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Research Article

Mapuche Herbal Medicine Inhibits Blood Platelet Aggregation

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12 plant species traditionally used by the Mapuche people in Chile to treat wounds and inflammations have been evaluated for their direct blood platelet inhibition. Seven of the 12 tested plant species showed platelet inhibitory effect in sheep blood, and four of these were also able to inhibit the ADP- (5.0 μM) and collagen- (2.0 μg/mL) induced aggregations in human blood. These four species in respective extracts (in brackets) were Blechnum chilense (MeOH), Luma apiculata (H2O), Amomyrtus luma (DCM: MeOH 1:1) and Cestrum parqui (DCM: MeOH 1:1). The platelet aggregating inhibitory effects of A. luma (DCM: MeOH 1:1), and L. apiculata (H2O) were substantial and confirmed by inhibition of platelet surface activation markers.

1. Introduction

Chile has an extraordinary variety of plants and animals, thanks to the latitudinal extent of the country and its great altitudinal range. The Chilean Winter Rainfall-Valdivian forest is one of the most exceptional and exposed biodiversity hotspots of the world. It encompasses approximately 40% of Chile’s land area and harbours both endemic flora and fauna. About 50% of the 4000 vascular plant taxa found in this area are endemic. Through collaboration, we have access to the traditional medicinal plants from this area [1–3]. The plants examined are traditionally used by the Mapuche people in Chile to treat wounds and associated infections, as shown in Table 1. This paper evaluates the platelet inhibitory capacity of 12 selected plant species.

Platelet receptors on the surface of the platelets determine the reactivity of platelets and have a wide range of agonists and adhesive proteins [7]. Current antiplatelet therapies target key pathways of platelet activation, including surface receptors and signalling molecules. Aspirin has been the foundation of antiplatelet therapy for over 50 years, and it inhibits platelets by irreversibly acetylating Ser529 of cyclooxygenase 1 (COX1), thereby inhibiting thromboxane A2 formation by the platelets. Aspirin has been shown to reduce vascular death in high-risk patients by 15% and non-fatal vascular events by 30%, as evidenced by meta-analysis of over 100 randomized trials [8–10]. Several medicinal plants have direct or indirect antiplatelet effects, many through inhibition of COX1 or 2. Likewise, a variety of fruit extracts have been tested in vitro for their antiplatelet property, and tomatoes have been found to have a very high activity [11]. It was showed that tomato extract inhibited both ADP- and collagen induced aggregation by up to 70% but not AA-induced platelet aggregation. Various fruit juices have also been tested, and some flavonoids have been established as inhibitors of collagen-induced platelet activity [12, 13]. The effect of flavonoids is well established, and for coffee, it was showed that the caffeine is not the inhibitor [14] but rather the phenolics that was also found inside the platelets. Many of the effects observed are often due to synergistic effects, which is also seen on tomato and grape juice, and the effect can be expected to be lower for the individual compounds [11–14].
Table 1: Overview of the plants examined for blood aggregation inhibition, including voucher number, Latin name, local name, and use. All the plants have been collected in region X in the Valdivian Coastal Range Forest. L: leaf, S: stem, R: root, W: whole plant, T: thorn, and F: flower.

| Family       | Voucher number | Latin plant name                              | Collected part | Common name   | Local use                                                                 |
|--------------|----------------|-----------------------------------------------|----------------|---------------|---------------------------------------------------------------------------|
| Araliaceae   | PM01-44        | *Pseudopanax laetevirens* (Gay.) Baill.       | L, S           | Sauco         | Leaves, fruit and bark are used for wound healing, as anti-inflammatory, laxative and anti-diuretic [3] |
| Asteraceae   | PM01-28        | *Baccharis absinthioides* Hook. & Arn.        | L              |               | *Baccharis* leaves are used for wound healing, as anti-pyretic and analgesic [4] |
| Blechnaceae  | PM01-18        | *Blechnum chilense* (Kaulf.) Mett.            | L, S, R        | Costilla de vaca | The whole plant is used towards gonorrhoea and wound and eye infections [3] |
| Gunneraceae  | PM01-09        | *Gunnera chilensis* Lam.                      | L, S           | Nalca         | Stem and root are used against uterus pains, as haemostatic and anti-inflammatiorial [3] |
| Lamiaceae    | PM06-38        | *Satureja multiflora* Briq. in Engl & Prantl  | L              | Oreganillo    | Leaves used for digestive problems [5]                                    |
| Malvaceae    | PM01-10        | *Corynabutilon vitifolium* (Cav.) Kearney    | L, S           | Huella        | Bark, stem and leaves are used for liver diseases and uterus contractions [3] |
| Myrtaceae    | PM03-24        | *Amomyrtus luma* (Molina) D. Legrand & Kausel | L, S           | Luma          | Leaves are used to decrease blood pressure and cholesterol levels, and to treat liver diseases [3] |
| Myrtaceae    | PM01-40        | *Luma apiculata* (DC.) Burret                 | L, S           | Arrayán, Quetri | Leaves are used to treat diarrhea, dysentery, ingestion [6]                |
| Myrtaceae    | PM01-16        | *Ugni molinae*                                | L, S           | Murta         | The fruit is stimulating and refreshing [3]                                |
| Onagraceae   | PM+1-19        | *Fuchsia magellanica* Lam.                    | L, S           |               | Leaves are used as antipyretic, blood pressure regulator, diuretic and wound healing [3] |
| Poaceae      | PM03-32        | *Anthoxanthum utriculatum* (Ruiz & Pav.) Y. Schouten & Veldkamp | L              | Ratonera      | Roots are used traditionally [3]                                          |
| Proteaceae   | PM03-25        | *Lomatia ferruginea* (Cav.) R.Br.             | L, R           |               | *Lomatia* leaves and bark are used as laxative, expectorant and as anti-inflammatory [3] |
| Solanaceae   | PM05-35        | *Cestrum parqui* L’Hér                      | L              | Palqui        | Leaves are used to relief fevers, and towards skin diseases [3]                |
| Winteraceae  | PM07-05        | *Drimys winteri* J.R. & G. Forster           | L, B           |               | Leaves are used as antipyretic, in wound healing, as diuretic anti-inflammatory agent, and against ulcers [3] |

