in 5%
191 CO_2 atmosphere in *Roswell Park Memorial Institute medium 1640* (RPMI 1640) medium
192 containing 100 g/l fetal bovine serum (FBS) and 10 g/l penicillin–streptomycin–glutamine
193 (PSG). However, the HeLa cells were harvested in *Minimal Essential Medium* (MEM)
194 containing L-glutamine and supplemented with 5% fetal bovine serum (Capricorn Scientific
195 GMBH, South America) and 1% gentamicin (Virbac, RSA) and the MCF-7 cells were seeded in

196 *Dulbecco's Modified Eagle Medium* (DMEM) containing 5% fetal bovine serum (Capricorn
197 Scientific GMBH, South America) and 1% gentamicin (Virbac, RSA). These cells were
198 suspended at a density of 2.5 to 5 x10⁵ cells/ml (200 µl) in 96-well micro-plates and incubated at
199 37 °C overnight to allow attachment and to reach 80% of cell confluence.

200 After 24 h of cells incubation, the medium was replaced with 200 µl medium containing
201 various doses of different extracts of *V. sericea* (1.0–1000.0 µg/ml). The microplates were
202 further incubated at 37 °C and 5% CO₂ for 72 hours. The medium with cells was used as a blank
203 where Paclitaxel was tested as a positive control. Then, the medium was aspirated from the cells,
204 which were then washed with 200 µl of phosphate-buffered saline (PBS). MTT solution of 0.5
205 mg/ml was then added to each well of micro-plate. After 4h of incubation, the medium was
206 carefully aspirated from the wells and the dimethylsulfoxide (DMSO) was added to solubilize
207 the formed formazan crystals and the plates were placed on an orbital shaker for about 2 min.
208 The absorbance was measured on a microplate reader at 570 nm.

209 The cytotoxicity effects of leaf extracts *V. sericea* was calculated as the ratio of absorbance
210 of the treated group divided by the absorbance of the control group, multiplied by 100 to express
211 the result as a percentage of cytotoxicity. A series of concentration of the extracts was prepared
212 to calculate the IC₅₀ values.

213 *2.5. Statistics analysis*

214 The analysis was executed by using the statistical package for the social sciences (SPSS
215 version 20.0 Armonk, NY: IBM Corp) SPSS IBM 20. All measurements were carried out in
216 triplicate and expressed as mean ± standard deviation. Statistical significance was determined
217 using one-way analysis of variance (ANOVA) with Turkey post-hoc multiple comparison test. p
218 values less than 0.05 were considered as significantly different (P ≤ 0.05).

219 **3. Results**

220 *3.1. Polyphenol content of leaf extracts of V. sericea*

221 Total phenolic and total flavonoids content were determined in the different extracts and the
222 results are presented in **Table 1**. The highest total phenolic contents were recorded with the
223 methanol, water, and ethanol extracts with values of 283.61 ± 0.58 , 182.84 ± 1.86 , and $164.89 \pm$
224 $2.42 \mu\text{gGAE}/\text{mg}$ respectively. Unlike, the lowest contents were recorded with the petroleum
225 ether and chloroform extracts with contents of 58.23 ± 2.86 and $67.46 \pm 1.26 \mu\text{gGAE}/\text{mg}$,
226 respectively.

227 Ethyl acetate, aqueous residue, and chloroform extracts gave the highest flavonoids contents
228 (54.48 ± 1.29 , 44.56 ± 0.14 and $34.64 \pm 1.59 \mu\text{gQE}/\text{mg}$, respectively). Whereas the extracts
229 prepared with methanol, ethanol and water gave low contents ranging from 18.43 ± 1.11 to 22.11
230 $\pm 1.78 \mu\text{gQE}/\text{mg}$, followed by the petroleum ether extract.

231 3.2. Determination of antioxidant activity

232 The antioxidant potential of the different extracts from the leaves of *V. sericea* was
233 assessed using different methods and the results are reported as values of IC_{50} and $\text{A}_{0.5}$ (**Table 2**).

