Review

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Functional, Chemical and Phytotoxic Characteristics of Cestrum parqui L'Herit: An Overview

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Posted Date: 14 June 2024

doi: 10.20944/preprints202406.0979.v1

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 doi:10.20944/preprints202406.0979.v1

Review

Functional, Chemical and Phytotoxic Characteristics of *Cestrum parqui* L'Herit: An Overview

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Abstract: *Cestrum parqui* L'Herit. (Solanaceae family) is a species of forest shrub, self-incompatible and specialized in pollination, widespread in the subtropical area of the planet and now widely distributed also in the Mediterranean area. The constituents of its leaves have antimicrobial, anticancer, insecticidal, antifeedant, and herbicidal properties. The spread of this species represents a valuable source of compounds with high biological value. Research on potential uses, understanding of the nutritional and functional composition, and applications of *C. parqui* extracts is important and very promising for its commercial use. To date, there are some reports on the potential applications of *C. parqui* extracts as selective natural pesticides and for their potential phytotoxic role. Scientific knowledge and the use of extraction techniques for these components are essential for commercial applications. This article summarizes the research and recent studies available on the botany, phytochemistry, functional properties, and commercial applications of *C. parqui* as a biopesticide.

Keywords: *Cestrum parqui* L'Herit.; insecticidal and antifeedant activity; herbicidal activity; secondary metabolites; lignans; flavones; oxylipins

1. Introduction

Cestrum parqui L'Herit. (green cestrum, Figure 1) is a plant also known with different names, such as Chilean cestrum, Chilean flowering jessamine, Chilean jessamine, green cestrum, green poison berry, green poison-berry, green poisonberry, iodine bush, willow jasmine, willow leaved jessamine, willow-leaf jessamine, willow-leaved jasmine, willow-leaved jessamine [1].

Figure 1. Flowers of *Cestrum parqui* L'Herit.

 doi:10.20944/preprints202406.0979.v1

1.1. Taxonomy, Morphology, and Distribution

C. parqui belongs to Solanaceae, a family of dicotyledonous angiosperms of great importance to humans as it includes many species used worldwide as vegetable crops. Among them, species important for human consumption (potatoes, eggplants, tomatoes, peppers, and chili peppers), some from which pharmaceutical drugs for medicinal or recreational use are derived (belladonna for atropine, tobacco, goji berries), including poisonous plants as *Datura* spp. The family comprises 147 genera with about 2930 species. The genus that hosts the largest number of plants is *Solanum*, with about 1400 species; conversely, *Licianthes* includes 200 species, and *Cestrum* has 175 species. Solanaceae are represented in the wild on all continents, with a greater number of species in the American continent, and they adapt well to almost all ecosystems, although most of them prefer warmth rather than intense cold. *C. parqui* is native to Brazil, Bolivia, northern and central Chile, Peru, Paraguay, Uruguay, and northern and central Argentina, but nowadays, it is widely spread in southeastern and eastern parts of Australia, New Zealand, in some parts of the southern United States like California and Texas, and in much of Europe (Figure 2) [2].

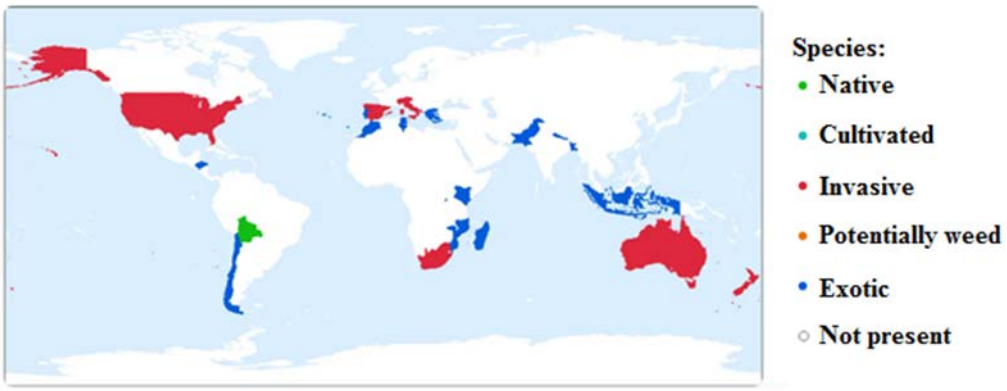

Figure 2. World distribution of *C. parqui*.

This plant, as one of the major weeds, is now widely distributed in the Mediterranean area. Its spread began as an ornamental plant, but today it is considered invasive in the warm temperate and subtropical regions because it well adapts to the edges of watercourses, and is also found in parks, old gardens, uncultivated areas, open woods, forest edges, pastures, and along roadsides. In Australia, *C. parqui* is considered an environmental weed, meaning it has no agricultural function, damaging and competing with existing plants, especially in New South Wales and Queensland [3]. For this reason, it is currently listed as a priority environmental weed in three regions and a sleeper weed in other parts of the country. The invasiveness of this plant is particularly evident when it forms dense stands along forest edges and watercourses, replacing native plants in these habitats and preventing their regeneration [3]. It is an erect, highly branched shrub that usually grows 1-3 m tall, but occasionally reaches up to 5 m in height. It has tubular flowers in clusters, yellow or greenish yellow in color, and stems and leaves that have an unpleasant odor when crushed. The taxonomy of the plant is described in Table 1.

Table 1. Phylogenetic taxonomy of *C. parqui*.

Kingdom	**Plantae**
Sub-kingdom	Tracheophytes
Division	Angiosperms
Class	Eudicots
Sub-class	Asterids
Order	Lamianae Takht
Sub-order	Solanales Juss. ex Bercht. & J.Presl

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Family	Solanaceae' Juss
Tribe	Cestreae
Genus	*Cestrum* L.
Species	*parqui*

2. Traditional Use and Properties

In the Chilean folk medicine, it was used as an antipyretic and for the treatment of fever and inflammation [4,5]. Extracts of the plant obtained with solvents of different polarity have shown moderate antimicrobial activity against the fungi Penicillium expansum and Candida albicans, and the bacteria Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, and Streptococcus pneumoniae [6]. The methanolic extract of the leaves has shown a possible anticancer activity on the human myeloid leukemia cell line (HL-60), suggesting that this may be due to the presence of ursolic and oleanolic acids, two pentacyclic triterpenes [7], as well as the ability to inhibit platelet aggregation induced by ADP and/or collagen, both in sheep and human blood [8]. Furthermore, the methanolic extract of C. parqui leaves has a strong effect on sperm motility in vitro. Electron microscopy studies on human sperm, incubated with concentrations ranging from 40 to 250 μg/mL of C. parqui leaf extract and at time intervals ranging from 5 to 240 minutes, have shown damage to the head and acrosomal membranes, with a maximum spermicidal effect at the highest tested concentration, generally dose and time-dependent [9]. A screening among Cestrum spp. reveals Aspergillus terreus as an endophytic fungus of C. parqui leaves. This microbe biosynthesizes camptothecin, a modified monoterpene indole alkaloid used in cancer chemotherapy [10,11].

