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Invited Review

The powerful colour of the maqui (*Aristotelia chilensis* [Mol.] Stuntz) fruit

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Abstract. Over the past 10 years, the research interest on maqui has increased due to the potential health benefits of the fruit, which are largely attributed to the high anthocyanin content and high antioxidant capacity. Furthermore, maqui fruit has earned the name of 'superfruit', and several products based on the dehydrated fruit and maqui juice are available on the international market. Although the maqui fruit is not frequently consumed by the Chilean population from urban areas, its use is deeply rooted in rural and native cultures (Mapuche and Huilliche). This review summarises the validation of the traditional uses of maqui and new evidence highlighting the principal role of anthocyanins in the antioxidant, anti-inflammatory and anti-diabetic activity of maqui fruit. The identification of a particular anthocyanin (delphinidin-3-glucoside-5-sambubioside) in maqui fruit and its anti-diabetic effect in *in vivo* models, in addition to its presence at higher concentrations in some maqui genotypes, encourages investigation into maqui genotypes that may have higher contents of particular anthocyanins. However, information concerning maqui domestication is still deficient.

Keywords: Anthocyanins, anti-inflammatory, antioxidant, anti-diabetic, Chile

1. Introduction

In recent years, international interest in South American berries has increased due to their potential health benefits and consumer demand for novel exotic fruit [1, 2]. Among these berries, maqui fruit (*Aristotelia chilensis* [Mol.] Stuntz, Elaeocarpaceae) from Chile has been singled out as an exceptionally rich source of anthocyanins and natural antioxidants [3]. Maqui fruits are collected from wild populations between December and February [4, 5] because commercial plantations of this species do not yet exist. Therefore, fresh fruits are available primarily in local markets. Although maqui berries are not frequently consumed by the urban population, the use of these berries is deeply rooted in rural and native cultures (Mapuche and Huilliche) [3]. Traditionally, in the summer, children from the countryside could be seen with purple-tinged smiles and tongues after eating maqui fruit [6]. For the Mapuche culture, the plant is a sacred symbol of "good intentions" [4]. Ethnopharmacological records recommend using the leaves as an infusion for the treatment of throat diseases and the fruits as an antidiarrheal treatment, whereas local populations prepare jams, a maqui wine called "tecu" and juice, which is used as a dye [7, 8].

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The earliest research on the maqui plant focused on the validation of traditional uses of both the leaves and fruits. In the last 6 years, new biological properties and health effects of maqui have been reported. Few studies have investigated maqui domestication [9]; however, R&D projects supported by Chilean public institutions regarding maqui domestication, selection and cultivation are currently underway. The objectives of this paper are to exhaustively review the evidence regarding the validation of traditional uses of maqui and to compile the new data concerning biological activities and health effects to propose future perspectives in the research and commercial development of maqui.

2. The maqui plant and fruit

Maqui, also known as 'clon', 'queldron' and 'maquei' [4], is in the Elaeocarpaceae family, which includes 10 genera and 400 species worldwide. In Chile, two genera (*Crinodendron* and *Aristotelia*) and three species (*C. hookerianum, C. patagua* and *A. chilensis*) are represented by this family [10]. Maqui is a dioecious species that grows naturally as an evergreen bush or tree from Limarí (30°S latitude, Coquimbo Region) to Aysén (45°S latitude, Aysén del General Carlos Ibáñez del Campo Region), Chile, and from the coast to 2000 m above sea level [10]. Maqui is also a native species of the Andean region of Argentina [11]. In Chile, it is a pioneer species that colonises varied environments and has a great capacity for regeneration in pine forests of the central and southern regions [12], forming wild populations called 'macales'. In native forests in Central Chile, maqui is a secondary species where populations of quillay (*Quillaja saponaria* Mol.), litre (*Lithrea caustica* [Mol.] Hook. et Arn.), and peumo (*Cryptocarya alba* [Mol.] Looser) are predominant [13]. In open areas, this species can reach 4–5 m tall, and, when grown in communities, it acquires a bushy form [4]. Both male and female flowers are arranged in clusters on separate individuals. Flowers compose a corymb inflorescence that arises from the leaf axils. The flowers (Fig. 1a), which appear in October and November, are pale yellow (5–6 mm diameter) and have a campanulate calyx of 5–6 lanceolate sepals [10].

