DETERMINATION OF REDUCING POWER AND PHYTOCHEMICAL PROFILE OF THE CHILEAN MISTLETOE "QUINTRAL" (*Tristerix corymbosus* (L) *Kuijt*) HOSTED IN "MAQUI" (*Aristotelia chilensis*), "HUAYÚN" (*Rhaphitamnus spinosus*) AND "POPLAR" (*Populus nigra*)

PATRICIO TORRES, CECILIA SALDAÑA, RUTH ORTEGA AND CHRISTOPHER GONZÁLEZ**

Escuela Agropecuaria y Agroindustrial, Sede Santiago Sur, Universidad Tecnológica de Chile, INACAP. Chile.

ABSTRACT

On the Chilean mistletoe "Quintral", there has been limited literature, despite the fact the numerous uses and ubiquity amongst the Mapuche natives in Chile and Argentina. Therefore, our research group has carried out an exhaustive extraction with solvents of increasing polarity to Quintral flowers and leaves hosted in three different host species. With the extracts of different polarities, a phytochemical screening was performed, finding the presence of glycosides, sterols, terpenoids and quinones which are documented to convey different applications in cardiovascular or gastric afflictions. Likewise, the content of total phenols, total flavonoids and the reducing power of all the extracts were determined, displaying significant differences for the three varieties. Leaf and flower of the "Huayún Quintral" in methanol extracts gave the highest values of reducing power, while leaf and flower methanol extracts of "Maqui Quintral" showed the highest values for total phenols. Also observed are the lower polarity extracts from Quintral, contributing to the reducing capability by means of non-phenolic compounds.

Keywords: Quintral; Mistletoe, Tristerix, Reducing power, Phytochemicals, Mapuche.

1. INTRODUCTION

Well-known is the primitive use of natural products, mostly as medicinal herbs by the ancient European or Asian civilizations[1], in addition to archaeological findings showing the use of medicinal plants in South America since approximately 14,500 years ago[2]. In Chile, the Mapuche people have stood out for their vast knowledge and use of plants, initially reported by the Spanish conquerors[3]. The ancestral knowledge of nature that the Mapuche people possess focuses on two spiritual authorities: Lawentucheve and Machi [4], and they are who have established in time the uses given to a certain plant, according to different conditions or diseases. At least 15 general uses have been described for more than 150 species of Mapuche plants[5], amongst where Quintral (*Tristerix corymbosus* (Linnaeus) Kuijt) stands out belonging to a hemiparasitic mistletoe that grows in association to the native forest as well as in some introduced species, and is used to treat stomach ulcers, high cholesterol and nervous disorders [6]. The genus consists of 11 species described, two of which are preferably found in Chile [7]. *Tristerix aphyllus* grows on cacti and is normally distributed between 30-34° S of Latitude, while *T. corymbosus* does so between 30-40° S. The latter also grows on a large variety of hosts, among the most common are Poplar, Maqui and Olivo. In Chile it is considered an agronomic pest in olive crops and is often eradicated through the pruning of the infected branch, generating economic losses to the industry [8].

In recent decades, the use of natural products in drugs has been rekindled in order to aid against a wide variety of diseases[9]–[14], mainly associated with the deregulation of reactive oxygen species at a cellular level. This imbalance has been shown to favor both the development, growth and migration of cancer tumor cells[15]. Likewise, vascular inflammation, hypertension and atherosclerosis are directly related to endothelial dysfunction caused by oxidative stress [16], [17]. Other common diseases linked to reactive oxygen species are asthma [18] and diabetes [19], that together with the previous ones, show a high incidence in the causes of death in Chile [20].

There are currently very few studies that describe the phytochemicals present in Quintral, highlighting a research on the phenolic compounds of Poplar Quintral [21] and its antibacterial activity [22]. Considering the traditional use of this plant and the ethnopharmacological approach, our interest focuses mainly on obtaining extracts of different polarities, as well as a phytochemical analysis and the reducing power of Quintral leaves and flowers (*T. corymbosus*) which parasitizes three different hosts: Poplar (*Populus nigra*), Maqui (*Aristotelia chilensis*) and Huayún (*Rhaphitamnus spinosus*).

The present research seeks to contribute to the knowledge of the previously mentioned plant establishing the presence of compounds that account for their traditional uses, to guide their future isolation and seek potential medicinal or food applications for the complementary use by Chilean population.

2. MATERIALS AND METHODS

2.1. Plant material

The plant and its flowers were collected in Ranquilco. Bio-Bio Region (37°37′00″S/73°39′00″O.), and Puente Alto, Metropolitan Region (33°36'36" S/70°31'25" W). Vouchers of each variety were deposited in the Herbarium CONC of the University of Concepción with the numbers 185661, 185662 y 185663. The plant material was separated into leaves and flowers. These were dried in a stove with an air current at 40°C for 72 hours. Once dry, the samples were crushed with mortar and pestle, stored under vacuum at -20°C until extraction. The authors keep samples for possible consultations.

The extraction was carried out separately on 20 g of leaves and 20 g of dried flowers, using 200 mL of solvents in an increasing polarity: hexane, dichloromethane, ethyl acetate and methanol sequentially, in a Soxhlet apparatus for 8 hours. Four extracts were obtained this way per leaf sample and 4 extracts per flower sample, giving 24 extracts in total. The obtained extracts were vacuum filtered, dried in a rotary evaporator under reduced pressure and kept under a nitrogen atmosphere at -20°C until further analysis.