3. Potential Effects of *C. parqui*

3.1. Insecticidal and Antifeedant Activity

The genus Cestrum is rich in saponins, and most species exhibit toxicity that supports their use as potential insecticides, herbicides, molluscicides, antimicrobial agents, and antitumor agents. In fact, as far back as the early 1950s, the discovery of gitogenin and digitogenin in the green berries of C. parqui [12], or tigogenin and digallogenin in dried leaves, has been documented [13].

The leaves of C. parqui are the most studied organ of the plant. This is likely related to the observation that many animals that had eaten its leaves were severely intoxicated. For example, several cases of cattle poisoning occurred in Chile between 1992 and 1998. Necropsies of the animals showed pulmonary edema, congestion, hemorrhages in various organs, and hepatic dysfunction [14]. Few years later, Babouche et al. [15] demonstrated that the saponin-rich fraction obtained from the hydroalcoholic extract of the plant could interfere with insect metabolism by lowering the amount of cholesterol needed for ecdysone production, a molting hormone [16].

Thus, over the past twenty years, numerous research groups have tested the insecticidal and antifeedant activity of hydroalcoholic leaf extracts. These extracts are mostly produced from leaves dried at 40 °C for four days and then finely powdered, first extracted with petroleum ether to remove fats, and subsequently with methanol. The methanolic extract is then washed with ethyl ether, resulting in the precipitation of the saponin-containing fraction. This extract has also been tested for molluscicidal activity, using the snail Theba pisana [17] as the target organism. Several types of experiments were set up in this regard. In some, the reaction mixture was deposited on the bottom of containers where the snails were free to move, or the mixture was deposited directly on the bodies of the target organisms: these responded to the presence of the saponin-containing mixture with a strong production of mucus, which caused their dehydration and, ultimately, death. In other experiments, the saponin-containing mixture was added to corn bran or deposited on cabbage leaves, which snails normally feed on, or dissolved in water in quantities ranging from 2 to 8 mg/mL. In these last three cases, the effects were minimal and mostly reduced to a slight weight loss in the animal, which stopped eating or drinking, evidently having recognized the presence of the toxic substance [17]. However, it is likely that by delivering the saponin mixture in different and more palatable foods, better results could be obtained than through simple contact.

 doi:10.20944/preprints202406.0979.v1

The insecticidal activity of aqueous extracts of C. parqui has also been evaluated on Ceratitis capitata, commonly known as the Mediterranean fruit fly, at different concentrations [18]. C. capitata, widespread in Africa, the Mediterranean basin, and South America, is a highly polyphagous species whose larvae develop in a wide range of fruits and are responsible for significant economic damage to the agricultural sector. The aqueous extract at 0.6% (w/w) of the plant completely inhibited the pupation process of the neonate larvae, while less concentrated extracts (0.2 and 0.4%, w/w) slowed larval development and reduced the percentage of formed pupae. In the case of adult target organisms, extracts with increasing polarity obtained with organic solvents such as n-hexane, acetone, and hydroalcoholic solutions were also tested. Aqueous extracts at 0.9% and 0.4% (w/w) of the plant, dissolved in water containers, caused the death of 50% of the insects after 3 and 6 days, respectively. Extracts obtained with organic solvents were almost harmless, except for the most polar extract obtained with methanol/water (80:20, 1% w/w), which was lethal to 12.5% of flies after 3 days and 55% after 6 days. Ingestion of the extracts can cause damage to the reproductive system of adults, contrary to simple contact, which seems to have no effect on eggs or directly on insects.

These results were confirmed a few years later by Chaieb et al. [19,20]. The authors evaluated the entomotoxic activity on: -two phytophagous insects such as Schistocerca gregaria, a polyphagous and voracious grasshopper that feeds on leaves, flowers, shoots, fruits, and seeds of various plant species, including numerous species of primary importance to humans such as rice, barley, corn, sorghum, sugarcane, cotton, date palm, banana, and Spodoptera littoralis, an insect that can attack numerous economically important crops such as turnips, tomatoes, hemp, hibiscus, purslane, mint, clover, tobacco, mallow, apple, grapevine, and many others; -Tribolium confusum, an insect that mainly feeds on natural products such as cereals and flour, rice, dried fruit, powdered milk, mouse baits, spices, and corn; -Theba pisana, a gastropod mollusk introduced into numerous areas including northern Europe, North America, parts of Africa, Asia, and Australia, where it has often become an invasive species, posing a serious problem for agriculture; and -Culex pipiens, the most common mosquito in the Northern Hemisphere, hematophagous and harmful to health. Chaieb and colleagues [19,20] performed toxicity tests based on the species, through simple contact, injection, forced ingestion, or addition to the food substrate. In the case of contact tests, the results were modest, probably because the saponins were unable to penetrate the waxy cuticle of the target organisms, evidently due to their hydrophilicity; while in ingestion tests, the food substrate was probably unpalatable to the target animal. The best results were obtained with injection, which is obviously impractical in daily practice. However, the results show greater activity on T. pisana, followed by S. littoralis, C. pipiens, and slightly less on S. gregaria and T. confusum. In any case, the chances of using the crude material as it is to be added to the diet of the target organism seem slim. Apparently, the added product had lost its palatability, suggesting the need to isolate the saponins present in the crude material for individual use. It is not excluded that the problem can be overcome by delivering the saponins through softer and more palatable foods preferred by the target insects.

The antifeedant effect of the aqueous extract of C. parqui has also been measured on Pieris brassicae, a butterfly that mainly feeds on cultivated varieties of Brassicaceae, especially Brassica oleracea (cabbage), and plants of the genus Tropaeolum [21]. The effect of increasing amounts of extract, added in percentages of 2, 4, 8, 16, and 32% to the lepidopteran's diet, was measured, showing a delay in larval growth at lower concentrations, abnormal metamorphosis at intermediate concentrations, and death at the highest concentration.

It is interesting to note that it has been proven that the activity significantly decreases with the loss of the sugar bound to the steroid nucleus [22], much like what happens in the case of α-chaconine and α -solanine. Thus, it is not surprising that the saponins of C. parqui are completely ineffective against the phytopathogenic fungi Fusarium solani and Botrytis cinerea, which probably can secrete detoxifying enzymes capable of hydrolyzing the sugar chains [23].

Since the best results were obtained on T. pisana, experimentation on this organism was continued to understand if there were differences in the toxicity of the saponin-rich crude material on the juvenile or adult form of the mollusk [24]. Two tests lasting 24 hours each were used and each repeated three times. In the first test, the saponin-containing fraction was deposited on the surface

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where the snails moved at concentrations of 10, 100, 500, 1000, and 2000 ppm, respectively. A mortality rate of 100% was found with a concentration of the analyzed fraction equal to 315 μg/cm² for adults and 157 μg/cm² for juveniles, with LD_{50} values of 36 and 6 ppm, respectively. In the second test, the saponin-containing fraction was placed in direct contact with the back of the target organism in quantities of 1, 5, and 10 mg, respectively. A mortality rate of 100% was found with a crude quantity of 10 mg for adults and 5 mg for juveniles, with LD_{50} values of 2.6 and 1.0 mg/animal, respectively.

