

1 **Seasonal changes in photosynthesis, phenolic content, antioxidant activity and anatomy**
2 **in of apical and basal leaves from of *Aristotelia chilensis* (Mol.) Stuntz**

3
4 **Abstract**

5
6 *Aristotelia chilensis* (Mol.) Stuntz is an evergreen antioxidant species endemic ~~from to~~ Chile. It grows
7 in open areas or under ~~the~~ tree canopy, and its leaves emerge in early spring and summer. ~~This-The~~
8 ~~objective of this study's study objective~~ was to determine annual station influence (winter, spring, and
9 summer) on photosynthetic activity, total phenol content (TPC), antioxidant activity, and anatomy of
10 apical and basal leaves of *A. chilensis*. ~~We determined photosynthesis~~ Photosynthesis performance was
11 ~~determined~~ by measuring electron transport rate (ETR), the quantum efficiency of photosystem II
12 (Fv/Fm), photochemical quenching (qP), and non-photochemical quenching (NPQ) with a fluorimeter.
13 ~~We analysed leaf~~ Leaf extracts were analysed to determine total phenol content (TPC) and antioxidant
14 activity by DPPH and ABTS methods. The maximum ETR and qP were recorded in spring and
15 summer when the photosynthetically active radiation (PAR) at midday was higher (1901 ~~$\mu\text{mol m}^{-2}\text{s}^{-1}$~~
16 ~~and~~ 1968 $\mu\text{mol m}^{-2}\text{s}^{-1}$, respectively). Fv/Fm ~~recorded had~~ typical physiological values in both types
17 of leaves, ~~around (about~~ 0.8 in all ~~the~~ seasons). The behaviour of NPQ was not influenced by the kind
18 of leaves and season of the year. In concordance, the basal spring leaves (~~42.8 mg GAE g⁻¹dw~~) was ~~had~~
19 higher TPC values (~~42.8 mg GAE g⁻¹dw~~). In contrast, the ~~higher-highest~~ values of antioxidant activity
20 were recorded in basal winter leaves followed by basal spring leaves. The results suggested ~~that~~ an
21 increase in light intensity (spring) ~~positively~~ affected ~~positively~~ the antioxidant activity and TPC,
22 which correlated with higher ETR and qP values. In ~~the~~ main anatomy results, apical leaves ~~display~~
23 ~~displayed~~ morphological adaptations as area intercellular spaces and parenchyma palisade areas were
24 ~~bigger larger~~ than ~~in the~~ basal leaves.

25
26 **Keywords:** *Aristotelia chilensis*, total phenols content, antioxidant activity, chlorophyll
27 fluorescence, ~~winter, spring, summer~~.

28
29
30 **INTRODUCTION**

31
32 ~~Some plant species, mainly of woody type, grow under adverse environmental conditions as it w just~~
33 ~~like as the ease of~~ *Aristotelia chilensis* (Mol.) Stuntz, a dioic evergreen tree ~~which is commonly known~~
34 ~~as "maqui" and native of to~~ Chile, ~~commonly known as "maqui". These This plants plant develop~~
35 ~~develops~~ preferably in humid and drained soils of the central valley, in the slopes of both mountain
36 ranges, streams and margins of forests, from near sea level to 2500 ~~meters of~~ altitude (Fredes et

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37 al., 2012). *A. chilensis* grows up ~~until to~~ 4 to 5 ~~meters-mhigher~~ high, it has a soft and smooth bark, and
38 ~~plentiful~~abundant, thin and flexible ramifications. Leaves are simple, oval-lanceolate form with
39 dentate edges and ~~size-range~~ from 4 to 9 cm ~~in size~~. The leaves veins are marked with a long reddish
40 petiole and stems are characterized by an intense red colour. Flowering occurs in the beginning of
41 spring- ~~and the fruit-fruitsis-are~~ harvested once ~~per-a~~ year, from December to February (Turchetti and
42 Paz, 2019). In addition, *A. chilensis* plants tolerate drought periods of less than one month. This
43 species appears in succession as a colonizer of newly burned or exploited soils, forming dense and
44 monospecific groups known as "macales" ~~which-fulfilwith~~ the function of reducing erosion and
45 generating the conditions for establishing other species forming secondary shrubs (Benedetti, 2012).

46 Variations in environmental factors such as temperature, light radiation, water availability,
47 among others, can cause stress and therefore, changes in plant metabolism. In fact, the photosynthetic
48 rate decreases due to an alteration in the electron transport mechanism and CO₂ assimilation, which
49 ~~finally-is~~ ~~finally~~ reflected in a decrease in carbohydrate production (Sáez et al., 2012). Plants, in their
50 interaction with the environment, produce a high number of secondary metabolites, which ~~normally~~
51 are ~~normally~~ not essential for ~~the-their~~ primary metabolism. The synthesis of these compounds is
52 enhanced under stress conditions and many of them have biological ~~activity-activities~~ which are
53 beneficial ~~for-to~~ human health, being used as biologically active compounds (Scossa and Fernie, 2020).