2.2. Reagents

The Folin-Ciocalteu (F-C), Butylhydroxytoluene (BHT) and (L)-Ascorbic Acid reagents were purchased from Merck. S.A; Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), Quercetin and Gallic Acid, were purchased from Sigma-Aldrich Co; The spectrophotometric measurements were made in a UV-Vis spectrophotometer, Rayleigh UV-1800, China.

2.3. Phytochemical Screening

The crude extracts of leaves and flowers of the three varieties of Quintral were analyzed qualitatively to determine the presence of cardiac glycosides, sterols/triterpenoids, saponins, alkaloids, tannins, steroids, quinones and flavonoids using methods well described in the literature [23]–[30]:

2.3.1. Test for cardiac glycosides: 2 mL of glacial acetic acid containing 1 drop of iron (III) chloride 5% w/v and 1 mL of concentrated sulfuric acid were added to 2 mL of the extract in ethanol. A brown ring at the interface indicates the presence of cardiac glycosides [31].

2.3.2. Test for sterol-triterpenoids: to 2 mL of the extract dissolved in isopropyl alcohol, 1 mL of acetic anhydride and 3 drops of concentrated sulfuric acid were added. After 5 minutes a ring is formed between the two phases, blue for sterols and pink-violet for triterpenes [25].

**Corresponding author email: ruth.ortega@inacapmail.cl, christopher.gonzalez05@inacapmail.cl*

2.3.3. Test for saponins: to 10 mL of water, 10 drops of the extract dissolved in isopropyl alcohol were added and the mixture was stirred vigorously until the formation of a foam. This mixture is allowed to stand for 10 minutes and the height of the foam column is measured [25].

2.3.4. Test for alkaloids: about 10 mg of the extract are dissolved in 2 mL of 5% hydrochloric acid. The mixture was filtered and divided into three portions. To each one, drops of the reagents of Mayer, Wagner and Dragendorff were added. The formation of yellow-white, red-brown and red-orange precipitates, respectively, indicate the presence of alkaloids [25].

2.3.5. Test for tannins: 1 mL of the extract dissolved in ethanol was mixed with 2 mL of distilled water and 5 drops of Iron (III) chloride 10% w/v. The appearance of blue or green indicates the presence of phenols [25].

2.3.6. Test for steroids: 10 drops of the extract dissolved in isopropyl alcohol were carefully added 2 mL of chloroform and 1 mL of concentrated sulfuric acid. The presence of a brown ring at the interface is indicative of sterols [25].

2.3.7. Test for quinones: 1 drop of concentrated sulfuric acid was added to 1 mL of isopropyl alcohol extract. The appearance of red indicates the presence of quinones [25].

2.3.8. Test for flavonoids: to 1 mL of the extract dissolved in isopropyl alcohol, 3 drops of 10% w/w sodium hydroxide were added. The formation of colors indicates the presence of various types of flavonoids: yellow-red for xanthones / flavones, coffee-orange for flavonols, purple-red for chalcones and blue for anthocyanins [25].

2.4. Content of Total Phenols

The content of total phenols was determined by using the Folin-Ciocalteu reagent, [32] with a few modifications. In summary, (500 - x) µL of reactive grade water was added in a test tube, together with x µL of the respective solution of extracts or standards or blank, followed by 2.5 mL of Folin-Ciocalteu reagent diluted 1:10 and finally 2 mL of sodium carbonate 7.5% w/v. The mixture was allowed to stand protected from light, at room temperature for 60 minutes. After the resting time, the absorbance at 760 nm was recorded in glass cuvettes. Gallic acid was used as standard and the results are presented as mg gallic acid equivalent for each 1 g of dry sample. All trials were carried out in triplicate.

2.5. Content of Total Flavonoids

The content of total flavonoids was determined by the formation of the yellow complex between aluminum and the carbonyl groups at C-4 and hydroxyl at C-3 and C-4 of the flavonoids [33] with some modifications. Briefly, in a test tube (x) µL of methanol solution of extracts or standards or blanks was added, followed by 500 µL of 2% m/v aluminum chloride in methanol and finally (500 - x) µL of reagent grade methanol. The mixture was allowed to stand in natural light and room temperature for 60 minutes. After the resting time, the absorbance at 420 nm was recorded in quartz cuvettes. Quercetin was used as standard and the results are presented as mg equivalent of quercetin for every 1 g of dry sample. All trials were carried out in triplicate.

2.6. Iron (III) Reducing Power

The reducing power of the extracts was evaluated by their ability to reduce iron (III) to iron (II) and thus form a colored complex known as "Prussian blue". A modified method was used to facilitate the measurement of lipophilic reducing compounds, with minor modifications [34]. Ethanolic solutions of the extracts were used in final concentrations of 70-80 µg / mL. The following reagents were successively added to test tubes: x mL of standard or extract, (1 - x) mL of 96% ethanol, 5 mL of water reactive grade, 1.5 mL of 1 M HCl, 1.5 mL of potassium ferricyanide solution 1% m/v, 0.5 mL of sodium dodecyl sulfate 1% and finally 0.5 mL of iron (III) chloride. The mixture was incubated in a water bath at 50°C for 20 minutes protected from light, allowed to cool to room temperature and the absorbance was recorded at 750 nm in 1 cm glass cuvettes. Three separate standards were used: Ascorbic acid (AA), Butylated hydroxytoluene (BHT) and Trolox. The results are presented as mg equivalent of AA/BHT/Trolox for every 1 g of dry sample. All trials were carried out in triplicate.

2.7. Statistic analysis

All determinations were made in triplicate and the results are presented as mean ± standard deviation. One-way ANOVA and Tukey test were performed considering significant differences for values of $p < 0.05$. All analyzes were performed using Microsoft Excel 2017 software (Microsoft Office 2017) and GraphPad Prism 6 (GraphPad Software Inc).