Botanically, the maqui fruit is a berry (5 mm diameter) with three or four seeds [10] that ripen (Fig. 1c–e) in 1,100 growing degree-days (91 d after fruit set) (Fig. 1b) in Central Chile [14]. Once mature, deep-purple fruits (Fig. 1e) have 18–19°Brix [14] and a weight of 21–24 g per 100 berries [11, 14]. The ripening processes in maqui fruit appears to have a significant effect on the total anthocyanin content and corresponding antioxidant capacity [14]. The reported mineral content (K, Ca, Ba, Br, Zn, Co, Cr, Fe, Na, Rb, Cs, and Sr) of maqui fruit shows that K (18,633 mg/kg), Ca (4,823 mg/kg), Fe (106 mg/kg), Na (49 mg/kg) and Zn (13 mg/kg) expressed on dry weight have the highest contents [11].

3. Validation of traditional uses of maqui

Different methods of biological activity have been used to validate the traditional uses of maqui. Some studies identified natural products such as alkaloids and polyphenols; although, in many cases it is difficult to correlate a specific compound to a specific biological effect. In addition, some studies lacked detailed information about the raw materials. This information is particularly important because the types and content of natural products differ according to the genotype, environment and time of harvest [15]. Generally, leaves and fruit are collected from wild habitats.

Fig. 1. Maqui flowering (a), fruit set (b) and different fruit maturity stages (c–e).

3.1. Maqui leaves

Alkaloids are the most well-studied natural products from the maqui leaf. Initial studies [16, 17] indicated the presence of indole alkaloids (aristotelinone, makonine, 8-oxo-9-dehydrohobartine and 8-oxo-9-dehydromakomakine) that were elucidated by high field NMR spectroscopy. In successive studies, these findings were complemented with the isolation of a quinoline alkaloid, the coumarin scopoletin [18] and other indole alkaloids such as makomakine, hobartine and serratoline [19]. Recently, Moreno et al. investigated the structure of indole alkaloids from maqui leaf [20].

Delporte et al. [21] studied different types of maqui leaf extracts based on the use of maqui leaf infusion for throat inflammation, pain and infection; these uses suggest anti-inflammatory, analgesic, and antimicrobial activities. A successive study [22] investigated the analgesic, anti-inflammatory and antioxidant activities using bioguided *in-vivo* assay to identify the main chemical constituents in maqui leaf and, thereby, scientifically validate its medicinal use. Aristoteline, aristone, serratoline and hobartinol were isolated from of a crude alkaloid residue of maqui leaf. Ursolic acid, friedelin and quercetin 5,3′-dimethyl ether were identified in the dichloromethane extract, while quercetin 3-*O*-β-D-glucoside and kaempferol were identified in the methanol extract. The aqueous leaf extract yielded protopine, aristoteline and caffeic and ferulic acids. Anti-inflammatory activity was studied using two animal models: 12-*O*-tetradecanoylphorbol-13-acetate (TPA)-induced inflammation in mice and carrageenan-induced paw oedema in guinea-pigs [22].

Based on use of maqui leaves in a poultice for the healing of wounds and associated infections by the Huilliche people of Chile, 40 plant species (included maqui) were evaluated against bacterial and fungal human pathogens, especially wound-associated pathogens [23]. The aqueous leaf extract showed antimicrobial activity against *Staphylococcus aureus* (ATCC 6538) and *Bacillus subtilis* (ATCC 6633) with a minimum inhibiting amount (MIA) <1 mg, whereas the DMC:methanol (1:1) leaf extract showed antimicrobial activity against *Staphylococcus aureus* (ATCC 6538) with a lower MIA (<0.5 mg). The DMC:methanol (1:1) and ethanolic (96%) leaf extracts inhibited *Escherichia coli* (EDL 933) with an MIA $\approx$ 1 mg. These findings were in agreement with previous work in which ethanolic (60%) leaf extracts inhibited the growth of *Pseudomonas aeruginosa* (ATTC 27653), *S. aureus* (ATTC 6538P), *Enterobacter aerogenes* (UC-1) and *Candida albicans* (UC-A) [24].