54 *A. chilensis* fruits have been ~~intensively~~ studied ~~intensively~~ (Masoodi et al., 2019) and they are
55 used for pharmacological (Céspedes et al., 2017; Fuentealba et al., 2012) and
56 nutraceutical purposes (Rubilar et al., 2011; Fredes et al., 2018). Their fruits are bright black edible
57 berries, with a high ~~presence-level~~ of anthocyanin, ~~so-thus~~, they have up to four times more
58 antioxidants properties than other berries (Fredes et al., 2014, 2018; Fuentes et al., 2019). However, the
59 national and international demand for these fruits is growing and so, affects ~~its-the~~ genetic heritage and
60 biodiversity ~~of this species~~. Faced with the drawbacks of the unregulated collection of fruits, the
61 proposal to use leaves as a source of phenolic compounds arises, constituting an alternative that can be
62 sustainable, permanent and that does not affect the state of conservation of the species.

63 *A. chilensis* leaves emerge in two periods of the year, one more abundant in early spring, and
64 another, in summer. As evergreen species, *A. chilensis* plants retain their leaves during ~~the-winter~~ and
65 they remain photosynthetically active, both in the days of moderate temperature in autumn and winter,
66 and during the early spring (Damascos and Prado, 2001). However, there are ~~searce-limited~~ studies ~~that~~
67 ~~report on~~ the behaviour of phenolic compounds in leaves in relation to leaf ontogeny and seasonal
68 period.

69 The present work relates the ontogenic age of the leaves of *A. chilensis* (Mol.) Stuntz ~~with-to~~
70 the photosynthetic capacity and the accumulation of phenolic compounds as well as their antioxidant
71 capacity under different natural growth conditions.

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73 **Materials and methods**

74
75 ~~The-This~~ research was conducted between August 2017 and January 2018, the period in which the
76 leaves of *A. chilensis* were collected, at the Universidad de Concepción, Biobío Region, Chile (36°
77 50'02.6 "S, 73° 01'54.3" W). The influence of climatic conditions on annual seasons in which this
78 study was carried out, is shown in Table 1 Suppl. (data from Dirección Meteorológica de Chile, 2018).
79 In addition, ~~in the Table 1 present~~ the photosynthetically active radiation (PAR) measurements ~~realized~~
80 ~~obtained~~ at midday in the study seasons ~~are presented in Table 1~~.

81 Adult male *A. chilensis* plants ~~were used~~, which grow in a natural environment and reach a
82 uniform height of 3 m ~~were used~~. Six plants were selected within the university campus. The study ~~is~~
83 ~~was~~ carried out with fully expanded leaves, where adult and young leaves ~~are were~~ distinguished. The
84 samples were collected near the base of the tree where the ontogeny ~~is~~ less. Leaves were selected from
85 the upper third of the branch ~~corresponding to as the~~ young leaves (apical leaves) and leaves from the
86 lower third of the branch ~~or were~~ adult leaves (basal leaves).

87
88 **Light response curves**

89 The chlorophyll fluorescence was evaluated through light responses. The leaves were previously put in
90 the ~~darkness-dark~~ for 30 min and then, exposed to different light intensities of 12.13, 25.62, 51.39,
91 86.64, 241.50, 479.08, 878.08 and 1280.83 $\mu\text{mol photons m}^{-2}\text{s}^{-1}$. Fluorescent signals were measured
92 with a pulse amplitude fluorimeter (FMS 2, Hansatech Instrument, U.K). According to the
93 terminology of Murchie and Lawson (2013), the minimum value for chlorophyll fluorescence (F_0) in
94 the dark-adapted state was determined by applying a weak pulse of modulated light, and the maximum
95 fluorescence (F_m) was induced by a short pulse (0.8 s) of saturating light. The fluorescence signals
96 were followed until they reached a steady state (F_s). To determine the maximal fluorescence in light
97 (F_m'), various pulses of saturating light were applied. The minimal value for chlorophyll fluorescence
98 (F_0') in the light-adapted state, was determined by turning off the actinic light, and immediately
99 applying a 2 s far-red pulse.

100 The maximum photochemical efficiency of photosystem II (ΦPSII), F_v/F_m (variable
101 fluorescence/maximal fluorescence) was calculated considering $F_v = F_m - F_0$. Once data from
102 fluorimeter were obtained, the efficiency of photosystem II ($\Phi\text{PSII} = (F_m' - F_s)/F_m'$), and the electron
103 transport rate ($\text{ETR} = 0.8 \times \Phi\text{PSII} \times \text{PAR} \times 0.5$) were calculated. The factor 0.8 is the average value of
104 the absorbance for the green leaves, and the factor 0.5 assumes that the efficiency of both
105 photosystems is equal, and that the radiation is distributed equally among them. In addition,
106 photochemical quenching ($q_P = (F_m' - F_s)/(F_m - F_0')$) and non-photochemical quenching ($\text{NPQ} = (F_m -$
107 $F_m')/F_m'$) were calculated (Sáez et al., 2012).

108 ~~In the different seasons of the year (winter, spring and summer), Branches-branches~~ of *A.*
109 *chilensis* were collected, ~~and identifying~~ apical and basal leaves ~~were identified, in the different~~

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110 ~~seasons of the year (winter, spring, summer).~~ The samples were collected at midday where the light
111 intensity is higher. At this time, the photosynthetically active radiation (PAR) was measured, and the
112 light condition of the exposed environment of the study plants was established based on the criteria
113 described by Zhen and Bugbee (2020). The PAR data ~~was~~is shown in Table 1.