234 For the DPPH assay, the ethanol extract presented the most scavenging activity with an IC_{50}
235 value of $146.73 \pm 1.22 \mu\text{g}/\text{ml}$ while chloroform and methanol extracts exhibited a good similar
236 antiradical activity against $\text{DPPH}\cdot$ of 155.18 ± 2.78 and $157.16 \pm 2.52 \mu\text{g}/\text{ml}$, respectively. A
237 slight effect was showed by petroleum ether and aqueous residue extracts.

238 As reported in **Table 2**, all leaf extracts of *V. sericea* showed an interesting effect against the
239 superoxide radical scavenging (Pyrogallol). Aqueous residue extract was the most active with an
240 IC_{50} value of $20.37 \pm 1.69 \mu\text{g}/\text{ml}$, followed by ethyl acetate, ethanol, petroleum ether, methanol,
241 and water extracts, in decreasing order 40.77 ± 2.67 , 43.85 ± 068 , 45.69 ± 2.44 , 81.96 ± 2.57 and
242 $88.51 \pm 1.26 \mu\text{g}/\text{ml}$, respectively with significant difference ($P \leq 0.05$). Chloroform extract
243 exhibited the lowest capacity in the scavenging of the superoxide anion with IC_{50} of $359.49 \pm$
244 $2.48 \mu\text{g}/\text{ml}$.

245 On the other hand, a strong β -carotene bleaching inhibition was showed by water and ethyl
246 acetate extracts with IC_{50} values of 36.78 ± 0.46 and 49.16 ± 0.24 $\mu\text{g/ml}$, respectively, followed
247 by methanol, ethanol and chloroform extracts with IC_{50} values of 68.06 ± 3.47 , 68.18 ± 5.06 and
248 93.53 ± 0.21 $\mu\text{g/ml}$, respectively ($P \leq 0.05$).

249 The capacity of different extracts to reduce iron ions property was assessed using reducing
250 power assay. The different extracts exhibit a slight reductive potential of iron ions at the tested
251 concentrations ($>200\mu\text{g/ml}$). Interestingly, α -tocopherol, ascorbic acid and tannic acid used as
252 standards showed a strong reducing activity of iron $A_{0.5}=34.93 \pm 2.38$, 6.77 ± 1.15 and 5.39 ± 0.9
253 $\mu\text{g/ml}$, respectively ($P \leq 0.05$).

254 Furthermore, the determination of the chelating copper ability (CCA) of leaf extracts of *V.*
255 *sericea* was presented in **Table 2**. The water and methanol extracts showed a moderate chelating
256 ability with IC_{50} values of 395.53 ± 0.16 and 396.79 ± 0.56 $\mu\text{g/ml}$, respectively, followed by the
257 ethanol and chloroform with values of 408.73 ± 2.32 and 465.38 ± 0.56 $\mu\text{g/ml}$, respectively ($P \leq$
258 0.05). The EDTA showed a strong effect with an IC_{50} value of 8.80 ± 0.47 $\mu\text{g/ml}$.

259 3.3. Determination of the cytotoxicity effect

260 The results for cytotoxicity effects were represented in **Table 3**. The IC_{50} values of extract
261 greater than $100 \mu\text{g/ml}$ were not considered to be active.

262 The petroleum ether, ethyl acetate and aqueous residue extracts were the most effective and
263 they inhibited the HeLa, MCF-7 and HL60 proliferation cells with IC_{50} values varying between
264 16.15 ± 0.79 to 211.96 ± 3.38 $\mu\text{g/ml}$, after 72 hours of incubation. The chloroform and ethanol
265 extracts revealed a potential antiproliferative effect against HL60 cells with IC_{50} values of 36.29
266 ± 5.82 and 43.25 ± 4.89 $\mu\text{g/ml}$, respectively ($P \leq 0.05$). In contrast, no cytotoxicity activity was
267 presented by methanol, water, ethanol and chloroform extracts against the MCF7 and HeLa cells.