3. RESULTS AND DISCUSSION

3.1. Yield

In order to obtain extracts of different hydro/lipophilic affinities, a conventional liquid solid extraction technique was used, with a Soxhlet apparatus and solvents of increasing polarity, such as hexane, dichloromethane, ethyl acetate and methanol[35], consecutively. The yields of the extraction are displayed in Table 1.

Table 1. Extraction yields for the leaves and flowers of the three varieties of mistletoe studied.

Variety of Quintral	Solvent	ID Extract[1]	% dry mass yield
Maqui Quintral Leaves	Hexane	HHQM	2,46 ± 0,13[a]
	Dichloromethane	HDQM	0,68 ± 0,05[b]
	Ethyl Acetate	HEQM	1,30 ± 0,08[c]
	Methanol	HMQM	24,31 ± 1,02[d]
Maqui Quintral Flowers	Hexane	FHQM	10,68 ± 0,91
	Dichloromethane	FDQM	0,77 ± 0,07[c]
	Ethyl Acetate	FEQM	4,07 ± 0,33[f]
	Methanol	FMQM	34,78 ± 3,15[g]
Huayún Quintral Leaves	Hexane	HHQH	2,69 ± 0,13[a]
	Dichloromethane	HDQH	0,72 ± 0,06[b]
	Ethyl Acetate	HEQH	0,93 ± 0,08[c]
	Methanol	HMQH	21,86 ± 0,65[d]
Huayún Quintral Flowers	Hexane	FHQH	5,40 ± 0,38
	Dichloromethane	FDQH	0,55 ± 0,05[e]
	Ethyl Acetate	FEQH	4,98 ± 0,19[f]
	Methanol	FMQH	37,23 ± 1,10[g, h]
Poplar Quintral Leaves	Hexane	HHQA	1,69 ± 0,09
	Dichloromethane	HDQA	0,76 ± 0,04[b]
	Ethyl Acetate	HEQA	3,05 ± 0,09
	Methanol	HMQA	15,62 ± 1,31
Poplar Quintral Flowers	Hexane	FHQA	2,47 ± 0,06
	Dichloromethane	FDQA	0,79 ± 0,07[c]
	Ethyl Acetate	FEQA	1,47 ± 0,13
	Methanol	FMQA	48,35 ± 4,28[h]

[1]. The identification of the extracts corresponds to the following order: The first letter represents the part of the plant, H for leaves and F for flowers. The second letter corresponds to the solvent used, H for hexane, D for dichloromethane, E for ethyl acetate and M for methanol. The last two letters represent the variety of Quintral, QM for Maqui Quintral, QH for Huayún Quintral and QA for Poplar Quintral.

[a, b, c, d, e, f, g, h]. Equal letters indicate that no significant differences are observed.

The results in Table 1 suggest that for the three varieties studied, both in leaves and flowers, the methanol solvent produces a yield at least three times higher than the other solvents, accounting for a high prevalence of polar compounds in Quintral. Moreover, a pattern is observed in which the flowers show higher yields than the leaves and the solvent dichloromethane presents the lowest yield values. Likewise, the yields for hexane solvent could be accounted for the lipophilic compounds that may be responsible for the capacity to reduce cholesterol levels attributed by the traditional Mapuche customs[6]. Flower Maqui Quintral contains about 10% dry mass of lipophilic components, including cardiac glycosides and sterols. These results, together with its wide distribution, allow Quintral to be viewed as a high availability source for obtaining compounds with biological activity.

3.2. Phytochemical screening

For a plant such as Quintral, with only very few phytochemical studies, establishing the presence of compounds with biological activity in order to guide separation methods and correlate the observed antioxidant activity has become interesting. The results obtained for the qualitative phytochemical study are shown in Table 2.

Table 2. Results of qualitative phytochemical analysis of Quintral leaf and flower extracts (*T. corymbosus*) hosted in Maqui (*A. chilensis*), Huayún (*R. spinosus*) and Poplar (*P. nigra*).

ID Extract	Cardiac glycosides	Tannins	Flavonoids	Steroids	Quinones	Saponins	Steroids/Triterpenes
HHQM	-	+	Flavone/Xanthone	+	-	-	-
HDQM	-	+	Flavonol	-	-	-	Sterols
HEQM	-	+	Flavonol	+	+	+	Sterols
HMQM	-	+	Flavonol	+	+	-	-
FHQM	+	+	Flavone/Xanthone	+	+	+	Triterpenes
FDQM	+	+	Flavone/Xanthone	+	+	-	-
FEQM	-	+	Flavone/Xanthone	+	-	-	-
FMQM	-	+	Flavone/Xanthone	+	+	-	-
HHQH	+	+	Flavone/Xanthone	+	-	-	Sterols
HDQH	+	+	Flavonol	+	+	+	Sterols
HEQH	+	+	Flavonol	+	-	+	Sterols
HMQH	-	+	Flavonol	+	+	-	-
FHQH	+	-	Flavone/Xanthone	+	+	+	Triterpenes
FDQH	-	+	Flavone/Xanthone	-	-	+	-
FEQH	-	+	Flavonol	-	+	-	Triterpenes
FMQH	-	+	Flavonol	+	+	+	-
HHQA	-	+	-	+	-	-	Sterols
HDQA	-	+	Flavonol	+	-	+	Sterols
HEQA	-	+	Flavonol	+	+	-	-
HMQA	-	+	Flavonol	+	+	+	Sterols
FHQA	-	+	-	+	+	-	-
FDQA	-	+	-	+	+	-	Sterols
FEQA	-	-	Flavonol	+	+	-	Sterols
FMQA	-	-	Flavonol	+	+	-	-