115 **Preparation of *A. chilensis* samples for anatomical studies**

116 For anatomical studies, the tissue was selected from the central portion of the leaf, cut quickly, and
117 fixed in 37% formalin, acetic acid, and 70% ethanol (FAA₇₀). The samples were dehydrated through
118 serial solutions in ethanol and *n*-butyl acetate, and embedded in paraplast. The cuts were made with a
119 Jung Biocut 2035 microtome and stuck in glass sheets with Hatsup and Bissmut adhesives. After
120 removing the paraplast by dipping in butyl acetate and washing with ethanol, the samples were
121 coloured with safranin and Astra blue. Subsequently, they were re-dehydrated in a series of dilutions
122 of ethanol and finally in butyl acetate. Glass sheets were visualized in a Leica ICC50 HP optical
123 microscope (dos Santos Isaias et al., 2011). Apical and basal leaves were selected from the three seasons
124 of the year

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126 **Preparation of *A. chilensis* samples for chemical study**

127 Fresh basal and apical leaves (40 g) were dried at 37°C for two days. Dried leaves were crushed to get
128 obtain powder and then, maceration was performed by exhaustion in methanol-HCl 0.1%. The total
129 extract was concentrated in a rotavapor at 37°C and lyophilized for 24 h. The extraction yield was
130 defined as the amount of extract (mg) recovered by leaf dry weight (mg), for each sample.

132 **Determination of total phenolic content (TPC)**

133 The total phenolic content (TPC) in each extract/sample was determined using TPC method described
134 by Mongkolsilp et al. (2004), with minor-slight modifications. The dried extract/sample was dissolved
135 in distilled water to a concentration of 200 µg mL⁻¹. The calibration curve was established using gallic
136 acid (10 to 200 µg mL⁻¹). The reaction mixture contained: distilled water (400 µL), sample or gallic
137 acid solution for the standard curve (20 µL), Folin-Ciocalteu reagent (40 µL) and 15% sodium
138 carbonate (200 µL). The reaction mixture was incubated at room temperature for 60 min in darkness,
139 with intermittent shaking for favouring colour development. Absorbance was measured at 750 nm
140 using UV-Vis spectrophotometer (BioTeK ELx800, Winooski, USA). TPC was expressed in mg of
141 gallic acid equivalents per gram of dry weight (GAE mg g⁻¹dw).

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145 **Determination of the antioxidant activity**

146 **DPPH radical scavenging assay**

147 The free radical scavenging activity of *A. chilensis* leaf extracts and standard solution Trolox (\pm -6-
148 Hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) were analysed using 1,1-diphenyl-2-
149 picrylhydrazyl (DPPH) radical scavenging method as reported by Morales et al. (2012). The assay
150 mixture contained 270 μ L of 0.06 mM DPPH radical solution, prepared in methanol, and 30 μ L of
151 Trolox at different concentrations (10- to 200 μ g mL⁻¹) or *A. chilensis* leaf extracts. The reaction
152 mixtures were quickly mixed and incubated in darkness at 37°C for 20 min. The decrease in
153 absorbance of each sample was measured at 515 nm using UV/Vis spectrophotometer. Trolox, a well-
154 known antioxidant, was used as positive control, while DPPH radical solution with 1 mL methanol
155 was taken as blank. All determinations were performed in triplicate (n=3).

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157 ABTS radical scavenging assay

158 For ABTS assay, the procedure followed was that described by Kuskoski et al. (2004) with some
159 modifications. The stock solutions included 3.5 mM ABTS (2,2'-azino-bis 3-ethylbenzothiazoline-6-
160 sulfonate) and 1.22 mM potassium persulfate. The working solution was then prepared by mixing the
161 two stock solutions in equal quantities and allowing them to react for 16 h at room temperature in
162 darkness. Once the ABTS^{•+} radical was formed, the solution was then diluted by mixing 1 mL ABTS^{•+}
163 solution with 14 mL distilled water to obtain an absorbance value of 0.70 \pm 0.01 units at 750 nm.
164 ABTS^{•+} solution was freshly prepared for each assay. The reaction (60 min, in dark conditions)
165 contained ABTS^{•+} radical (180 μ L) and 20 μ L of the samples at different concentrations. For standard
166 Trolox and samples, dilutions of 10 to 200 μ g mL⁻¹ were prepared. All determinations were performed
167 in triplicate (n=3). The antioxidant activity for both methodologies was expressed as percentage of
168 inhibition, which corresponds to the amount of radical (DPPH and ABTS) neutralized by the extract at
169 a certain concentration, as described in the following equation

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$$170 \quad \% \text{ inhibition} = ((A_s - A_c) / A_c) \times 100$$

171
172 Where, A_c is the absorbance of control and A_s , the absorbance of the samples. The antioxidant activity
173 was expressed as IC₅₀, which was defined as the final concentration (μ g extract mL⁻¹) of the tested
174 sample required for the inhibition of radical by 50% (Kulisic et al., 2004, Rubilar et al., 2011).