268 These results were compared with Paclitaxel, as a reference compound, that showed a strong
269 inhibitory activity against the three cells with HeLa cell ($IC_{50} = 0.1 \pm 0.04$ $\mu\text{g/ml}$), MCF7 cell

270 (IC₅₀ = 0.1 ± 0.02 µg/ml) and HI60 cell (IC₅₀ = 0.7 ± 0.36 µg/ml), after 72 hours of incubation (P
271 ≤ 0.05).

272 **4. Discussion**

273 *V. sericea* is a medicinal plant from the pharmacopeia of the Algerian Sahara, used to treat a
274 wide range of diseases. The leaves of this plant have been reported to possess antispasmodic and
275 analgesic activities, thus used as a therapy in traditional medicine [31].

276 In the present study, for the first time, we tested the effect of different extracts on the
277 cytotoxicity effect beside the antioxidant activity to prospect for possible bioactive
278 molecules.

279 The results of total phenolic and total flavonoids content (**Table 1**) were better when
280 extracted by polar solvents, methanol and ethyl acetate than by solvents with low polarity,
281 petroleum ether and chloroform, that gave only small amounts of phenolic compounds. In
282 addition, the ethyl acetate and aqueous residue extracts presented the highest flavonoids
283 content.

284 Qualitative phytochemical screening of different chemical classes in extracts of *V.*
285 *sericea* revealed the presence of the alkaloids, tannins, and coumarin, which may contribute
286 to the antioxidant activities of the leaf extracts of *V. sericea* [32].

287 In a study carried out by Al-Dabbas et al. [44], the ethanol extract of *Varthemia*
288 *iphionoides* recorded the highest amount of phenolic compounds with 54.13 mg GAE/g,
289 followed by the water, ethyl acetate, chloroform and hexane extracts, in decreasing order. In
290 addition, the content of flavonoids varied between 1.40 mg/g and 75.14 mg/g. This was the
291 highest in the ethyl acetate extract, whereas markedly low in the hexane extract.

292 Our findings are in agreement with a study carried out by Alam et al. [45], confirming
293 that solvents such as ethanol, water, methanol and water/ethanol (V/V) possess a good

294 polarity, allowing extraction of polar molecules such as phenolic compounds and
295 flavonoids, which are antioxidants of interest.

296 Our findings agreed also with the study of Akkol et al. [46] who found the highest values
297 of the total phenolic and flavonoids content in the ethyl acetate extract while the lowest
298 amounts were obtained with the hexane and chloroform extracts from *Salvia halophila* and
299 *Salvia virgate* (Lamiaceae).

300 It should be noted the importance of using many assays to measure the antioxidant
301 properties through various mechanisms like the inhibition of chain initiation, binding of
302 transition metal ion catalysis, peroxide decomposition, stoppage of continued hydrogen
303 abstraction, reductive ability and radical scavenging taking into consideration the composition
304 of extracts [47].

305 In our study, we explore the antioxidant properties of *V. sericea* extracts and we found out
306 that ethanol, chloroform, methanol, ethyl acetate and aqueous residue extracts, in decreasing
307 order, exhibited significantly higher scavenging percentages that correlate with their phenolic
308 compound contents ($P \leq 0.05$). Our findings are in agreement with a study carried out by
309 Alam et al. [45], confirm that ethanol, aqueous, methanol and aqueous-ethanol have good
310 polarity and therefore are solvent of choice to extract polar molecules such as phenolic
311 compounds and flavonoids, which are antioxidants of interest.