All the analyzed extracts reveal the presence of flavonoids, which in this analysis are restricted to flavones, xanthones and flavonols. The presence of steroids, quinones and tannins in leaves and flowers of all studied varieties becomes something outstanding. Despite being present in fewer extracts, cardiac glycosides are interesting metabolites with properties well described in the literature [36]–[41], showing Huayún Quintral leaf extract has a strong response to the presence of these compounds, mainly in the less polar extracts, this being consistent with their chemical structures. The hexane extracts of Maqui and Huayún Quintral flower also show the presence of these compounds. Finally, the presence of saponins was evaluated, being present mostly in Huayún Quintral extracts.

Studies in other species of the genus show the presence of apolar compounds with hypocholesterolemic activity, or as gastric protection [42], [43], which positively correlates with our results, being the hexane extract mainly the source of these compounds. In this way an agreement is observed between the results and the traditional use of *T. corymbosus*.

3.3. Content of Phenols and Total Flavonoids

The properties of phenolic compounds and flavonoids, as well as their possible applications have been extensively described [44]–[46]. In this study the colorimetric method with the Folin-Ciocalteu reagent was used to quantify the total phenolic compounds of the 24 obtained extracts, showing a great variability in the results, ranging from 0.02 to 150 mg EAG / g dry sample. The results of all the extracts are shown in Table 3. In the three Quintral varieties studied, it was found that the content of total phenols tend to increase with the polarity of the solvents used in the extraction, being always higher in the methanol extracts. Within this last group of extracts, those corresponding to the flowers were about twice as large as the leaves in the Maqui and Huayún Quintral, while in the extract from Poplar Quintral this difference becomes almost 20-fold. The Maqui Quintral flower and leaf stand out as having the highest total phenols, with 150.09 and 89.18 mg EAG / g dry sample, respectively. These values are similar to those obtained for ethanolic extracts of Murtilla [47], a Chilean berry with a high content of antioxidants, and even higher than those reported for leaves and fruits

of the same Maqui [48]. The extracts of ethyl acetate for Maqui and Huayún Quintral flowers show a content of total phenols more than three times greater than their analogue Poplar Quintral. Our results show that Quintral flower extracts have higher total phenols than leaf extracts, unlike the only published results for this species [21], which could be attributed to the different extraction methods used. There are reported data for *Viscum album*, a European mistletoe used in oncological drugs, showing lower values of total phenols than those obtained for the Chilean mistletoe varieties here-described. [49].

Table 3. Content of phenols and total flavonoids of the different Quintral leaf and flower extracts.

ID Extract	Total Phenols (mg EAG/g m.s)	Total Flavonoids (mg EQ/g m.s)
HHQM	0,27 ± 0,02	2,32 ± 0,20[a]
HDQM	0,40 ± 0,02	2,22 ± 0,19
HEQM	4,13 ± 0,22	2,97 ± 0,22
HMQM	89,18 ± 3,27	10,31 ± 0,74
FHQM	1,04 ± 0,01	0,43 ± 0,02
FDQM	0,34 ± 0,03	0,27 ± 0,02
FEQM	16,71 ± 0,40[b]	0,85 ± 0,03
FMQM	150,09 ± 2,73	6,59 ± 0,45[b]
HHQH	0,53 ± 0,04	2,22 ± 0,15[a]
HDQH	0,36 ± 0,01	3,18 ± 0,06
HEQH	2,85 ± 0,04	1,68 ± 0,05
HMQH	73,40 ± 4,15	4,79 ± 0,29
FHQH	0,45 ± 0,04	0,37 ± 0,01
FDQH	0,17 ± 0,02[a]	0,20 ± 0,01
FEQH	17,78 ± 0,72[b]	0,99 ± 0,03
FMQH	127,12 ± 6,15[c]	9,30 ± 0,54
HHQA	0,18 ± 0,01	0,79 ± 0,04
HDQA	0,17 ± 0,01	1,23 ± 0,03
HEQA	1,53 ± 0,05	6,47 ± 0,29
HMQA	4,88 ± 0,44	22,60 ± 0,57
FHQA	0,02 ± 0,001	0,12 ± 0,01
FDQA	0,22 ± 0,005[a]	0,02 ± 0,002
FEQA	5,27 ± 0,18	0,16 ± 0,01
FMQA	118,22 ± 5,32[c]	6,69 ± 0,27[b]

[a, b, c]. The same letters in a column indicate that there are no significant differences.

The content of total flavonoids was determined by the formation of a colored complex with an aluminum salt. The results are shown in Table 3. Unlike the content of phenols, a relationship with the polarity of the solvents is not observed for flavonoids, although a common pattern is seen. The leaf extracts are in all cases of greater quantity of flavonoids than the flower extracts, being the methanol leaf extract for Poplar Quintral the one with the highest flavonoid content, with 22.60 mg EAG / g dry sample. For flowers, the methanol extract of Huayún Quintral presents the highest value with 9.30 mg EAG / g dry sample. Leaf analysis of other members of the Loranthaceae family [44] show similar values in the content of flavonoids to those of Quintral, as well as the data reported for the same mistletoe [21], and other Mapuche medicinal plants [50].

The statistical examination shows significant differences concerning the content of total phenols in all the extracts. However, in some solvents there are pairs of extracts that are equivalent, highlighting FDQH-FDQA, FAQM-FAQH, FMQH-FMQA.