The plants collected for this study have been chosen based on their use in the treatment of wounds and inflammatory diseases [1, 2]. Many inflammatory mechanisms are involved in wound healing. Especially, platelets plays a crucial role in haemostasis and thrombosis, and they also play an important role in wound healing, inflammation, antimicrobial host defence, angiogenesis, and tumour growth and metastasis [15]. Therefore, plants used against these or related diseases have been collected. The plants examined in here are collected based on ethnopharmacological data from the Region de Los Lagos in southern Chile, part of the Chilean Winter Rainfall-Valdivian forest [3, 16, 17]. Deforestation threatens this area, and the evaluation of traditional medicine might help to preserve the area with its natural richness. Furthermore, the evaluation also contributes to the preservation of the Mapuche culture, and a sustainable production and/or collection of plants may create an economic foundation as an alternative to the felling of the rainforest.

The aim of the study was a screening of a variety of Mapuche herbal medicine for platelet inhibitory effects. Inhibition on platelet aggregation in sheep blood was chosen as an initial screening method due to the large volumes of blood needed. Plant extracts with activity in sheep blood were subsequently investigated for inhibitory effects on human blood platelets.

2. Material and Methods

2.1. Plant Material. The plant species in this study are traditionally used to treat wounds, wound infections, and/or inflammatory ailments by the Mapuche people. The collection have been conducted in February in the years 2001, 2003, 2005, 2006, and 2007 under the supervision of Alfonso

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Guzman [18]. All plants have been collected in Region de Los Lagos located in Chile’s region X. Available plant parts were collected without destroying the population, for example, leaves, stems, flowers, and roots though mainly leaves are used for teas, the preferred preparation in Mapuche traditional medicine (Table 1) [17]. After collection, the plant material was immediately dried at room temperature and transported to Denmark for further studies, where it was kept dry and in darkness until use. Voucher specimens are stored at the Botanical Garden and Museum, University of Copenhagen (C); see Table 1 for voucher specimen number.

2.2. Extraction and Sample Preparation. Dried material from 12 different species of Chilean plants was subjected to extraction. 5 mL DCM: MeOH 1:1 was added to 0.5 g dry plant material and exposed to ultrasonication for 30 minutes and filtration. This was repeated twice, and the combined plant material was immediately dried at room temperature and transported to Denmark for further studies, where it was kept dry and in darkness until use. Voucher specimens are stored at the Botanical Garden and Museum, University of Copenhagen (C); see Table 1 for voucher specimen number.

2.3. Preparation of Samples for Aggregation and Flow Cytometry Assays. The dried extracts were dissolved in DMSO : EtOH 1:4 in order to reach a concentration of 20 mg/mL, only extracts that was fully redissolved where taken forward. Tested extracts are listed in Table 2. The DMSO : EtOH samples were diluted in sterile filtered HEPES-tyrode’s buffer pH 7.4 (137 mM NaCl, 2.7 mM KCl, 1.0 mM MgCl₂, 12 mM NaHCO₃, 0.4 mM Na₂HPO₄, 5.5 mM glucose, 10 mM HEPES) for aggregation assays, and in HEPES-tyrode’s buffer with 0.5% BSA for flow cytometry assays to a final concentration of 1 mg/mL. The DMSO : EtOH samples diluted in HEPES-tyrode’s buffer was added to PRP as a 1:10 dilution. HEPES-tyrode’s buffer containing 0.5% DMSO : EtOH 1:4 was used as vehicle control. Sample and vehicle control were incubated in PRP with 0.5% BSA for flow cytometry assays to a final concentration of 1 mg/mL. The aggregation response was recorded using the Aggrolink software (Chronolog Corp.). Maximal aggregation (MA) was recorded in order to obtain a % inhibition of plant extract, comparing the vehicle control (HEPES-tyrode’s buffer including BSA) with that of the plant extract % inhibition = (MA vehicle control − MA extract)/MA vehicle control × 100%.

The experiments were approved by the Animal Experiments Inspectorate under the Danish Ministry of Justice. All human blood used was drawn from the authors themselves.