Avello et al. [25] investigated the use of a leaf infusion for the treatment of various ailments in folk medicine by evaluating antioxidant capacity (AC) with the TBARS (thiobarbituric acid reactive substances) method in plasma before and after ingestion of maqui leaf infusions (1%). The total phenolic content (TPC) in the infusion was 0.074 mM of gallic acid equivalent (GAE). In this study, healthy non-smoking volunteers with body mass within the normal range drank this infusion two times a day for three days according to the doses recommended by folk medicine. The results indicated an average increase of AC after 24 hours (30.27%).

Current research regarding the bioactive compounds of maqui leaf is focused on the stabilisation of the compounds. Vidal et al. [26] studied the microencapsulation of maqui leaf ethanolic extract (1%) by water-oil (W/O) emulsion. The extract included flavonoids (0.061 mg/mL) such as gallic acid (47.6%), catechin (21.6%) and pelargonidin (14.45%) and the AC was 189.5 and 165.7 mM GAE according to the DPPH (extract at 1%) and ABTS methods (extract at 1%), respectively. Encapsulation technology can be applied to maqui leaf extract in a ratio of 20% aqueous phase and 80% oil phase depending on the properties of the component to be encapsulated, droplet diameter, gum arabic content, and surfactant concentration.

3.2. Maqui fruits

Anthocyanins are the primary natural products studied in maqui fruit. Initial studies characterising the anthocyanin profile in maqui fruit by HPLC-DAD-MS [27] identified eight anthocyanins: delphinidin-3-sambudioside-5-glucoside (del-3-sa-5-glu), delphinidin-3,5-diglucoside (del-3,5-diglu), cyanidin-3-sambudioside-5-glucoside (cy-3-sa-5-glu), cyanidin-3,5-diglucoside (cy-3,5-diglu), delphinidin-3-sambudioside (del-3-sa), delphinidin-3-glucoside (del-3-glu), cyanidin-3-sambudioside (cy-3-sa) and cyanidin-3-glucoside (cy-3-glu). The delphinidin derivatives (73%) predominated over the cyanidin derivatives (37%), and del-3-sa-5-glu was the most abundant anthocyanin (34% of total anthocyanins). The average total anthocyanin content was 137.6 mg del-3-glu equivalent/100 g of fresh fruit. Similar anthocyanin profiles have been reported by Céspedes et al. [28], Ruiz et al. [29] and Gironés-Vilaplana et al. [30].

However, Rojo et al. [31] reported that del-3-glu (34.1% of total anthocyanins) was the most abundant anthocyanin in maqui fruit. Similarly, Schreckinger et al. [32] identified seven anthocyanins in which cyanidin-3,5-diglucoside was not included. Gironés-Vilaplana et al. [33, 34] also identified cyanidin-3-glucoside-5-rhamnoside in maqui fruit. Fredes et al. [35] evaluated the anthocyanin contents of 18 maqui genotypes during two growing seasons in which two genotypes (from the same geographical area) had cy-3-sa and del-3-sa at trace levels. Three new anthocyanin peaks with absorption at 520 nm were also present in quantifiable amounts in some of the genotypes. Del-3-sa-5-glu was not the most abundant anthocyanin in any of the genotypes, whereas del-3,5-diglu and del-3-glu were the most abundant anthocyanins in 50% of the genotypes. The authors indicated that variations and ranges recorded in these results were expected given the different genotypes and environments studied.