The content of flavonoids in leaves and flowers of the three varieties of Quintral is statistically different in all the solvents used. As in the case of phenols, there are also pairs of extracts that do not show significant differences, as they are HHQM-HHQH, FMQM-FMQA.

Considering the same species is analyzed residing in 3 different hosts, the variances observed in the content of phenols and total flavonoids, could be explained by a transference relationship of parasite-host solutes [51], showing a clear influence of the host in its content of phytochemicals, as has been established in studies of other hemiparasitic mistletoes of the same order [49].

3.4. Iron (III) Reducing Power

The interest in finding compounds capable of regulating the physiological levels of reactive oxygen and nitrogen species has increased in recent decades, in order to combat oxidative stress associated with high-risk diseases and incidence in the population. Therefore, several methods have been developed for the analysis of the antioxidant activity of compounds of natural origin [52]–[57]. Amongst the methods based on electron transfer, we found those based on the reduction of the iron (III) ion by the compounds capable of donating a single electron. The reducing power of a substance then lies with its ability to reduce an oxidant, thus generating less reactives species as a byproduct. In this way, it is possible to associate the reducing power with the antioxidant potential of natural products. Under this premise, the reducing power of the 24 extracts was determined using a modified method that allows the analysis of reducing compounds with less polarity [34], in order to include the extracts of Hexane and Dichloromethane, as well as less polar standard antioxidants such as BHT and Trolox. The results are shown in Table 4.

Table 4. Yield Results, Reducing Power, Total Phenolic Content and Total Flavonoid Content of Quintal leaf and flower extracts (*T. corymbosus*) hosted in Poplar, Maqui and Huayún expressed as standard equivalent mg per gram of dry plant.

ID Extract	Reducing Power (mg EAA/g m.s)	Reducing Power (mg EBHT/g m.s)	Reducing Power (mg ETrolox/g m.s)
HHQM	5,98 ± 0,10[a]	5,89 ± 0,14	3,84 ± 0,19[a]
HDQM	1,82 ± 0,08	1,91 ± 0,11	1,38 ± 0,15
HEQM	6,80 ± 0,15	8,40 ± 0,09	8,96 ± 0,29[b]
HMQM	142,62 ± 7,23	180,88 ± 12,19	199,31 ± 13,70
FHQM	2,91 ± 0,16	3,18 ± 0,23	2,78 ± 0,30
FDQM	0,39 ± 0,01	0,48 ± 0,02	0,51 ± 0,02
FEQM	23,19 ± 0,87	28,60 ± 1,23	31,59 ± 1,65
FMQM	213,31 ± 12,53[b]	268,87 ± 17,76	307,16 ± 23,73
HHQH	5,76 ± 0,12[a]	5,29 ± 0,18	2,59 ± 0,24
HDQH	2,59 ± 0,08	2,92 ± 0,11	2,72 ± 0,15
HEQH	5,90 ± 0,32	7,38 ± 0,46	8,35 ± 0,62[b]
HMQH	163,58 ± 1,42	209,51 ± 2,02	245,16 ± 2,70
FHQH	1,44 ± 0,04	1,47 ± 0,05	1,07 ± 0,07
FDQH	0,28 ± 0,01	0,35 ± 0,01	0,37 ± 0,02
FEQH	29,90 ± 1,52	38,62 ± 2,16	45,75 ± 2,89
FMQH	256,57 ± 10,84	324,99 ± 15,37	374,07 ± 20,54
HHQA	3,12 ± 0,12	3,53 ± 0,17	3,34 ± 0,22[a]
HDQA	0,56 ± 0,02	0,59 ± 0,03	0,47 ± 0,05
HEQA	4,68 ± 0,21	5,04 ± 0,30	4,24 ± 0,40
HMQA	20,60 ± 0,68	21,50 ± 0,96	16,73 ± 1,28
FHQA	0,17 ± 0,01	0,26 ± 0,01	0,23 ± 0,02
FDQA	0,07 ± 0,004	0,10 ± 0,01	0,10 ± 0,01
FEQA	1,45 ± 0,05	4,62 ± 0,22	3,77 ± 0,30
FMQA	193,35 ± 7,98[b]	223,26 ± 11,31	219,24 ± 15,11

[a, b]. The same letters in a column indicate that there are no significant differences.

The leaf and flower of Huayún Quintral show the highest values of reducing power in the three standards used, highlighting the methanol extract of the flower with 374.07 mg ET / g dry sample. In the three varieties studied, both in leaves and flowers, the methanol extracts show the highest values of reducing power. However, the ethyl acetate extracts for flower of Maqui and Huayún Quintral also show high values compared with the other used solvents, indicating that the components of greater polarity would be largely responsible for the greater reducing power. The dichloromethane extracts show in all cases the lowest values of reducing power, correlating well with the yields and the content of the obtained phenols and flavonoids. Interestingly, the flower extracts for the three varieties show a linear relationship between the reducing power and the content of phenols and flavonoids, so it can be established than the greater amount of phenols and flavonoids, greater the reducing power. This relation is greater in Huayún Quintral for the Phenols and in Maqui Quintral for the Flavonoids.

However, having lower values in reducing power, the extracts of leaves in hexane for the three varieties show values similar to those of ethyl acetate, which do not directly correlate with the content of phenols and flavonoids, which could be attributed to the presence of non-phenolic components with reducing capacity, such as cardiac glycosides, steroids and terpenoids found in phytochemical screening.

An investigation with the mistletoe *Phthirusa pyrifolia* [58] shows that the reducing power increases with the polarity of the extraction solvent, same trend as that observed in our investigation.