For economic and especially environmental reasons (reassuring an increasingly reluctant civil society to the use of chemical products), many research groups are committed to identifying specific natural insecticides. For example, pine wood is particularly susceptible to colonization by organisms of the genera Leptographium spp. Ophiostoma and Ceratocystis, which, by invading the vessels, block the passage of sap and cause deterioration phenomena, as well as the death of plants. The activity of these pathogens, which is only visible by stripping the trunks of dead or suffering plants, is preceded by that of their vectors, mostly coleopteran insects like Hylurgus ligniperda, which, by invading plants weakened by various stresses, allow the fungus to penetrate the sapwood, following their galleries. The symptoms of the infection consist of a wilting of the canopy, with needles quickly turning from pale green to brown and drying up. Wood assortments undergo considerable depreciation due to aesthetic defect. To date, H. ligniperda is controlled using methyl iodide, a chemical product dangerous for users and the environment. Huanquilef et al. [25] tested various fractions of the ethanolic extract of C. parqui precisely on H. ligniperda. Thus, the dried leaves of the plant were first degreased with dichloromethane and then extracted with ethanol using a Soxhlet apparatus. The alcoholic solution was dried, reconstituted with water, and the aqueous phase was shaken and extracted with chloroform, ethyl acetate, and normal butanol, to obtain three fractions. The extracts obtained were then added in known quantities to what the insects ate, consisting of a mixture of water, cellulose, pine extract, glucose, and agar. The resulting mixture was placed in contact with the insects, which could eat it ad libitum. The insects were weighed before and after being left in the presence of the meal for seven days at room temperature. The results obtained showed that the extracts influenced the feeding behavior of the target organism both in adult organisms and larvae, with a dose-dependent effect. In particular, the chloroform extract was the most active, with the ability to reduce weight by 1000% in the case of adult males and by 196% in the case of larvae, considering the concentration of the extract at 0.4% (w/v). This demonstrates that the chloroform extract of C. parqui leaves, even at low concentrations, could be considered a potent tool for controlling the target organism to achieve an economical and relatively simple commercial application [25].

3.2. Herbicidal Activity

From the fresh leaves of C. parqui, a total of 76 compounds were isolated (Table 2), which after purification and structural determination were tested to evaluate their phytotoxic activity. In particular, the fresh leaves were finely chopped and then infused with methanol, methanol/water: 9/1 (v/v), water/ethanol: 1/1 (v/v) + 1% NH_4OH, and water, respectively. The last three infusions were dried and then extracted with solvents of increasing polarity as indicated in Scheme 1. Thus, after numerous chromatographic steps, the following compounds were isolated: the C13 norisoprenoids 1-18 (Figure 3) [26,27]; the sesquiterpenes 19 and 20, the spirostan 21, the pseudosapogenin 22 (Figure 4) [27]; the lignans 23-42 (Figure 5) [28,29]; the aromatic compounds 43-59 (Figure 6) [30,31]; the flavones 60-62 (Figure 7) [30]; and the oxylipins 63-76 (Figure 8) [32].

Table 2. Secondary metabolites isolated from the leaves of *C. parqui* and tested for their potential phytotoxic activity in different studies.

No.	Name	Ref
C13 Norisoprenoids		
1	(6R,9R)-9-Hydroxy-4-megastigmen-3-one	
2	(2R,6R,9R)-2,9-Dihydroxy-4-megastigmen-3-one	
3	Byzantionoside A	

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4	Annuionone E	
5	(3R,6R,7E,9R)-3,9-Dihydroxy-4,7-megastigmadiene	
6	(3R,6R,7E)-3-Hydroxy-4,7-megastigmadien-9-one	
7	(6R,7E,9R)-9-Hydroxy-4,7-megastigmadien-3-one	
8	Byzantionoside B	
9	Corcoionolo C	
10	(3S,5R,6R,7E,9R)-3,5,6,9-Tetrahydroxy-7-megastigmene	26,27
11	(3S,5R,6R,7E,9R)-5,6,9-Trihydroxy-3-isopropyloxy-7-megastigmene	
12	4-Oxo-β-ionol/(7E)-9-Hydroxy-5,7-megastigmadien-4-one	
13	(3S,7E,9R)-3,9-Dihydroxy-5,7-megastigmadiene	
14	(3R,7E)-3-Hydroxy-5,7-megastigmadien-9-one	
15	(3S,5R,6S,7E)-5,6-Epoxy-3-hydroxy-7-megastigmen-9-one	
16	(3S,5R,6S,7E,9R)-5,6-Epoxy-3,9-dihydroxy-7-megastigmene	
17	(6E,9S)-9-Hydroxy-4,6-megastigmadien-3-one	
18	(6Z,9S)-9-Hydroxy-4,6-megastigmadien-3-one	
Sesquiterpenes		
19	12-Hydroxy-α-cyperone	27
20	1,2,2a,3,6,7,8,8a-Octahydro-7-hydroxy-2a,7,8-trimethylacenaphthylen-4(4H)-one	33,34
Spirostane		
21	5α-Spirostan-3β,12β,15α-triol	27
Pseudosapogenine		
22	26-O-(30-Isopentanoyl)-β-D-glucopyranosyl-5α-furost-20(22)-ene-3β,26-diol	27
Lignans		
Diepoxylignans		
23	(+)-Pinoresinol	
24	(+)-Mediaresinol	
25	(+)-Syringaresinol	28
26	β-D-Glucopyranoside, 2,6-dimethoxy-4-[(1S,3aR,4S,6aR)-tetrahydro-4-(4-hydroxy-3,5-dimethoxyphenyl)-1H,3H-furo[3,4-c]furan-1-yl]phenyl	
Epoxylignans		
27	(+)-Lariciresinol	
28	(+)-Justiciresinol	
29	5′-Methoxylariciresinol	28
30	(-)-Berchemol	
Neolignans		
31	*cis*-Dehydrodiconiferyl alcohol	
32	*trans*-Dehydrodiconiferyl alcohol	28
33	(-)-Simulanol,	
Sesquilignans		
34	rel-(7Z,7′β,7′′β,8′α,8′′α)-4′′,9,9′,9′′-Tetrahydroxy-3,3′,3′′-trimethoxy-4,7′:4′,7′′-diepoxy-5,8′:5′,8′′-sesquilign-7-ene	
35	Herpetotriol	
36	rel-(7Z,7′α,7′′α,8′β,8′′β)-4′′,9,9′,9′′-Tetrahydroxy-3,3′,3′′-trimethoxy-4,7′:4′,7′′-diepoxy-5,8′:5′,8′′-sesquilign-7-ene	28
37	rel-(7E,7′α,7′′α,8′β,8′′β)-4′′,9,9′,9′′-Tetrahydroxy-3,3′,3′′-trimethoxy-4,7′:4′,7′′-diepoxy-5,8′:5′,8′′-sesquilign-7-ene	
40	threo-4′,4′′,7′′,9′′-Tetrahydroxy-3,3′,3′′,5′-tetramethoxy-4,8′′-oxy-7,9′:7′,9-diepoxylignan	