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176 Statistical Analysis

177 For the light response curves, eight samples for six plants were used for each type of leaf in relation to
178 the season of the year studied. For the determination of TPC, six plants-plant samples were used for
179 each type of leaf in relation to the season of the year studied. For the foliar anatomy measurements,
180 five samples with fifteen repetitions were used. The analyses were carried out with the AxioVision LE
181 4.8.2.0 software. The assays for the determination of antioxidant activity were carried out in triplicate.
182 The data obtained were analysed statistically with an using analysis of variance (ANOVA), and the
183 differences between the means were determined through the Tukey test ($P \leq 0.05$). Statistical analyses

184 were performed using the InfoStat/L software (FCAUNC, Argentina) and the graphic representations
185 were made using SigmaPlot software version 10.0 (SPSS; Chicago, IL, USA).

186

187

188 Results

189

190 Effect of seasonal conditions on fluorescence parameters of both basal and apical leaves

191 According to the chlorophyll fluorescence data ~~in for~~ *A. chilensis*, ~~we it was~~ observed that they were
192 affected by ~~seasons of the year's season year. Indeed~~ Certainly, the season of the year had a significant
193 effect on ETR (Table 3 Suppl.). On the other hand, quantum efficiency of the PSII (Fv/Fm) ~~;~~ was
194 affected by the leaf type but not by the environmental conditions or their interactions. However, qP and
195 NPQ ~~was were~~ significantly affected by the leaf type and the season, and an interaction between both
196 variables (season and leaf type) was observed (Table 3 Suppl.).

197 Light response showed that the maximum values of ETR were ~~found obtained~~ in the seasons
198 of the year with greater luminosity (spring and summer) in both types of leaves (basal and apical); ~~as~~
199 ~~shown in~~ Table 2). In fact, the minimum values were observed in winter, regardless of the type of leaf,
200 showing a strong correlation of ETR/PAR during this season, as ~~it is~~ described for this the last
201 parameter in the M&M Materials and Methods section. The parameter Fv/Fm remained with values
202 ~~around of about~~ 0.80 in all the seasons of the year analysed, without significant differences among
203 them. In the same way, Fv/Fm values showed no significant differences between basal and apical
204 leaves (Tables 2 and ~~Table~~ 3 Suppl.).

205 Photochemical quenching (qP) is the ratio of excitation energy trapped by open reaction
206 centres that has been used for electron transport (Moreno et al. 2008). ~~We It was~~ observed that the
207 highest qP values were recorded in spring and summer, in both types of leaves, and they all showed
208 ~~significance significant~~ differences with respect to the data ~~collected obtained~~ during the winter (Table
209 2). However, there were no significant differences in qP values in both apical and basal leaves during
210 winter, where the lowest values of qP were found.

211 Non-photochemical quenching (NPQ) ~~represents indicates~~ the influence of non-photochemical
212 processes on the fluorescence emission of chlorophyll from a darkness to light state (Moreno et
213 al. 2008). The type of leaf used and the seasons of the year did not influence the behaviour of NPQ,
214 although a trend to rise increase the NPQ values with the increase in the light intensity during spring
215 and summer, especially in apical leaves was observed (~~Table Tables~~ 2 and ~~Table~~ 3 Suppl.).

216

217 Foliar Anatomy

218 Fig. 1 Suppl. shows cross sections of basal (Fig. 1A) and apical (Fig. 1B) fully expanded leaves.
219 Important anatomical differences were observed, mainly in the conformation of palisade and spongy
220 parenchyma of both types of leaves. In apical leaves (Fig. 1B), the area of parenchyma palisade was

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221 ~~bigger~~larger than in the basal leaves. Also, the basal leaves had smaller intercellular spaces than the
222 apical leaves. However, no significant changes were recorded in the epidermis in both types of leaves
223 (Table 4).

224 **Yields of the extraction of phenolic compounds**

226 Fig. 2Suppl. shows the yield of the extraction process of phenolics, ~~which was~~ obtained from basal
227 and apical leaves of *A. chilensis*, in the different seasons of the year (mg extract mg⁻¹dw). When
228 comparing the yields, both apical and basal leaves in winter were lower than in spring and summer.
229 Basal and apical leaves in spring and summer, respectively, showed the highest yield levels.

230 **Determination of Total Phenol Content**

232 TPC values showed significant differences in both basal and apical leaves, and in the different seasons
233 (Fig. 3). The highest values were recorded for extracts obtained from basal leaves in spring, followed
234 by those of apical leaves in winter and summer. The lowest value of TPC was ~~recorded~~ ~~detected~~-in
235 extracts of basal leaves in winter and in those obtained from apical leaves in spring. According to TPC
236 data from leaves of *A. chilensis*, they were not affected by the type of leaf and interaction between
237 both factors ~~were was~~ observed (Table 5 Suppl.).

238 **Determination of the Antioxidant Activity**

240 The *F*-values from two-way ANOVA for the antioxidant activities obtained by both methodologies
241 (ABTS and DPPH), were influenced by the season of the year, the type of leaf and ~~by~~ the interaction
242 between both factors as ~~can be observed~~ ~~shown~~ in Table 5 Suppl. In addition, the antioxidant activity
243 for ABTS assay expressed as a function of IC₅₀ is shown in Table 6. Data described in this Table
244 strongly support significant differences in extracts of basal leaves sampled in winter, with respect to
245 the extracts obtained in the other two seasons, as well as significant differences between both types of
246 leaves as ~~it can be seen~~ ~~by from~~ their *F* values. The best result of this ABTS antioxidant activity was
247 found in basal winter leaves, which corresponds to the lower value of IC₅₀, followed to spring and
248 summer basal leaves. The lowest antioxidant capacity which corresponds to the higher value of IC₅₀
249 was found in apical spring leaves by ABTS assay.