Finally, the results obtained in function of the three standards show that those of lower polarity gave the highest values of reducing power, indicating the presence of polar compounds with high reducing power both in Quintral leaves and flowers. This allows to establish that Quintral hemiparasitic plant is a rich source of reducing compounds and therefore antioxidants, accounting for its traditional use.

CONCLUSIONS

The leaves and flowers of three varieties of Quintral, hosted in different hosts, were analyzed, finding significant differences in the content of phenols, flavonoids and reducing power, which account for the influence of the host on the phytochemical composition of the plant.

The leaves and flowers of Huayún and Maqui Quintral show the highest values of phenols, flavonoids and reducing power, being the extracts of greater polarity those with greater activity and antioxidant potential.

The content of phenols is higher in the flowers than in Quintral leaves and increases in the following order for both leaves and flowers: Poplar Quintral < Huayún Quintral < Maqui Quintral.

The content of flavonoids is higher in leaves than in flowers and increases in the following order for leaves: Huayún Quintral < Maqui Quintral < Poplar Quintral, while for flowers the order is completely opposite: Poplar Quintral < Maqui Quintral < Huayún Quintral.

The presence of cardiac glycosides, sterols, terpenoids and quinones was determined, which could be responsible for their cholesterol-lowering activity and gastric affections, according to their traditional uses.

The reducing power for extracts of this plant was determined for the first time, showing that the Huayún Quintral has the greatest reducing activity. Moreover, it is established that not only the polar components contribute to the reducing power, but also the components of less polarity make a remarkable contribution to their antioxidant potential.

For the flowers of Quintral it is established that the reducing power is strongly influenced by the content of phenols and flavonoids, in such a way that, the greater is the quantity of these compounds, the greater is the reducing power.

Finally, our results show that Quintral is a plant with a high antioxidant potential and with a phytochemical composition that correlates directly with its traditional uses, so it is essential to continue its study in order to isolate and characterize the biologically active compounds present on this plant.

ACKNOWLEDGMENT.

To my family and students who works with us. To Proyect PI8STSUTCIDP03 from Vicerrectoría de Investigación y Postgrados, Inacap.

REFERENCES

1. S. Bernardini, A. Tiezzi, V. Laghezza Masci, and E. Ovidi, “Natural products for human health: an historical overview of the drug discovery approaches,” *Nat Prod Res*, vol. 6419, no. November, pp. 1–25, 2017.
2. T. D. Dillehay *et al.*, “New archaeological evidence for an early human presence at Monte Verde, Chile,” *PLoS One*, vol. 10, no. 11, pp. 1–27, 2015.
3. C. E. Burdick, “The remedies of the machi : visualizing Chilean medicinal botanicals in Alonso de Ovalle’s Tabula geographica (1646).,” *Colon Lat Am Rev*, vol. 26, no. 3, pp. 313–334, 2017.
4. A. Díaz Mujica, M. V. Pérez Villalobos, C. y González Parra, and J. Simon, “Conceptos de enfermedad y sanación en la cosmovisión mapuche e impacto de la cultura occidental,” *Cienc Enferm*, vol. X, no. 1, pp. 9–16, 2004.
5. P. J. Houghton and J. Manby, “Medicinal plants of the Mapuche,” *J Ethnopharmacol*, vol. 13, no. 1, pp. 89–103, Mar. 1985.
6. MINSAL: Ministerio de Salud de Chile, *MHT: Medicamentos Herbarios Tradicionales - 103 especies vegetales*, Primera Ed. MINSAL, 2010.
7. J. Kuijt, “Revision of Tristerix (Loranthaceae),” *Syst. Bot. Monogr.*, vol. 19, no. 29, pp. 1–61, 1988.
8. J. Ormeño, “Review Dodder (Cuscuta suaveolens Syr.), Mistletoe (Tristerix corymbosus L.) y Broomrape (Orobanche ramosa L.): Parasitic weeds of economic importance in Chile,” *Chil J Agric Anim Sci*, vol. 26, no. 2, pp. 109–119, 2010.
9. D. J. Newman and M. Cragg, Gordon, “Natural Products as Drugs and Leads to Drugs: The Historical Perspective,” in *Natural Product Chemistry for Drug Discoveryr*, 2010, pp. 3–27.
10. T. Arif *et al.*, “Natural products - Antifungal agents derived from plants,” *J Asian Nat Prod Res*, vol. 11, no. 7, pp. 621–638, 2009.
11. G. Appendino and F. Pollastro, “Plants: revamping the oldest source of medicines with modern science.,” in *Natural Product Chemistry for Drug Discovery*, 2010, pp. 140–173.
12. E. European Food Safety Authority, “Compendium of botanicals reported to contain naturally occuring substances of possible concern for human health when used in food and food supplements,” *EFSA Journal*, vol. 10, no. 5, p. 2663. [60 pp.], 2012.
13. OMS, *Estrategia de la OMS sobre medicina tradicional 2014-2023*. Ediciones de la OMS, 2013.
14. K. Sanders, Z. Moran, Z. Shi, R. Paul, and H. Greenlee, “Natural Products for Cancer Prevention: Clinical Update 2016,” *Semin. Oncol. Nurs.*, vol. 32, no. 3, pp. 215–240, 2016.
15. S. Chikara, L. D. Nagaprashantha, J. Singhal, D. Horne, S. Awasthi, and S. S. Singhal, “Oxidative stress and dietary phytochemicals: Role in cancer chemoprevention and treatment,” *Cancer Lett*, vol. 413, pp. 122–134, 2018.
16. K. A. Ahmad *et al.*, “Antioxidant therapy for management of oxidative stress induced hypertension,” *Free Radic. Res*, vol. 51, no. 4, pp. 428–438, 2017.
17. H. N. Siti, Y. Kamisah, and J. Kamsiah, “The role of oxidative stress, antioxidants and vascular inflammation in cardiovascular disease (a review),” *Vasc. Pharmacol.*, vol. 71, pp. 40–56, 2015.
18. P. Kleniewska and R. Pawliczak, “The participation of oxidative stress in the pathogenesis of bronchial asthma,” *Biomed Pharmacother*, vol. 94, pp. 100–108, 2017.
19. U. Asmat, K. Abad, and K. Ismail, “Diabetes mellitus and oxidative stress—A concise review,” *Saudi Pharm J*, vol. 24, no. 5, pp. 547–553, 2016.
20. MINSAL: Ministerio de Salud de Chile, “Indicadores Basicos de Salud Chile 2015,” 2015.
21. M. J. Simirgiotis, C. Quispe, C. Areche, and B. Sepúlveda, “Phenolic compounds in chilean mistletoe (quintral, Tristerix tetrandus) analyzed by UHPLC-Q/Orbitrap/MS/MS and its antioxidant properties,” *Molecules*, vol. 21, no. 3, pp. 1–15, 2016.
22. P. Mølgaard *et al.*, “Antimicrobial evaluation of Huilliche plant medicine used to treat wounds,” *J Ethnopharmacol*, vol. 138, no. 1, pp. 219–227, 2011.
23. S. Chowdhury *et al.*, “Phytochemical screening and evaluation of cytotoxic and hypoglycemic properties of Mangifera indica peels,” *Asian Pac J Trop Biomed*, vol. 7, no. 1, pp. 49–52, 2017.
24. G. J. Kaur and D. S. Arora, “Antibacterial and phytochemical screening of Anethum graveolens, Foeniculum vulgare and Trachyspermum ammi,” *BMC Complem Aletrn M*, vol. 9, no. 30, pp. 1–10, 2009.
25. R. María *et al.*, “Preliminary phytochemical screening, total phenolic content and antibacterial activity of thirteen native species from Guayas province Ecuador,” *J King Saud Univ Sci*, no. in press, 2017.
26. D. Rathee, P. Rathee, S. Rathee, and D. Rathee, “Phytochemical screening and antimicrobial activity of Picrorrhiza kurroa, an Indian traditional plant used to treat chronic diarrhea,” *Arab. J. Chem.*, vol. 9, pp. S1307–S1313, 2016.