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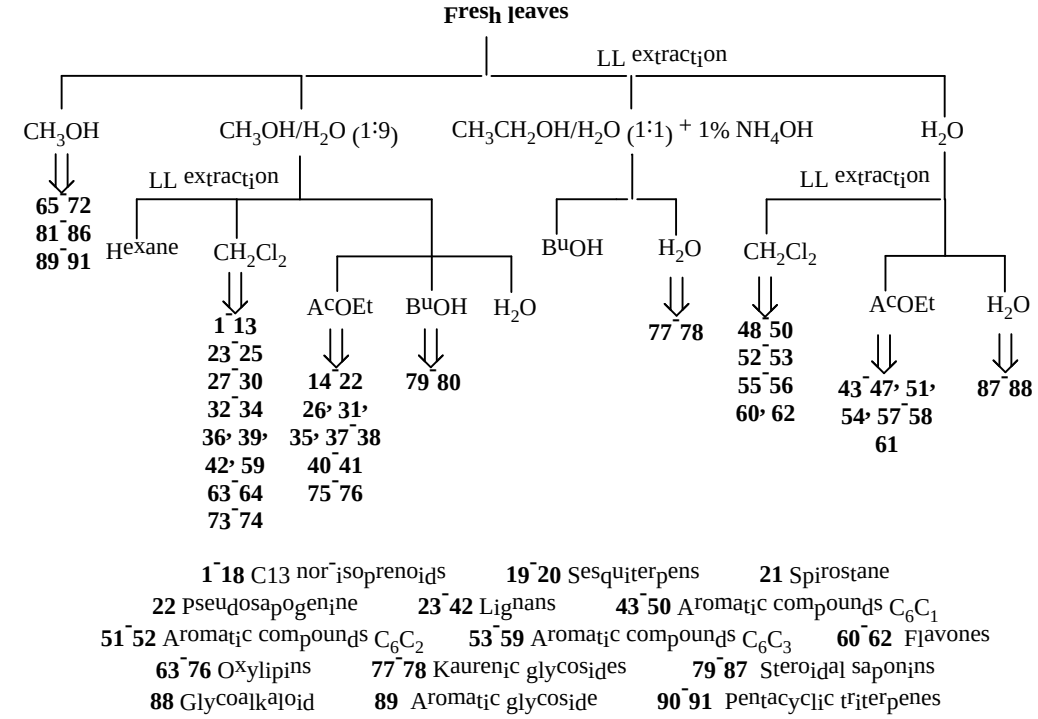
41	erythro-4′,4′′,7′′,9′′-Tetrahydroxy-3,3′,3′′,5′-tetramethoxy-4,8′′-oxy-7,9′:7′,9-diepoxylignan	
42	erythro-4′,4′′,7′′,9′′-Tetrahydroxy-3,3′,3′′,5,5′-pentamethoxy-4,8′′-oxy-7,9′:7′,9-diepoxylignan	
Oxyneolignan		
38	Dimethyl (7′E)-3,3′-dimethoxy-4,40-oxyneolign-7′-ene-9,9′-dioate	28,29
Norlignan		
39	9′-Nor-3′,4,4′-trihydroxy-3,5-dimethoxylign-7-eno-9,7′-lactone	28,29
Aromatic compounds		
43	4-Hydroxybenzaldehyde	30,31,35-37
44	3,5-Dimethoxybenzaldehyde	
45	4-Hydroxybenzoic acid	
46	Vanillic acid	
47	Syringic acid	
48	Methyl 4-hydroxybenzoate	
49	Methyl vanillate	
50	Methyl syringate	
51	Tirosol	
52	3′,5′-Dimethoxy-4′-hydroxy-2-hydroxy-acetophenone	
53	p-Coumaric acid	
54	Caffeic acid	
55	Methyl ferulate	
56	Methyl ester of caffeic acid	
57	p-Dihydrocoumaric acid	
58	Methyl ester of p-dihydrocoumaric acid	
59	Dihydrosynapic acid	
Flavones		
60	4′-Hydroxy-4-methoxychalcon	
61	Quercitin	30
62	N-(p-Carboxymethylphenyl)-p-hydroxybenzamide	
Oxylipins		
63	(8S,9R,10E,12R,14Z)-Heptadeca-10,14-diene-1,8,9,12-tetraol	
64	(8S,9R,10E,12S,14Z)-Heptadeca-10,14-diene-1,8,9,12-tetraol	
65	(9S,10R,11E,13R,15Z)-9,10,13-Trihydroxyoctadeca-11,15-dienoic acid	
66	(9S,10R,11E,13S,15Z)-9,10,13-Trihydroxyoctadeca-11,15-dienoic acid	
67	Methyl (9S,10R,11E,13R,15Z)-9,10,13-trihydroxyoctadeca-11,15-dienoate	
68	Methyl (9S,10R,11E,13S,15Z)-9,10,13-trihydroxyoctadeca-11,15-dienoate	32
69	(9S,10R,11E,13R)-9,10,13-Trihydroxyoctadec-11-enoic acid	
70	(9S,10R,11E,13S)-9,10,13-Trihydroxyoctadec-11-enoic acid	
71	Methyl (9S,10R,11E,13R)-9,10,13-trihydroxyoctadec-11-enoate	
72	Methyl (9S,10R,11E,13S)-9,10,13-trihydroxyoctadec-11-enoate	
73	(8S,9S,10R,11Z,14Z)-Heptadeca-11,14-diene-1,8,9,10-tetraol	
74	(9S,10S,11R,12Z,15Z)-9,10,11-Trihydoxyoctadeca-12,15-dienoic acid	

 doi:10.20944/preprints202406.0979.v1

75 Methyl 10-hydroxyoctadec-12-enoate

76 Methyl (9S,10E,12Z,15Z)-octadeca-10,12,15-trien-9-ol



Scheme 1. Isolation of C13 nor-isoprenoids, sesquiterpenes, spirostanes, pseudosapogenins, lignans, aromatic compounds, flavones, and oxylipins.