250 On the other hand, IC₅₀ values obtained from DPPH assays are shown in Table 6. Data
251 obtained from this assay were significantly different in both types of leaves as confirmed by their *F*
252 values. Although, the differences in antioxidant activity were small and significant, basal leaves during
253 winter had a higher antioxidant capacity than those ~~leaves~~-in spring and summer. Regarding apical
254 leaves, the extracts in all the seasons presented values statistically different, being lower in summer,
255 which means a higher antioxidant capacity, and higher levels in both winter and spring, which is
256 related to a lower antioxidant capacity.

258

259 Discussion

260

261 Weather conditions that characterize the different seasons of the year affect both the
262 morphology and ~~the~~ physiology of the plant, and therefore, modify the growth and
263 development of its organs, including the leaves. Consequently, the objective of this work was
264 to analyse the effect of climatic conditions ~~in-on~~ *A. chilensis* leaves. As maqui leaves emerge
265 in two periods of the year, one more abundant in spring, and another, in summer, ~~we selected~~
266 these two seasons ~~were selected~~ to carry out this study. In addition, ~~we also selected~~ a third season,
267 winter, ~~was also selected~~ because *A. chilensis* plants retain their leaves during this period, and ~~so thus~~,
268 they remain photosynthetically active. Another factor analysed was the level of growth of *A. chilensis*
269 leaves, in both apical and basal leaves, ~~due to because~~ these leaves have different morphological and
270 physiological characteristics, ~~as also, well as~~ apical leaves ~~are~~ continuously ~~growing-grow~~ leaves which
271 act as consumptive sinks while basal leaves, being adult leaves, are sources of photosynthetic products.

272 In fact, Damascos and Prado (2001) indicated that adult leaves of *A. chilensis* in winter,
273 ~~remain-remained~~ photosynthetically active during the spring and 15 days before the senescence, ~~and~~
274 ~~when~~ subjected to a low photonic flow density ($150 \text{ mmol m}^{-2} \text{ s}^{-1}$), ~~and~~ showed higher average values
275 of photosynthesis. Being an evergreen tree, its leaves emerge in two seasons of the year, spring, and
276 summer. These authors indicated that, the new leaves in the spring constitute sinks of mass and energy.
277 However, the formation and growth of the reproductive structures of the plant are processes with high
278 energy demand. In other studies, conducted ~~in-on~~ evergreen species, it was found that the conservation
279 of leaves from winter to spring was not associated with the translocation of foliar nutrients before the
280 formation of new leaves but to maintain a positive carbon balance in less favourable periods (Mendoza
281 ~~et al.~~ 2014).

282 Murchie and Lawson (2013) ~~described-carried out a revision-review-about-on~~ how fluorescence
283 parameters can be used to evaluate changes in photosystem II photochemistry, linear electron flux, and
284 CO_2 assimilation *in vivo*, and described the theoretical bases for the use of specific fluorescence
285 parameters. In relation to these parameters, apical leaves showed lower Fv/Fm values than basal leaves
286 during spring and summer, although, the data ~~recording-made~~ ~~obtained~~ was ~~always constantly~~ within
287 the normal physiological values (~~around-about~~ 0.8) throughout the year. This agrees with ~~our-the~~
288 results ~~of the current study~~ (Table 2) and ~~so thus~~, the decrease of Fv/Fm values cannot be interpreted
289 as a photoinhibition of the photosynthetic apparatus. This could be due to two reasons: either *A.*
290 *chilensis* tolerant to high light conditions (Lusk, 2004), or this plant is not under stress, ~~allowing~~
291 ~~making~~ it to achieve the optimum physiological performance in any season of the year (Molina-
292 Montenegro ~~et al.~~ 2012). Other studies ~~confirm-confirmed~~ that this species has a great phenotypic
293 plasticity in traits associated with carbon gain and water economy, which ~~allows-makes~~ it to survive

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294 both under habitats with low light and water availability (continuous forest), and with high light
295 conditions and water scarcity (Repetto-Giavelli et al., 2007).

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296 In the present study, ETR and qP values were well-correlated and ~~appear~~ appeared to be
297 dependent on light intensity. However, in *A. chilensis* basal leaves, both ETR and qP values were
298 slightly higher in spring and summer. Acosta-Motos et al. (2015a) studied the effect of high light
299 intensity on chlorophyll fluorescence parameters in apical and basal leaves ~~in~~ of myrtle plants. Under
300 high light irradiation, basal leaves ~~from~~ of myrtle plants ~~displayed~~ showed higher values for qP, Fv/Fm
301 and NPQ than apical leaves (Acosta-Motos et al., 2015a). These results partially ~~agreed~~ agrees with ~~our~~
302 ~~those of the present study results, due that because~~ Fv/Fm values of *A. chilensis* leaves were
303 influenced by leaf type, with higher Fv/Fm values in basal leaves than apical leaves under high light
304 conditions (spring and summer). This result can be due to a down-regulation mechanism of PSII in
305 high light conditions in apical leaves, which were more exposed to sunlight than basal leaves. A
306 similar response in Fv/Fm values has been observed in pea leaves subjected to high light irradiation,
307 ~~being that is,~~ the response ~~is~~ dependent on the exposure time to this high light intensity (Hernández et
308 al., 2004). On the other hand, higher NPQ means that much of the light energy absorbed is dissipated
309 by the protective mechanisms. However, in ~~our~~ the results of the current study, NPQ behaved
310 differently depending on the leaf type, but no significant ~~changes~~ change were recorded for NPQ
311 values during the different seasons. These stable NPQ values suggested the capacity of *A. chilensis*
312 leaves to use the excess ~~of~~ light energy into photosynthesis process. In contrast, in basal myrtle leaves,
313 high NPQ values were observed during periods of high light intensity, which could facilitate the safe
314 removal of excess light energy and minimize the generation of ROS (Acosta-Motos et al., 2015a).