27. S. C. Mandal, V. Mandal, and A. K. Das, "Qualitative Phytochemical Screening," in *Essentials of Botanical Extraction - Principles and applications*, 1st ed., Academic Press, 2015, pp. 173–185.
28. M. Saha and P. K. Bandyopadhyay, "Phytochemical screening for identification of bioactive compound and antiprotozoan activity of fresh garlic bulb over trichodinid ciliates affecting ornamental goldfish," *Aquaculture*, vol. 473, pp. 181–190, 2017.
29. M. T. Olivier, F. M. Muganza, L. J. Shai, S. S. Gololo, and L. D. Nemutavhanani, "Phytochemical screening, antioxidant and antibacterial activities of ethanol extracts of Asparagus suaveolens aerial parts," *S. Afr. J. Bot.*, vol. 108, pp. 41–46, 2017.
30. H. M. Ahmed, "Phytochemical screening, total phenolic content and phytotoxic activity of corn (Zea mays) extracts against some indicator species," *Nat Prod Res*, vol. 6419, no. November, pp. 1–5, 2017.
31. A. M. Ismail, E. A. Mohamed, M. R. Marghany, F. F. Abdel-Motaal, I. B. Abdel-Farid, and M. A. El-Sayed, "Preliminary phytochemical screening, plant growth inhibition and antimicrobial activity studies of Faidherbia albida legume extracts," *J. Saudi Soc. Agric. Sci.*, vol. 15, no. 2, pp. 112–117, 2016.
32. H. Speisky, C. López-Alarcón, M. Gómez, J. Fuentes, and C. Sandoval-Acuña, "First web-based database on total phenolics and oxygen radical absorbance capacity (ORAC) of fruits produced and consumed within the south andes region of South America," *J. Agric. Food Chem.*, vol. 60, no. 36, pp. 8851–8859, 2012.
33. R. G. Woisky and A. Salatino, "Analysis of propolis: Some parameters and procedures for chemical quality control," *J. Apic. Res.*, vol. 37, no. 2, pp. 99–105, 1998.
34. K. I. Berker, K. Güçlü, İ. Tor, and R. Apak, "Comparative evaluation of Fe(III) reducing power-based antioxidant capacity assays in the presence of phenanthroline, batho-phenanthroline, tripyridyltriazine (FRAP), and ferricyanide reagents," *Talanta*, vol. 72, no. 3, pp. 1157–1165, 2007.
35. G. Brusotti, I. Cesari, A. Dentamaro, G. Caccialanza, and G. Massolini, "Isolation and characterization of bioactive compounds from plant resources: The role of analysis in the ethnopharmacological approach," *J Pharm. Biomed*, vol. 87, pp. 218–228, 2014.
36. M. Diederich, F. Muller, and C. Cerella, "Cardiac glycosides: From molecular targets to immunogenic cell death," *Biochem Pharmacol*, vol. 125, pp. 1–11, 2017.
37. H. Todt and H. Fozzard, "Cardiac Glycosides," in *Principles of Medical Biology*, vol. 8, 1997, pp. 501–518.
38. R. C. Elderfield, "The chemistry of the cardiac glycosides," *Chem Rev*, vol. 17, no. 2, pp. 187–249, 1935.
39. H. P. Albrecht and K.-H. Geiss, "Cardiac Glycosides and Synthetic Cardiotonic Drugs," in *Ullmann's Encyclopedia of Industrial Chemistry.*, Wiley-VCH Verlag GmbH & Co. KGaA, 2000, pp. 1–18.
40. T. R. Sherrod, "The Cardiac Glycosides," *Hosp. Pr.*, vol. 2, no. 6, pp. 56–59, 1967.
41. L. Menger *et al.*, "Trial watch: Cardiac glycosides and cancer therapy," *Oncoimmunology*, vol. 2, no. 2, 2013.
42. F. Leitão, D. de Lima Moreira, M. Z. de Almeida, and S. Guimarães Leitão, "Secondary metabolites from the mistletoes Struthanthus marginatus and Struthanthus concinnus (Loranthaceae)," *Biochem. Syst. Ecol.*, vol. 48, pp. 215–218, 2013.