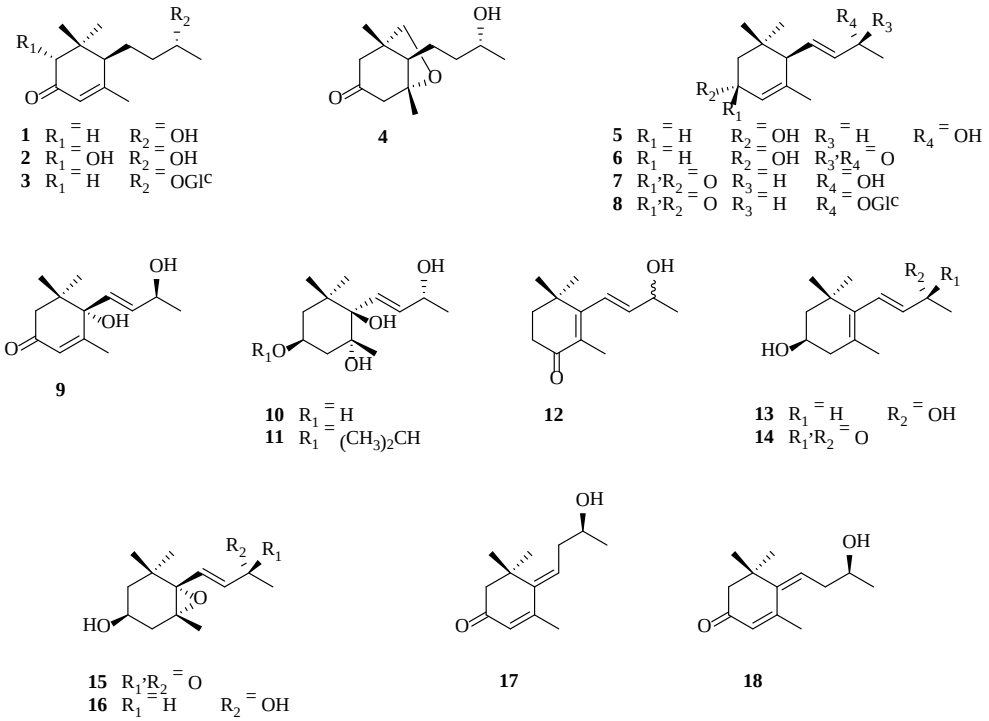


Figure 3. C13 nor-isoprenoids isolated from *C. parqui*.

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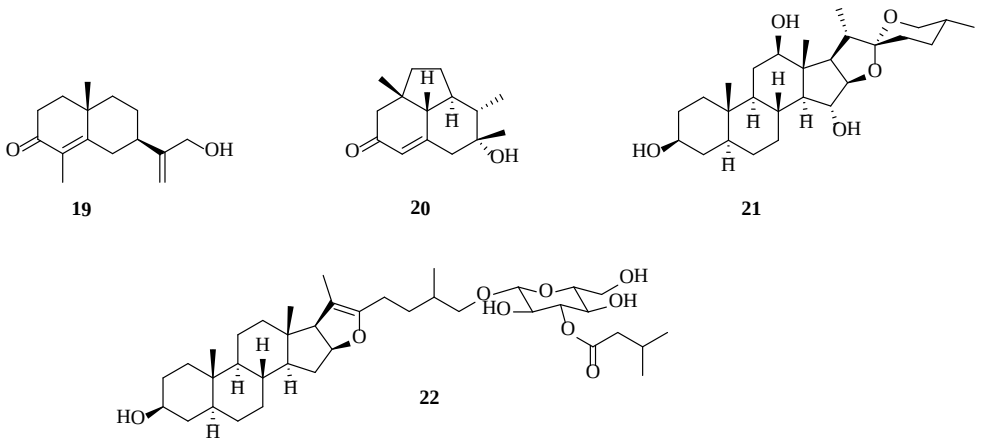

Figure 4. Sesquiterpenes (19-20), spirostane (21) and pseudosapogenin (22) isolated from *C. parqui*.

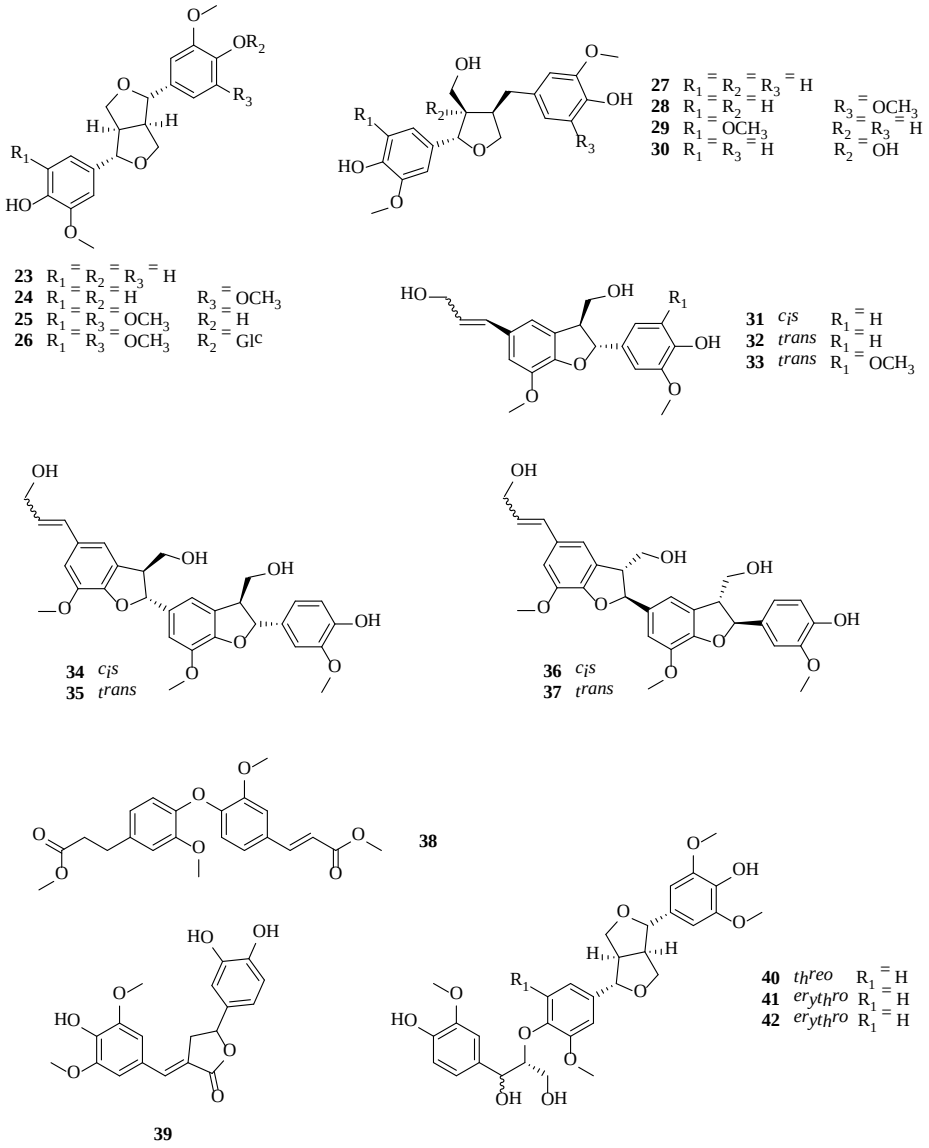

Figure 5. Lignans from *C. parqui*.