Céspedes et al. [28] identified other polyphenols in maqui fruit, namely, gentisic acid, ferulic acid, gallic acid, ρ-coumaric acid, sinapic acid, 4-hydroxybenzoic acid, vanillic acid, gallocatechin gallate, quercetin, rutin, myricetin, catechin, epi-catechin and proanthocyanidin B. In addition, Gironés-Vilaplana et al. [33] identified ellagic acid derivatives (granatin B, ellagic acid and ellagic acid rhamnoside), flavonols (myricetin 3-*O*-galoylglucoside, myricetin 3-*O*-galactoside, myricetin 3-*O*-glucoside, quercetin 3-*O*-rutinoside, quercetin 3-*O*-galactoside, quercetin 3-O-glucoside, quercetin 3-*O*-xyloside, quercetin 3-*O*-arabinoside, quercetin 3-*O*-rhamnoside) and 5-*O*-caffeoylquinic acid in maqui fruit.

Similar to the investigation of maqui leaf, biological activities of maqui fruit were evaluated to validate the traditional uses. Maqui fruit extracts exhibited strong anti-inflammatory activity in mouse ears, in the TPA-induced inflammation model [36], and in rat paws, in the carrageenan-induced inflammation model [37]. In these studies different extracts, fractions and sub-fractions were evaluated and dose-dependent anti-inflammatory activity was found. The anti-inflammatory activity of these extracts was similar to commercial drugs (indomethacin and ovatifolin) and some natural flavonoids (quercetin, myricetin, luteolin and diosmetin). This anti-inflammatory activity was attributed to phenolic acids, anthocyanins and other flavonoids present in maqui fruit. In another study [32], the anti-inflammatory properties were evaluated *in vitro* using lipopolysaccharide-stimulated (LPS) RAW 264.7 macrophages. The phenolic extracts of maqui fruit decreased inflammation *in vitro* by inhibiting LPS-induced iNOS/NO and COX-2/PGE_2 pathways in macrophages, and the *in vitro* potency of these extracts was dependent upon the phytochemical composition.

Methods that have been used to evaluate the *in vitro* and *in vivo* AC of maqui fruit include: FRAP (ferric reducing activity power) [38], TRAP (total radical-trapping antioxidant parameter), TAR (total antioxidant reactivity), TBARS (thiobarbituric acid reactive substances), radical DPPH (2,2-diphenyl-l-picrylhydrazyl) [39], and ORAC (oxygen radical absorbance capacity) [40].

Miranda-Rottmann et al. [41] compared the TPC and AC of different concentrated juices (blueberry, raspberry, strawberry, cranberry, blackberry and maqui) and red wine using TRAP and TAR. The AC was directly correlated to the TPC, and the maqui juice had significant higher AC compared to the samples. Furthermore, maqui juice inhibited *in vitro* copper-induced low-density lipoprotein (LDL) oxidation and protected endothelial cells from hydrogen peroxide-induced intracellular oxidative stress.

Araya et al. [42] determined the AC (FRAP method) of different fruits and vegetables consumed in Chile, and, again, maqui stood out significantly from the other samples analysed. AC ranged from 0.02 mM Fe/100 g for melon pear to 12.32 mM Fe/100 g for maqui fruit. High AC was observed in other berry-type fruits such as strawberry and blackberry (3.10 and 3.55 mM Fe/100 g, respectively). Intermediate AC was observed in fruits such as lemon and quince (0.25 and 0.23 mM Fe/100 g), and the lowest AC was found in apples (Fuji variety) and peaches. These results were in agreement with later studies in which maqui berries were compared with other fruits grown in Chile [43–45]. However, in a comparative study of more than 120 species/varieties of fruits, maqui had an ORAC value of 19,850 μmol TE/100 g whereas calafate (*Berberis microphylla*) — another native Chilean fruit — showed the highest ORAC value (25,662 μmol TE/100 g) [44]. Guerrero et al. [43] and Fredes et al. [45] incorporated the quantification of total anthocyanin content (TAC) into their studies using the pH differential method [46], and they reported that maqui fruit had the highest TAC of the polyphenol-rich fruits investigated, including the first comparison to pomegranate [45]. Berries primarily accumulate anthocyanins in the epicarp (peel) [47]. Botanically, maqui fruit is a true berry, and its high anthocyanin content may be attributed to its high surface/volume ratio, especially considering the low fruit weight compared to the other polyphenol-rich fruits (blueberries, raspberries, strawberries and blackberries) [45]. According to the same study [45], blackberries had the highest TAC per fruit (7.8 mg cy-3-glu FW), whereas red raspberry had the lowest TAC per fruit (0.8 mg cy-3-glu FW). Although red raspberry has a berry weight that is 11.5-fold higher than maqui berry, its TAC is 2.8-fold lower than that of maqui berry (1.4 mg cy-3-glu FW).