43. O. E. Ogechukwu *et al.*, "Steroids and triterpenoids from Eastern Nigeria mistletoe, Loranthus micranthus Linn. (Loranthaceae) parasitic on Kola acuminata with immunomodulatory potentials," *Phytochem Lett*, vol. 4, no. 3, pp. 357–362, 2011.
44. L. Zhang *et al.*, "Antioxidant and Anti-inflammatory Activities of Selected Medicinal Plants Containing Phenolic and Flavonoid Compounds," *J. Agric. Food Chem.*, vol. 59, no. 23, pp. 12361–12367, 2011.
45. H. Rui-Min *et al*., "Comparison of Flavonoids and Isoflavonoids as Antioxidants," *J. Agric. Food Chem.*, vol. 57, pp. 3780–3785, 2009.
46. G. D. Mogoșanu, A. M. Grumezescu, C. Bejenaru, and L. E. Bejenaru, "Natural products used for food preservation," in *Food Preservation*, A. M. Grumezescu, Ed. 2017, pp. 365–411.
47. M. Peña-Cerda *et al*., "Phenolic composition and antioxidant capacity of Ugni molinae Turcz. leaves of different genotypes," *Food Chem*, vol. 215, pp. 219–227, 2017.
48. M. Rubilar *et al.*, "Extracts of maqui (Aristotelia chilensis) and murta (Ugni molinae Turcz.): Sources of antioxidant compounds and α-glucosidase/α-amylase inhibitors," *J. Agric. Food Chem.*, vol. 59, no. 5, pp. 1630–1637, 2011.
49. S. I. Vicas, D. Rugina, and C. Socaciu, "Antioxidant Activity of European Mistletoe (Viscum album)," in *Phytochemicals as Nutraceuticals - Global Approaches to Their Role in Nutrition and Health*, V. Rao, Ed. InTech, 2012, pp. 115–134.
50. M. J. Simirgiotis, M. Silva, J. Becerra, and G. Schmeda-Hirschmann, "Direct characterisation of phenolic antioxidants in infusions from four Mapuche medicinal plants by liquid chromatography with diode array detection (HPLC-DAD) and electrospray ionisation tandem mass spectrometry (HPLC-ESI-MS)," *Food Chem.*, vol. 131, no. 1, pp. 318–327, 2012.
51. J. D. Smith, M. C. Mescher, and C. M. De Moraes, "Implications of bioactive solute transfer from hosts to parasitic plants," *Curr. Opin. Plant Biol.*, vol. 16, no. 4, pp. 464–472, 2013.
52. R. L. Prior, X. Wu, and K. Schaich, "Standardized Methods for the Determination of Antioxidant Capacity and Phenolics in Foods and Dietary Supplements," *J. Agric. Food Chem.*, vol. 53, no. 10, pp. 4290–4302, 2005.
53. K. M. Schaich and J. Xie, "Re-evaluation of the 2,2-Diphenyl-1-picrylhydrazyl Free Radical (DPPH) Assay for Antioxidant Activity," *J. Agric. Food Chem.*, vol. 62, pp. 4251–4260, 2014.
54. C. López-Alarcón and A. Denicola, "Evaluating the antioxidant capacity of natural products: A review on chemical and cellular-based assays," *Anal. Chim. Acta*, vol. 763, pp. 1–10, 2013.
55. I. Pinchuk, H. Shoval, Y. Dotan, and D. Lichtenberg, "Evaluation of antioxidants : Scope , limitations and relevance of assays," *Chem. Phys. Lipids*, vol. 165, no. 6, pp. 638–647, 2012.
56. T.-S. Chen *et al.*, "New Analytical Method for Investigating the Antioxidant Power of Food Extracts on the Basis of Their Electron-Donating Ability : Comparison to the Ferric Reducing / Antioxidant Power (FRAP) Assay," *J Agr Food Chem*, vol. 58, pp. 8477–8480, 2010.
57. T. Shibamoto and J.-K. Moon, "Antioxidant Assays for Plant and Food Components," *J. Agric. Food Chem.*, vol. 57, pp. 1655–1666, 2009.
58. R. M. P. B. Costa, A. F. M. Vaz, H. S. Xavier, M. T. S. Correia, and M. G. Carneiro-da-Cunha, "Phytochemical screening of Phthirusa pyrifolia leaf extracts: Free-radical scavenging activities and environmental toxicity," *S Afr J Bot*, vol. 99, pp. 132–137, 2015.