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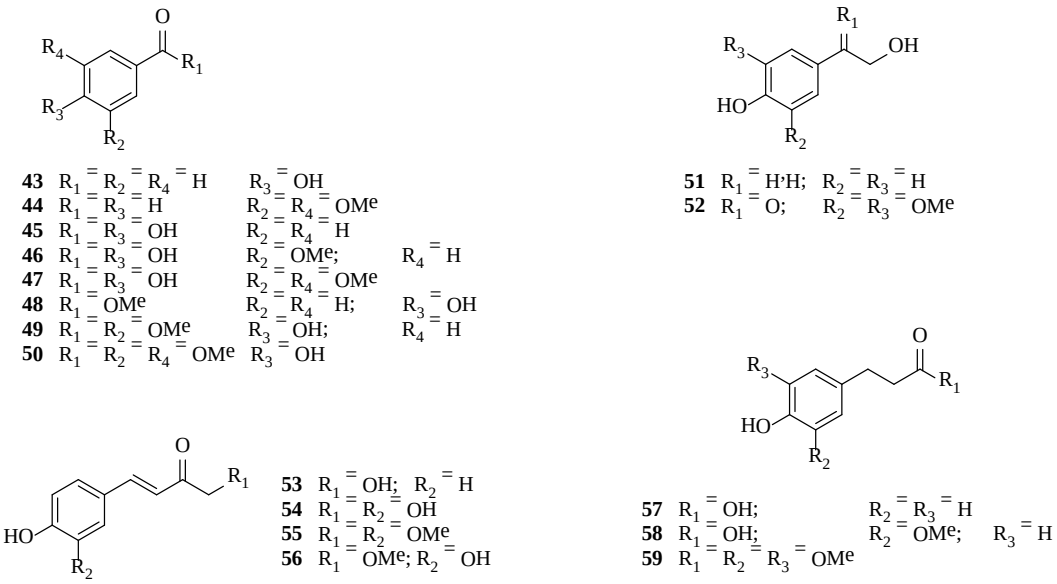

Figure 6. Aromatic compounds C_6C_1 (43-50), C_6C_2 (51-52), and C_6C_3 (53-59) from *C. parqui*.

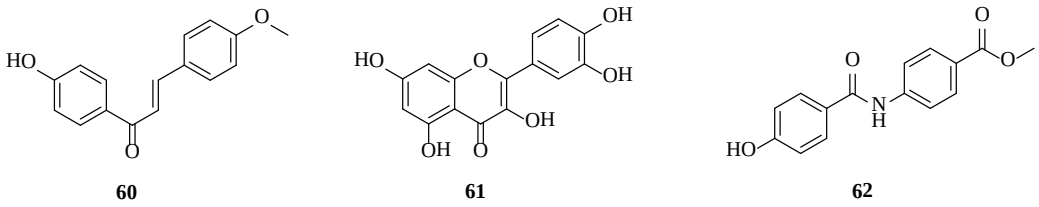

Figure 7. Flavonoids from *C. parqui*.

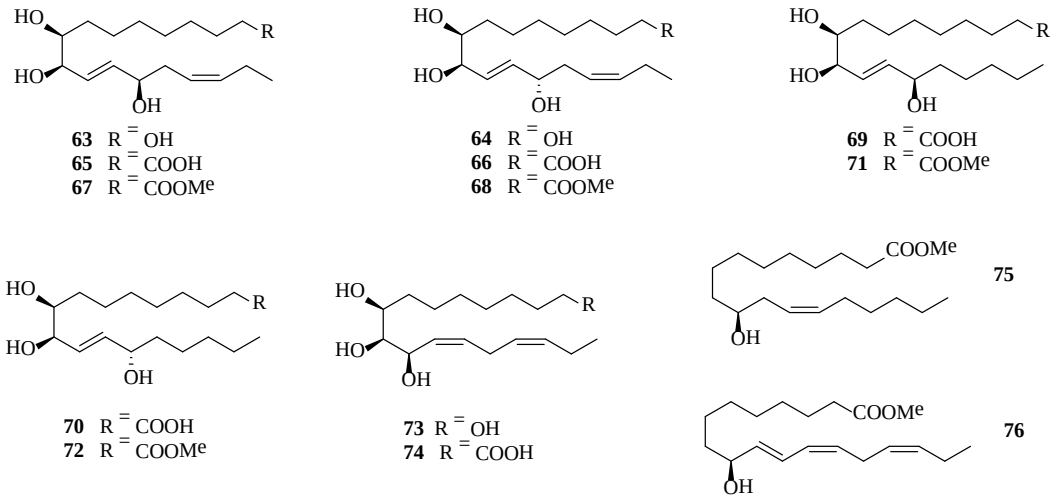

Figure 8. Polyol fatty acids 63-64 and 73, methylated oxylipins 67 and 68 and oxylipins 65-66, 69-72 and 74-76 (Note that 63/64, 65/66, 67/68, 69/70, 71/72 are five diastereomeric couples of compounds).

The compounds 1-76 were subjected to phytotoxicity assays to evaluate their effects on seed germination and the growth of roots and seedlings of various target organisms, including *Lactuca sativa*, *Solanum lycopersicum*, *Amaranthus retroflexus*, *Chenopodium album*, *Potamogeton oleracea*, and *Allium cepa* (Table 3). The assays were performed at different concentrations ranging from 10^{-4} to 10^{-9} M, following the protocol developed by Macias et al. [38], using the well-known herbicide Pendimethalin as a reference.

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Table 3. Range of tested concentrations and target organisms for the phytotoxicity tests of the compounds 1-76.

Compounds	Range of concentrations	Organism test					
		L. sativa	*S. lycopersicum*	*A. retroflexus*	*C. album*	*P. oleracea*	*A. cepa*
1-13	10^{-4} - 10^{-7} M	x					
14-18	10^{-5} - 10^{-7} M	x					
19-22	10^{-4} - 10^{-7} M	x					
23-35	10^{-4} - 10^{-8} M			x	x	x	
36-39	10^{-4} - 10^{-7} M	x	x				
40-42	10^{-4} - 10^{-8} M			x	x	x	
43-59	10^{-4} - 10^{-9} M	x	x				x
60-62	10^{-4} - 10^{-9} M	x	x				x
63-76	10^{-4} - 10^{-8} M	x					

3.2.1. Assay with C13 Nor-isoprenoids (1-18), Sesquiterpenes (19-20), spirostane (21) and pseudosapogenin (22)

Except for nor-terpenes 3, 21, and 22, the tested compounds had no effect on germination but showed moderate inhibitory activity on root and shoot growth. The activity of the glycosylated compound 22 is intriguing, considering that glycosylation is the main detoxification mechanism adopted by plants to defend themselves against phytotoxic substances they produce and store [39]. Among all the compounds tested, spirostane 21 was the most active, with root and shoot elongations reduced by up to 60% and germination by up to 30% at a concentration of 10^{-4} M. In general, the more polar compounds that are more soluble in water appear to be more active.