4. New research on maqui: The powerful colour of maqui fruit

In addition to the pigmentation properties of anthocyanins, these compounds have been associated with a wide range of biological, pharmacological, anti-inflammatory, anti-oxidative, and chemoprotective properties [48]. Anthocyanins are also beneficial against many chronic diseases [49, 50]. Accordingly, several studies have investigated the health effects of maqui anthocyanins over the last 6 years. Céspedes et al. [51], upon studying the methanolic extract of maqui fruits, observed antioxidant and cardioprotective activity against the acute ischemia/reperfusion of rat hearts *in vivo*. This extract prevented these harmful events in the animal's heart due to the decrease in lipid oxidation and the reduction of the TBARS concentration.

Ojeda et al. [52] evaluated the *in vitro* effect of concentrated maqui juice on the colorectal adenocarcinoma Caco-2 cell line, which exhibits a high level of cyclooxygenase-2 (COX-2) expression, and found that the maqui juice (50 ng/mL anthocyanin concentration) reduced the basal expression of COX-2 mRNA and protein. In addition, maqui juice did not modify cell viability of Caco-2 cells at the evaluated concentration.

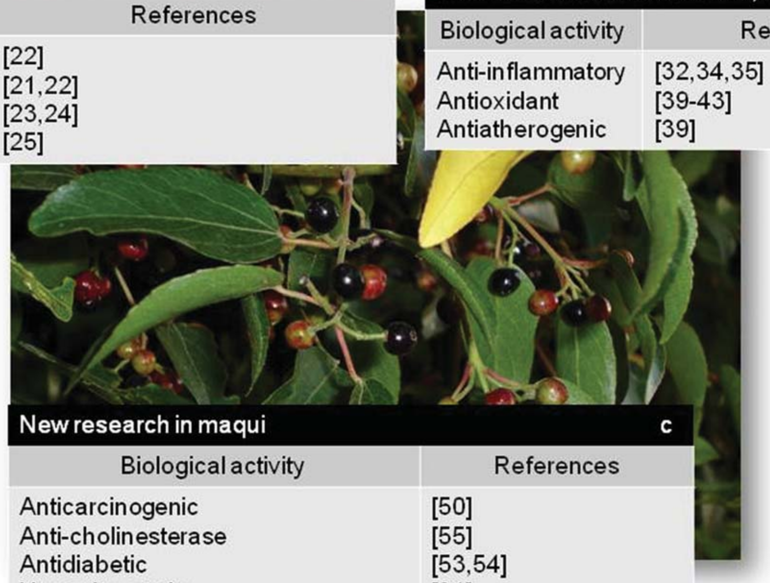
Maqui fruit polyphenols act as an antioxidant that can prevent diseases related to enhanced ROS production [53, 54]. In the case of diabetes, aqueous-ethanol extracts of maqui fruit and rutin had a protective effect against the functional impairment of endothelium-dependent vasorelaxation; this effect diminished the contractile response in aortic rings after phenylephrine treatment and the remaining contraction after acetylcholine application in rats where diabetes mellitus was induced by intraperitoneal injection. This activity has been associated with a reduction of nitric oxide bioavailability. Furthermore, with maqui treatment, the total cholesterol, LDL-cholesterol and triglycerides decreased, reaching similar levels to those of normal rats. Although, the level of serum glucose diminished with maqui treatment,

Leaves — Traditional uses: throat diseases (pain and infection) a

Biological activity	References
Analgesic	[22]
Anti-inflammatory	[21,22]
Antimicrobial	[23,24]
Antioxidant	[25]