312 In a recent study carried out by Dehimat et al, [32], the antioxidant ability of methanol
313 extract and its fractions, cold water and ethanol extracts of *V. sericea* leaves was evaluated,
314 through using different complementary *-in vitro-* tests, showed that all leaf extracts
315 exhibited a good antioxidant activity with the IC_{50} ranging between 32.44 to 715.68 $\mu\text{g/ml}$
316 for free radical scavenging assays (ABTS scavenging activity, Galvinoxyl radical (GOR)
317 scavenging assay and Hydroxyl ($\bullet\text{OH}$) radical scavenging activity), and values of $A_{0.5}$
318 ranged between 45.99 to 696.61 $\mu\text{g/ml}$ using the Cupric reducing antioxidant capacity

319 (CUPRAC) and the O-phenanthroline reduction assay. In addition, the Ferrous ions
320 chelating activity of the leaf extracts of *V. sericea* was assessed and the results found that
321 the IC₅₀ values were more than 800µg/ml.

322 The ethanol and water extracts of *Varthemia iphionoides* showed a pronounced DPPH
323 radical-scavenging activity, with inhibition of about 90% at a concentration of 100 µg/ml.
324 Unlike, the chloroform and hexane extracts that exhibited a weak effect [48]. In many
325 studies of the DPPH radical-scavenging activity of plant extracts, the activity of the
326 extracts is correlated to the amount of phenolic compounds [25, 27, 49].

327 The presence of antioxidants minimizes and prevents the β-carotene bleaching by acting
328 as reducing agents. Their presence seems to be strongly correlated with the phenolic
329 content. Thus, ethanol and ethyl acetate extracts from *Adansonia digitata* (Bombacaceae)
330 with substantial amounts of phenolic compounds exhibited the strongest antioxidant effect
331 [50].

332 Furthermore, the hexane extract of *Mentha spicata* (Lamiaceae) exhibited a significant
333 protecting effect of β-carotene despite its low phenolic content ($P \leq 0.05$). This observation
334 can be interpreted by the presence of lipophilic compounds in the hexane extract, which
335 participate in the neutralization of free radicals, as this test has a high specificity for
336 lipophilic compounds [51].

337 Nevertheless, the antioxidant activity did not correlate with the phenolic content when
338 assessed by acting a reducing power activity.

339 Ferrous ions reduction may be used as an indicator of electron-donating activity, which
340 displays a crucial mechanism of polysaccharide antioxidant action [52]. However, the
341 chelation capacity can be due to the presence of the nitrogen groups explaining the activity
342 of alkaloids [53].

343 Phenolic compounds were the most studied for their antioxidant activity and were reported

344 to protect from many diseases by their capacity to neutralize free radicals. Polyphenols can also
345 act as reducing agent by donating the electrons to regenerate an oxidized molecule or to stop the
346 free radical chain reaction [54].

347 Our findings are in agreement with Al-Dabbas et al. [48], where the methanol, ethanol
348 and water extracts were found to exhibit a strong antioxidant activity with a significant
349 correlation with the phenolic content in almost all assays ($P \leq 0.05$).

350 As it has been described that the phenolic content of plant materials is correlated with
351 their antioxidant property, the antioxidant potentials of natural compounds are referred to
352 their capacity to scavenging radicals and by chelating metal ions via functional groups on
353 their structure. In addition, it has been suggested that the antioxidant and lipid
354 peroxidation-inhibiting potential of flavonoid predominantly resides in the radical-
355 scavenging capacity rather than the chelation of metals [55,56].

356 The results of numerous current studies have established that the hydroxyl groups in
357 polyphenolic compounds may perform an important function in donating a hydrogen to free
358 radical and promoting inhibitory activity [57,58].

359 The cytotoxic studies for the leaf extracts of *V. sericea* were determined by using the MTT
360 colorimetric method. The *V. sericea* leaves extracted by water, ethanol, methanol and its
361 subsequent extracts were investigated for the first time for their cytotoxicity potential
362 against human cancer cell lines (MC-7, HeLa and HL60).

363 No detailed cytotoxicity study of *V. sericea* has been reported earlier. Based on the findings,
364 the current study supports the hypothesis that cytotoxicity and antioxidant activities are due to- at
365 least in part-to the presence of bioactive molecules in leaf extracts of *V. sericea*.