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315 Furthermore, in this study, some differences in foliar anatomy ~~can be~~ were found between
316 apical and basal leaves. One of the ~~more~~ evident ~~one~~ is the number of chloroplasts, more abundant in
317 the palisade parenchyma in basal leaves. ~~That~~ This means that photosynthetic activity must be higher
318 in basal than in apical leaves, which must still function as a sink organ. These differences can be
319 reflected in Fv/Fm values, ~~being in which higher~~ basal leaves ~~are~~ higher during spring and summer.
320 Interestingly, apical leaves had a higher percentage of area occupied by palisade parenchyma, an
321 adaption that could favour the photosynthetic process. Another interesting difference was the greater
322 intercellular spaces observed in ~~the~~ apical leaves in relation to basal leaves. This anatomical
323 modification in leaves, can improve the CO₂ diffusion, and facilitate its entry ~~to~~ into the chloroplast,
324 especially under stress conditions. A similar modification was previously described in myrtle and
325 Eugenia plants under salinity conditions (Acosta-Motos et al., 2015a,b). These authors observed a
326 decrease in the percentage of spongy parenchyma, and an increased percentage of intercellular spaces
327 under NaCl-stress. ~~These~~ They authors concluded that these anatomical changes may serve to facilitate
328 CO₂ diffusion in a situation of reduced stomatal aperture (Acosta-Motos et al., 2015a,b).

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329 *A. chilensis* is characterized by high ~~phenols~~ phenol content ~~as~~ compared to other berries, and
330 ~~the~~ nutritional and pharmacological effects are attributed to them. The TPC observed in this study

331 were lower ~~to than that those~~ reported by Vidal et al. (2013) for *A. chilensis* leaves collected in Región
332 del Biobío. These authors carried out the extraction in ethanol-water 50% v/v, and obtained TPC values
333 of 40 ± 0.57 mmolL⁻¹GAE, which is equivalent to 136 mg of phenols g⁻¹ dw values, and higher when
334 compared with samples of apical leaves of spring. A similar response was reported by Rubilar
335 et al.(2011) where TPC of *A. chilensis* leaves was higher than ~~A. chilensis leaves on those of~~ this study
336 (69.0 ± 0.9 mg GAE g⁻¹dw). These results could indicate that the extraction method used influenced ~~on~~
337 the TPC quantification.

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338 In addition, phenolic compounds exhibit a wide range of biological effects. Some of them are
339 powerful free radical scavengers (and so, have antioxidant activity). For this reason, they are useful in
340 the prevention of arteriosclerosis, cancer, diabetes, neurodegenerative diseases and arthritis
341 (Gonçalves and Romano, 2017). Depending on the extraction procedure, the antioxidant activity can
342 vary, being in some cases, higher in leaves than in fruits (IC₅₀ values, leaves: 8.0 ± 0.1 mg extractL⁻¹,
343 fruit: 399.8 ± 17.5 mg extract L⁻¹)(Rubilar et al., 2011). In ~~our the case of the present study,~~ the
344 antioxidant activity recorded in leaves was similar to that observed by Rubilar et al.(2011) in stems
345 (IC₅₀ value: 43.1 ± 1.7 mg extractL⁻¹).

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346 The mechanism by which an antioxidant compound interacts with a radical molecule depends
347 on the structural conformation of the antioxidant (Mishra et al., 2012). Granato et al. (2018) indicate
348 ~~indicated~~ that no single antioxidant activity assay will reflect the total antioxidant capacity. The
349 antioxidant activity methods have particularities depending on mechanisms of action, types of radical,
350 pH, time of exposition, and temperature. Another important factor is the use of standards to build
351 analytical curves, which generate the sample's quantitative results (Granato et al., 2018). One of the
352 tests used in this study was the DPPH assay, which is simply due to its stable nitrogen radical, but has
353 problems with many antioxidants by reacting with different kinetics or not reacting at all (Mishra et
354 al., 2012). DPPH assay is reversible due to the reaction's low reading of antioxidant capacity of some
355 antioxidants. It was also indicated that the DPPH assay is pH-dependent, and the final result could be
356 influenced by the deprotonation of the phenolic group (Mishra et al., 2012; Tirzitis and Bartosz, 2010).
357 The other method used in this study was the ABTS assay, which consists of an oxidation reaction of
358 the coloured cation ABTS^{•+}. The ABTS assay can be applied to lipophilic and hydrophilic
359 compounds (Huyut et al., 2017). ~~Thereby Thus, our the results of the current study determine~~
360 ~~determined~~ in the DPPH assay, an increase in antioxidant activity in summer in both types of leaves,
361 coinciding with the greater luminosity, without the same trend in ABTS assay.