3.2.2. Assay with Lignans (23-35, 40-42)

Compounds 23-35 and 40-42 were tested on *A. retroflexus*, *P. oleracea*, and *C. album*, in a concentration range varying from 10^{-4} to 10^{-8} M. Lignans 23-26 were the most active on *A. retroflexus*, inhibiting its germination even at the lowest concentration, while compounds 29-30 showed anti-germination activity on *P. oleracea* and anti-radical activity on *A. retroflexus*. All compounds were slightly stimulating on shoot elongation of *C. album* and *P. oleracea*.

3.2.3. Assay with Lignans (36-39)

These compounds were tested on *L. sativa* and *S. lycopersicum*, showing low phytotoxic activity on both. Of the four compounds in question, only compound 39 was able to inhibit the shoot length of *S. lycopersicum* by about 50% at a concentration of 10^{-4} M.

3.2.4. Assay with Aromatic Compounds (43-59) and Flavones (60-62)

The aqueous infusion of *C. parqui* leaves was tested on the germination, root length, and shoot length of *L. sativa*, *S. lycopersicum*, and A. cepa [25]. The interesting results obtained suggested dividing the entire extract into three fractions, two obtained by extraction with methylene chloride and ethyl acetate, while the third was the remaining aqueous part (Scheme 1). From the first organic

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fraction, compounds 48-50, 52-53, 55-56, 60, and 62 were isolated, while from the second fraction, compounds 43-47, 51, 54, 57-58, and 61 were isolated. Compounds 43-62 were tested on the same target organisms used for the phytotoxic evaluation of the aqueous extract, and some of them were far more active than the herbicides used as reference standards.

Only aromatic compounds 55 and 56 on *L. sativa* and chalcone 60 on *A. cepa* showed weak inhibitory effects on germination, while all others were practically inactive. Results on root elongation showed that some compounds, such as product 45, could have a phytotoxic effect on *S. lycopersicum* but even have a stimulating effect on *A. cepa*, or compound 44, stimulating for *A. cepa* but inhibiting for L. sativa. Compounds 45 and 48 were able to inhibit the shoot length of *S. lycopersicum* and *A. cepa* by 66% and 60%, respectively, at a concentration of 1 nM [30].

3.2.5. Assay with Oxylipins (63-76)

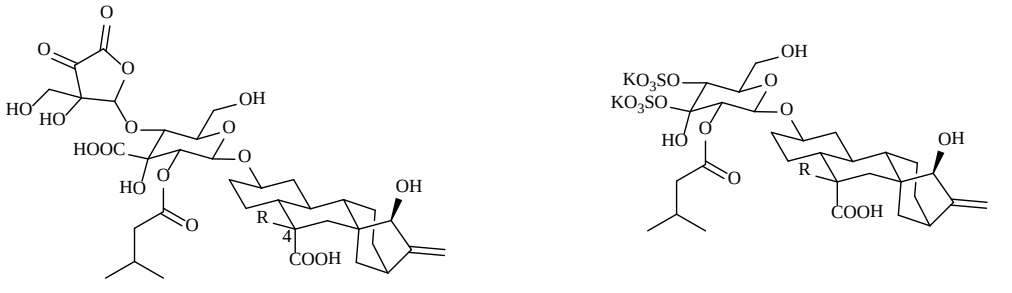
The oxylipins were also tested on *L. sativa* seeds but in a narrower concentration range, specifically between 10^{-4} and 10^{-8} M. It is not easy to rationalize the results of the phytotoxicity of these compounds. For example, at a concentration of 10^{-4} M, compounds 63-68 showed weak inhibitory action on germination, with values around 10%, and on the elongation of the hypocotyl and root with inhibition values around 20%. However, at a concentration 100 times lower, only compounds 65 and 66 remained weak inhibitory activity on root growth, while the corresponding alcohols 63 and 64, or the corresponding methyl esters 67 and 68, were even slightly stimulatory. Alternatively, compounds 69 and 71 stimulate germination and inhibit radical elongation, while compounds 70 and 72 inhibit germination and stimulate radical elongation. In general, it seems that the compounds present phytotoxicity values closely related to their degree of unsaturation, as for compounds 63-68, 73, and 74, or phytotoxicity values dependent on the number of hydroxyl functions, as for compounds 75 and 76.

It is interesting to note that oxylipins seem to play a crucial role in intra- and extracellular communication in vertebrates, fungi, and plants. In microorganisms, these metabolites are involved in the regulation of cell growth and differentiation, while in plants, their role in defense mechanisms based on apoptosis processes in response to infections caused by pathogens seems to be proven.

3.3. Other Isolated Metabolites

3.3.1. Kaurenic Glycosides (77-78) with Strychnine-like Action

Two kaurenic glycosides named Carbossiparquin (77) and Parquin (78) have been isolated from the leaves of *C. parqui*, whose structures have been determined using NMR techniques and mass spectrometry [40] (Figure 9 and Table 4).



77 R = COOH Carboxyparquin
78 R = H Parquin

A R = COOH Carboxyatractyloside
B R = H Atractyloside

Figure 9. Kaurenic glycosides (77-78).

These compounds are structurally very similar, differing only in the presence of a second carboxylic function at carbon C-4 of the first compound. It is noteworthy that compounds 77 and 78 are quite like two toxins with strychnine-like action, namely carboxyatractyloside (A) and

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atractyloside (B), isolated from *Atractylis gummifera* [41]. In mice, Carbossiparquin (77) has an LD_{50} value of 4.3 mg kg^{-1} and is over 50 times more toxic than crude extracts of *C. parqui* leaves. It is interesting to note that this toxin causes lesions in both the kidneys and the liver, like those observed in animals intoxicated after consuming *C. parqui*. The second compound (78) is relatively non-toxic and considered essentially a co-metabolite.

3.3.2. Cytotoxic Secondary Metabolites

Four new steroid saponins have also been isolated, three of which are monodesmosidic, called Parquisoside A (79) e B (80) [42] e Parquispiroside (83) [43], along with compound 84, named Parquifuroside [43]; together with the known steroid saponins: Neotigogenin (81) [44] and (25R)-Isonuatigenin (82) [45], Capsicoside D (85) [46], 22-*O*-Methyl-capsicoside D (86) [43] and Digitogenin (87) [13,46]; the glycoalkaloid Solasonine (88) [46] and the aromatic glycoside Benzyl primeveroside (89) [43] (Figure 10 and Table 4). If compounds 79 and 80 are likely capable of inhibiting carrageenan-induced edema, there is no definitive evidence to support this. However, compounds 81-83 and 86-89 were tested for their cytotoxicity on four human cell lines: HeLa, HepG2, U87, and MCF7. Of these latter 5 compounds, only compound 81 showed moderate activity, with IC50 values of 7.7, 7.2, 14.1, and 3.3 μM, respectively. These values are quite promising considering that Cisplatin, an antineoplastic chemotherapeutic agent used in the treatment of numerous tumors but with significant side effects, has much higher LC_{50} values of 39.2, 14.6, 7.3, and 23.0 μM, respectively [42].