Fruits — Traditional uses: antidiarrheal, dye b

Biological activity	References
Anti-inflammatory	[32,34,35]
Antioxidant	[39-43]
Antiatherogenic	[39]



New research in maqui c

Biological activity	References
Anticarcinogenic	[50]
Anti-cholinesterase	[55]
Antidiabetic	[53,54]
Hypoglycaemic	[31]
Cardioprotective	[49]

Fig. 2. Summary of the biological activities of leaves (a) and fruits (b) that validate the traditional uses of maqui and the new research in maqui (c).

it did not reach normal levels [55]. Rojo et al. [31] evaluated the hypoglycaemic effect of maqui anthocyanin enriched-formulations using a standard diet-induced murine model of type II diabetes where mice (C57BL/BJ) with a genetic predisposition to develop characteristic features of type II diabetes were studied. The results showed that treatment with an anthocyanin-rich formulation resulted in a significant reduction of fasting glucose levels in hyperglycaemic obese mice, and this reduction was similar to metformin. In addition, the anthocyanin-rich formulation improved glucose tolerance in obese hyperglycaemic mice, suggesting a potential insulin sensitisation by maqui anthocyanins. Del-3-sa-5glu was at least partially responsible for the *in vivo* anti-diabetic effects of maqui anthocyanins. In addition, maqui fruits, leaves and stems were evaluated for the inhibition of α-amylase and α-glucosidase activity [56]. The most active inhibitors of α-amylase and α-glucosidase were the fruit and stem, respectively. The inhibition of these enzymes potentially delays carbohydrate digestion and reduces glucose absorption.

Recently, maqui anthocyanins have been used in the design of new beverages [30, 33] and isotonic drinks [34, 57] with lemon juice and maqui as a potential functional and/or healthy beverage. In these beverages, lyophilised maqui fruit is added to lemon juice at levels of 2 and 5%. High antioxidant capacities (ABTS, DPPH, superoxide and hydroxyl radicals) were found as well as *in vitro* α-glucosidase and lipase inhibition. In addition, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitory activity was evaluated because cholinesterases (AChE and BChE) are the principal enzymes involved in the hydrolysis of acetylcholine.

5. Conclusions and future trends

Sufficient information should be provided to validate the traditional uses of maqui leaves and fruit as antioxidant and anti-inflammatory products. New evidence highlights the potential use of maqui fruit as a source of bioactive compounds, primarily anthocyanins, in food and nutraceutical industries. However, the use of maqui extracts as functional and healthy foods is limited due to the low stability of anthocyanins to the environmental conditions (heat, oxygen, and light) [58]. The stabilisation of anthocyanins could be improved by using microencapsulation technologies [58–61]. Thus, encapsulated anthocyanins potentially protect the anthocyanins (preservation of their bioactive properties) until they are consumed within a food vehicle.

Although, there are efforts in Chile to domesticate maqui, little information about its cultivation is available. Therefore, the impacts of genetic and environmental effects on the chemical composition in maqui fruit are poorly understood. The recent identification of anthocyanin profiles in maqui fruit from different genotypes aids in the search for potentially beneficial anthocyanins [35]. For example, del-3-sa-5-glu is a promising anthocyanin because of its superior anti-diabetic effect *in vivo* [31]. This delphinidin glucoside has only been reported in few species [27]; therefore, maqui genotypes with high contents of this anthocyanin may be a desirable trait in the selection of maqui genotypes. Due to the wide distribution of maqui in Chile [10], it is also important to evaluate the best geographical areas for its cultivation, including locations that promote high productivity and high anthocyanin content in the fruit. Thus far, studies have indicated that fruit grown in areas with moderate air temperature ($\sim$25°C) and high temperature oscillation (12–17°C) during fruit development have higher anthocyanin content [35].

Finally, maqui domestication and subsequent cultivation are required for maqui commercial development to generate an adequate supply of raw material for the food and nutraceutical industries as well as to ensure the sustainable use of this Chilean botanical resource.

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