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362 On the another hand, Harnly (2017) described how the measurement of *in vitro* antioxidant
363 activity and total phenolic content using the Folin-Ciocalteu reagent are not suitable. The author
364 indicated no standard mechanism or method to quantify antioxidant activity, and scientific research
365 will only use advanced techniques to identify antioxidants. In this regard, chromatography techniques
366 used to identify and quantify phenolic compounds in foods, beverages, and herbal extracts have
367 sufficient accuracy or precision. It is also necessary to highlight what the author indicates about the

368 method's results, *a* are (usually) not comparable with the data of method *b* or even between laboratories
369 (Harnly, 2017). Therefore, as indicated by Granato et al. (2018), it is evident that "antioxidant activity"
370 involves complex interactions. However, screening spectrophotometric methods to characterize the
371 samples ~~and~~ give an idea of total phenolic content in the matrix.

372 As a general conclusion, in this study, it was determined that the *A. chilensis* basal leaves showed a
373 better photosynthetic performance as ~~observed—indicated~~ by higher Fv/Fm values, ~~that~~ which
374 ~~correlated~~ correlates with higher total phenol content in high light conditions. In both types of leaves,
375 the increase in light intensity was accompanied by ~~a~~ an increase ~~rise~~ in NPQ values, reflecting a safe
376 mechanism to dissipate excess light energy. In addition, apical leaves display some morphological
377 adaptations, such as the increase in intercellular spaces in order to facilitate the entry of CO₂ inside the
378 chloroplasts, as a mechanism to protect the photosynthetic process. Finally, in order to take advantage
379 of the research, it is necessary to know in the future, the performance of the activity of the different
380 antioxidant enzymes and compare these antioxidants mechanisms in both types of leaves.

381 **Acknowledgements**

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Table 1. Climatic conditions of Región del Biobío, Concepción, Chile, where *A. chilensis* is growing. Each parameter corresponds to the monthly average for the three seasons of the year.

Season	Winter (August)	Spring (November)	Summer (January)
Minimum temperature (°C)	0	8	9
Maximum temperature (°C)	14	22	29
Average temperature (°C)	9	14	17
Average rainfall (mm)	586.73	849.72	1.45
Humidity (%)	85	81	75
Average wind speed (km h ⁻¹)	13.7	13.6	14.8
Maximum wind speed (km h ⁻¹)	85.2	50	50
PAR at midday (μmol photons m ⁻² s ⁻¹)	435.98 ± 10.4	1901.7 ± 3.6	1968.7 ± 7.2

*Source: Dirección Meteorológica de Chile, 2018.

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Table 2. Effect of the season of the year on fluorescence parameters in basal and apical leaves of *A. chilensis*.

Parameters	Winter	Spring	Summer	F
Basal leaf				
ETR	64.02 ± 6.47a	129.71 ± 13.69b	108.83 ± 4.83b	13.38**
qP	0.25 ± 0.03b	0.47 ± 0.04a	0.42 ± 0.02a	13.81**
Fv/Fm	0.83 ± 0.01a	0.84 ± 0.005a	0.84 ± 0.01a	0.97ns
NPQ	3.42 ± 0.11a	3.79 ± 0.15a	3.84 ± 0.22a	1.85ns
Apical leaf				
ETR	85.75 ± 5.25a	123.08 ± 12.58b	122.19 ± 3.64b	6.84*
qP	0.35 ± 0.02b	0.48 ± 0.04a	0.51 ± 0.01a	13.35**
Fv/Fm	0.83 ± 0.004a	0.82 ± 0.01a	0.82 ± 0.004a	1.16ns
NPQ	3.85 ± 0.14a	3.89 ± 0.14a	4.27 ± 0.07a	3.44ns

420 Data represent the mean ± SE from eight samples ~~from of~~ each season of the year. Different letters in
421 the same row indicate significant differences according to Tukey's test ($P \leq 0.05$). F values from
422 ANOVA for the different season of the year and type of leaf.

423 *F values were significant at 95% ~~levels-level of~~ probability.

424 **F values were significant at 99% ~~level levels-of~~ probability.

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425 ***F values were significant at 99.9% levels of probability.
 426 Non-significant values are indicated by "ns".

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433 Table 3. Relationship between type of leaf (A) and season of the year (B) as well as interaction of both
 434 (AXB) on fluorescence parameters in *A. chilensis*.

Source of variation	ETR	qp	Fv/Fm	NPQ
Type of leaf (A)	1.76ns	8.01*	7.19*	7.08*
Season (B)	19.22**	25.07**	0.27ns	4.06*
AXB	13.4**	19.38**	2.58ns	5.06*

435 F-values from two-way ANOVA for ETR, qP, Fv/Fm and NPQ. F-values significant at 99.9% (***),
 436 99% (**), or 95% (*) levels of probability. Non-significant values are indicated by "ns".