366 The current findings suggest that ethyl acetate and aqueous residue extracts are rich in
367 flavonoids, and it was reported earlier that secondary metabolites like flavonoids could be
368 responsible for cytotoxicity activities [59].

369 In addition, all extracts from the leaves of *V. sericea* showed a strong effect at concentrations
370 tested against HL60 cell line.

371 Despite the low flavonoid content in the petroleum ether extract, it showed a strong
372 cytotoxicity effect against all the cell lines tested. This may be due to the presence of other
373 molecules acting separately or in synergy.

374 Our findings are in agreement with Al-Dabbas et al. [44], where the cytotoxic activity against
375 HL-60 cells seemed to be dose-dependent, because the inhibition activity of the hexane, ethanol
376 and chloroform extracts of *Varthemia iphionoides* were 89%, 68% and 62% respectively, at the
377 concentration of 200 µg/ml.

378 Flavonoids from *Varthemia* species have been reported to exhibit a strong cytotoxicity
379 activity [44]. Based on several studies, the flavonoid from the natural source has been reported as
380 the most potent compounds as antitumor agents, and show cytotoxic effects on various human
381 cell lines, for example, leukemia cells [60, 61], ovarian cancer cells [62].

382 Other plants that belong to the same family Asteraceae, *Jasoinia Candidans*, *Centaurea lipii*,
383 and *Pulicaria undulata*, are rich in flavonoids and terpenoids, showed a different cytotoxic as
384 well as antioxidant activities [63,64,65,66,67,68].

385 **5. Limitations of the study**

386 These preliminary tests need extra advanced strategies to formulate therapeutic compounds
387 from the leaves of *V. sericea*.

388 **6. Conclusion**

389 The present study was carried out to explore, for the first time, antioxidant and
390 cytotoxicity properties of leaf extracts of *V. sericea*. Phenolic compounds and flavonoids
391 seem to assure the antioxidant activity of the methanol and its extracts, water and ethanol
392 extracts and could be the responsible agents for the growth inhibition of cancer cells. From the
393 different obtained results, we can consider *V. sericea* as a potential source of molecules with dual

394 action in the development of new drugs to protect cells from oxidative stress and cancer
395 complications. Further work is in progress on *Varthemia sericea* leaves extracts to isolate the
396 bioactive compounds.

397 **-Authors contribution:**

398 Author contribution statement, Abdelouahab Dehimat: literature search, study design, data
399 communication, data analysis, data interpretation, writing and tables.

400 Abdelouahab Dehimat and Ines Azizi have carried out the experiments.

401 Abdelouahab Dehimat wrote the manuscript.

402 Abdelouahab Dehimat, Veronique Barragan-Montero (PhD co-adviser) and Bachra Khettal (PhD
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408 **- Declaration of Competing Interests**

409 We desire to verify that there are not any recognized conflicts of interest related to this
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415 **-Data availability**

416 It can be acquired from the author on request

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Table 1. Total phenolic content and total flavonoids content of leaf extracts of *V. sericea*.

Extracts	Total phenolic content	Total flavonoid content
	($\mu\text{g GAE/mg}$)	($\mu\text{g QE/mg}$)
Petroleum ether	58.23 \pm 2.86 ^j	20.66 \pm 1.50 ^d
Ethyl acetate	112.84 \pm 2.41 ^e	54.48 \pm 1.29 ^a
Aqueous residue	126.69 \pm 2.99 ^d	44.56 \pm 0.14 ^b
Chloroform	67.46 \pm 1.26 ^f	34.64 \pm 1.59 ^c
Methanol	283.61 \pm 0.58 ^a	22.11 \pm 1.78 ^d
Water	182.84 \pm 1.86 ^b	18.43 \pm 1.11 ^e
Ethanol	164.89 \pm 2.42 ^c	20.42 \pm 1.42 ^d

- Results are expressed as means \pm standard deviation (n = 3) of three measures (Tukey test, $P \leq 0.05$).
- Results with different superscript letters are significantly different ($P \leq 0.05$) and the decrease order (a>b>c>d>e>f>j>h>..)