Table 4. Other metabolites isolated from the leaves of *C. parqui*.

No.	Common name/IUPAC name	Ref.
77	Carbossiparquin	40
78	Parquin	
79	Parquisoside A/(3β,24S,25S)-spirost-5-ene-3,24-diol 3-O-{[α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)]-β-D-glucopyranoside	42
80	Parquisoside B/(3β,24S,25S)-spirost-5-ene-3,24-diol 3-O-{[α -L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→2)]-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranosyl-(1→4)}-β-D-glucopyranoside	42
81	Neotigogenin	44
82	(25R)-Isonuatigenin	44
83	Parquispiroside/25(R)-3β-[(O-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)-O-[β-D-xylopyranosyl-(1→3)-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy]-5α,15β,22R,25R-spirostan-3,15-diol	43
84	Parquifuroside/25(R)-26-[(β-D-Glucopyranosyl)oxy]-(3β[(O-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→2)-O-[β-D-xylopyranosyl-(1→3)-O-β-D-glucopyranosyl-(1→4)-β-D-galactopyranosyl)oxy],5α,15β,22R,25R)-furostane-3,15,22-triol	43
85	Capsicoside D	46
86	22-O-Methylcapsicoside D	43
87	Digitogenin	13,46
88	Solasonine	46
89	Benzyl primeveroside	43
90	Ursolic acid	43
91	Oleanolic acid	46

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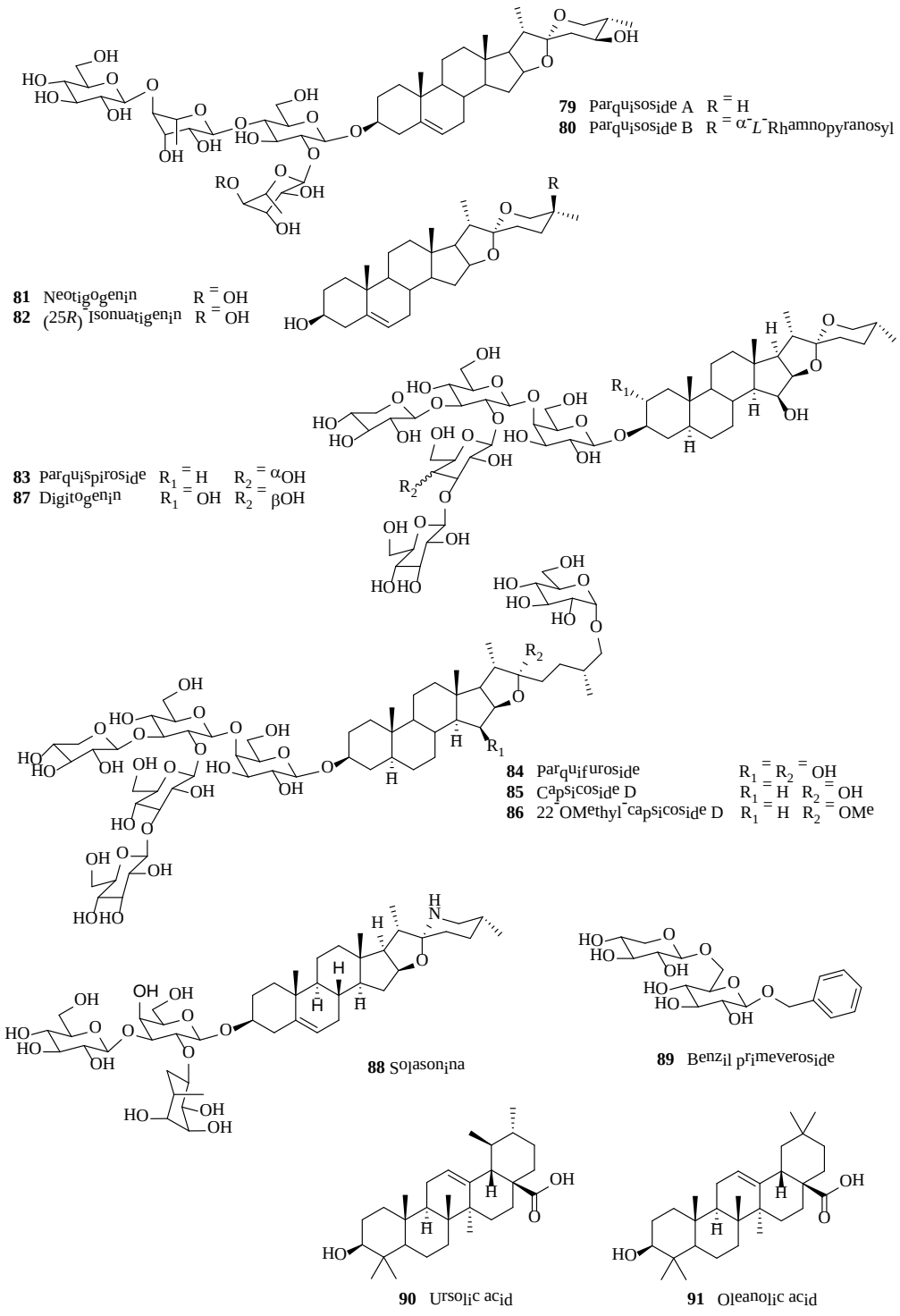

Figure 10. Saponins and aromatic glycosides.

4. Conclusions

As well as all Solanaceae, *Cestrum parqui* L'Herit is an extremely toxic plant, which even with intense exposure to its leaves can cause respiratory difficulty, nausea, headache, and other unpleasant symptoms. There are numerous accounts of its toxicity even with the flowers or fruits. Several studies have allowed for the isolation and determination of the structure of many constituents of the plant, such as C13 norisoprenoids, sesquiterpenes, lignans, aromatic compounds, flavones, kaurenic glycosides, saponins, and alkaloids. Many of these compounds, but not all, have been studied to assess their entomotoxic activity on various phytophagous insects and/or mollusks, and their phytotoxicity on different target organisms. The results to date are not definitive because it is not yet

 doi:10.20944/preprints202406.0979.v1

clear whether it is preferable to use a crude extract of the plant, generally an alcoholic or hydroalcoholic one, especially from the leaves, or to use isolated individual metabolites. All of this suggests and justifies the significant interest in this plant from both a toxicological and phytochemical perspective, with the prospect of commercial application of its derivatives.

Author Contributions: Conceptualization, M.C.D.M. and C.D.M.; resources, A.R.B. and S.P.; writing, P.N. and D.P.; supervision, A.D.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: Not applicable.

Acknowledgments: This study was supported by AIPRAS Onlus (Associazione Italiana per la Promozione delle Ricerche sull'Ambiente e la Salute umana).

Conflicts of Interest: The authors declare no conflicts of interest.

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