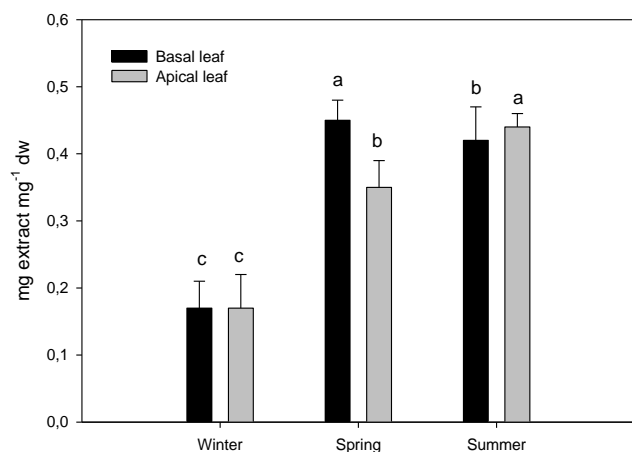
440 Fig. 1. Cross section of basal (A) and apical (B) leaves of *A. chilensis*. Black bars: 50µm. EP:
 441 epidermis. PP: palisade parenchyma. SP: spongy parenchyma.

444 Table 4. Measurements of areas (µm²) in cross sections of basal and apical leaves of *A. chilensis*.

	Area (µm ²)		
	Basal leaf	Apical leaf	F
Adaxial epidermis	243.27 ± 8.24a	257.25 ± 7.51a	1.57ns
Palisade parenchyma	266.78 ± 7.16a	336.98 ± 5.33b	61.81**
Spongy parenchyma	203.5 ± 7.97a	214.33 ± 4.91a	2.52ns
Abaxial epidermis	135.29 ± 5.28a	140.85 ± 3.38a	0.79ns
Intercellular space	888.71 ± 23.26a	1165.04 ± 31.42b	49.97**

445 Data represent the mean ± SE from 75 measurements. Different letters in the same row indicate
 446 significant differences according to Tukey's test ($P \leq 0.05$). F values from one-way ANOVA for the
 447 different type-types of leaf.

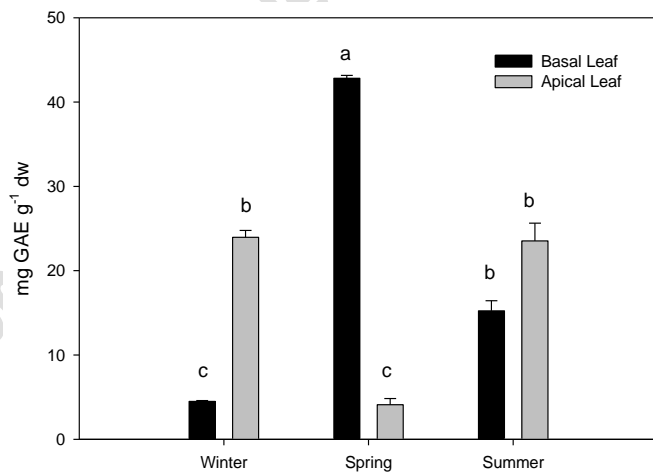
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456 Fig. 2. Yield of extracts of basal and apical leaves of *A. chilensis* to determine phenolic content in three
457 seasons of the year (mg extract mg⁻¹ dw). Data represent the mean \pm SE from of three samples from
458 each season of the year. Different letters in the same row indicate significant differences according to
459 Tukey's test (one-way ANOVA, $P \leq 0.05$).

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463 Fig. 3. TPC (expressed as mg GAE g⁻¹ dw) in basal and apical leaves of *A. chilensis* in the different
464 seasons of the year. Data represent the mean \pm SE from of six samples from each season of the year.
465 Different letters indicate significant differences according to Tukey's test (one-way ANOVA, $P \leq 0.05$).

466

467 Table 5. Relationship between type of leaf (A) and season of the year (B), as well as the interaction of
 468 both (AXB), expressed as *F*-values from two-way ANOVA, for TPC and antioxidant activity (ABTS
 469 and DPPH assays) in *A. chilensis*.

Source of variation	TPC	ABTS	DPPH
Type of leaf (A)	0.65ns	25.15**	11.04*
Season (B)	1.39*	9.15*	3.97*
AXB	1.14ns	14.48*	6.32*

470 *F*-values from two-way ANOVA for TPC, ABTS and DPPH. *F*-values significant at 99.9% (***)
 471 (**), or 95% (*) levels of probability. Non-significant values are indicated by “ns”.

472
 473 Table 6. Antioxidant activity (ABTS assay and DPPH assay assays) expressed as a function of the IC₅₀
 474 obtained for each type of leaf and season of the year (µg extract mL⁻¹).

Parameters	Winter	Spring	Summer	<i>F</i>
Basal leaf				
ABTS	45.05 ± 0.30a	49.97 ± 2.24b	49.91 ± 0.97b	11.88*
DPPH	34.68 ± 0.58a	35.92 ± 0.90ab	38.08 ± 1.65b	6.93*
Apical leaf				
ABTS	51.81 ± 4.59a	69.02 ± 4.21b	56.05 ± 4.21a	12.81*
DPPH	59.49 ± 1.87c	47.37 ± 0.63b	33.89 ± 1.53a	236.03***

475 Data represent the mean ± SE of three samples from each season of the year. Different letters in the
 476 same row indicate significant differences according to Tukey’s test ($P \leq 0.05$). *F* values from one-way
 477 ANOVA for the different type-types of leaf.

478