Table 2. Antioxidant activity of leaf extracts of *V. sericea* (IC₅₀ and A_{0.5}) µg/ml ±SD.

Extracts	Radical scavenging activity IC ₅₀ (µg/mL)		Antioxidant IC ₅₀ (µg/mL)	Reducing power A _{0.5} (µg/mL)	Chelating assay IC ₅₀ (µg/mL)
	DPPH	Pyrogallol	β-carotene bleaching	FRAP	CCA
Petroleum ether	608.82±1.41 ^b	45.69±2.44 ^d	Na	Na	Na
Ethyl acetate	201.71±2.06 ^e	40.77±2.67 ^d	49.16 ± 0.24 ^e	Na	Na
Aqueous residue	492.30±0.86 ^e	20.37±1.69 ^f	Na	Na	Na
Chloroform	155.18±2.78 ^f	359.49±2.48 ^a	93.53±0.21 ^c	Na	465.38±0.56 ^a
Methanol	157.16±2.52 ^f	81.96±2.57 ^c	68.06±3.47 ^d	Na	396.79±0.56 ^d
Water	603.12±2.17 ^a	88.51±1.26 ^b	36.78±0.46 ^f	Na	395.53±0.16 ^c
Ethanol	146.73±1.22 ^d	43.85±0.68 ^d	68.18±5.06 ^d	Na	408.73±2.32 ^b
Standards					
BHA	6.14±0.41 ^h	NT	99.66±0.52 ^a	NT	NT
BHT	12.99±0.41 ^j	NT	95.58±0.19 ^b	NT	NT
EDTA	NT	NT	NT	NT	8.80 ± 0.47 ^e
α-Tocopherol	13.02±5.17 ^j	NT	NT	34.93±2.38 ^a	NT
Ascorbic Acid	NT	32.33±1.17 ^e	NT	6.77±1.15 ^b	NT
Quercetin	NT	NT	NT	NT	NT
Tannic acid	NT	NT	NT	5.39±0.91 ^b	NT

- Na: No activity; NT: Not tested. Values expressed were means ± S.D. of three parallel measurements (P ≤ 0.05).
- Results with different superscript letters are significantly different (P ≤ 0.05) and the decrease order (a>b>c>d>e>f>j>h>..).
- DPPH· radical scavenging, Superoxide radical scavenging (Pyrogallol) assay, β-carotene/linoleic acid bleaching assay, Ferric reducing power (FRAP) assay, Copper Chelating Ability (CCA).

Table 3. Cytotoxicity effect of leaf extracts of *V. sericea* (IC50) $\mu\text{g/ml} \pm \text{S.D.}$

Cell type	Petroleum ether	Ethyl acetate	Aqueous residue	Chloroform	Methanol	Water	Ethanol	Paclitaxel
HeLa	75.79 \pm 1.12 ^d	153.06 \pm 4.02 ^b	42.17 \pm 2.2 ^c	>1000	>1000	>1000	824 \pm 8.56 ^a	0.1 \pm 0.04 ^e
MCF-7	61.83 \pm 0.49 ^c	16.15 \pm 0.79 ^b	211.96 \pm 3.38 ^a	>1000	>1000	>1000	>1000	0.1 \pm 0.02 ^d
HL60	41.85 \pm 4.77 ^f	17.33 \pm 0.42 ^j	16.33 \pm 0.46 ^j	36.29 \pm 5.82 ^e	143.96 \pm 3.20 ^b	284.40 \pm 3.15 ^a	43.25 \pm 4.89 ^d	0.707 \pm 0.36 ^c

- Cancer cell lines: MCF-7: Breast cancer cell line, HeLa: Cervical cancer cell line, HL60: Leukemia cancer cell line.
- Values expressed are means \pm S.D of three parallel measurements ($P \leq 0.05$).
- Results with different superscript letters are significantly different ($P \leq 0.05$) and the decrease order (a>b>c>d>e>f>j).