2.5. Initial Experiments in Aggregometer. Arachidonic acid (AA), ADP and collagen were tested in several concentrations in sheep blood in order to find the most suitable agonists and the appropriate concentrations of these.

Based on the results from the initial experiment it was decided to test AA in 500 μM, ADP in 5.0 μM, and collagen in 2.5 μg/mL in an initial experiment, and from that, it was decided to use ADP and collagen to all future experiments in the aggregometer. The tested ADP concentration at 5.0 μM was suitable, whereas the collagen concentration was increased from 2.5 μg/mL to 5.0 μg/mL. The aggregation percentage in the collagen-induced reaction with vehicle control was 61%, and by increasing the concentration of agonist, the aggregation percentage would hopefully also increase in order to get closer to the desired 70%. AA was not used in any further experiments.

ADP and collagen were used as agonists based on the initial experiments. For human blood, it was decided to lower the collagen concentration to 2.0 μg/mL due to the observed aggregation. The ADP concentration was the same (5.0 μM) as in the experiment with sheep blood.

To establish the plant extract testing concentration, the MeOH extract of the pharmacologically well described of Drimys winteri [6] was tested in three different concentrations, 10, 1, and 0.1 mg/mL. All three gave high inhibition...
Table 2: Mapuche medicinal plant extracts tested in sheep blood. The agonists are ADP (5.0 μM) and collagen (5.0 μg/mL) and the obtained aggregations are shown in percentage. Extracts are tested in 0.1 mg/mL end concentrations. The aggregation is shown for both extract and vehicle control. If the extract aggregation is 20% lower than that of the vehicle control inhibition is observed.

| Plant                        | Extract       | Extract Yield (% dw) | Agonist | % Aggregation (extract) | % Aggregation (vehicle control) | % Inhibition | Inhibition |
|------------------------------|---------------|----------------------|---------|-------------------------|---------------------------------|--------------|------------|
| *Amomyrtus luma* (leaf)      | DCM : MeOH 1:1| 6.8                  | ADP     | 47                      | 88                              | 47           | Yes        |
|                              | Collagen      |                      |         | 25                      | 85                              | 71           | Yes        |
|                              | ADP           |                      |         | 61                      | 60                              | -2           | No         |
|                              | Collagen      |                      |         | 30                      | 83                              | 64           | Yes        |
|                              | ADP           |                      |         | 45                      | 68                              | 34           | Yes        |
|                              | Collagen      |                      |         | 23                      | 86                              | 73           | Yes        |
| *Anthoxanthum utriculatum* (leaf) | DCM : MeOH 1:1| 5.2                  | ADP     | 38                      | 49                              | 22           | Yes        |
|                              | Collagen      |                      |         | 106                     | 124                             | 15           | *1         |
|                              | ADP           |                      |         | 33                      | 68                              | 51           | Yes        |
|                              | Collagen      |                      |         | 22                      | 77                              | 71           | Yes        |
|                              | ADP           |                      |         | 50                      | 51                              | 2            | No         |
|                              | Collagen      |                      |         | 39                      | 75                              | 48           | Yes        |
| *Blechnum chilense* (leaf)   | DCM : MeOH 1:1| 2.8                  | ADP     | 52                      | 62                              | 16           | No         |
|                              | Collagen      |                      |         | 26                      | 44                              | 41           | Yes        |
|                              | ADP           |                      |         | 38                      | 52                              | 27           | Yes        |
|                              | Collagen      |                      |         | 1                       | 18                              | 94           | Yes        |
| *Cestrum parqui* (leaf)      | DCM : MeOH 1:1| 7.6                  | ADP     | 49                      | 81                              | 40           | Yes        |
|                              | Collagen      |                      |         | 27                      | 91                              | 70           | Yes        |
|                              | ADP           |                      |         | 44                      | 77                              | 43           | Yes        |
|                              | Collagen      |                      |         | 30                      | 83                              | 64           | Yes        |
|                              | ADP           |                      |         | 73                      | 82                              | 11           | *1         |
|                              | Collagen      |                      |         | 52                      | 82                              | 37           | Yes        |
| *Corynabutilon vitifolium* (leaf) | DCM : MeOH 1:1| 3.6                  | ADP     | 48                      | 54                              | 11           | No         |
|                              | Collagen      |                      |         | 44                      | 35                              | -26          | No         |
|                              | ADP           |                      |         | 39                      | 59                              | 34           | *1         |
|                              | Collagen      |                      |         | 48                      | 49                              | 2            | No         |
| *Fuchsia magellanica* (leaf) | DCM : MeOH 1:1| 12.2                 | ADP     | 45                      | 45                              | 0            | No         |
|                              | Collagen      |                      |         | 10                      | 12                              | 17           | No         |
|                              | ADP           |                      |         | 30                      | 52                              | 42           | Yes        |
|                              | Collagen      |                      |         | 32                      | 38                              | 16           | No         |
| *Gunnera chilensis* (leaf + stem) | DCM : MeOH 1:1| 13.1                 | ADP     | 50                      | 59                              | 15           | No         |
|                              | Collagen      |                      |         | 49                      | 64                              | 23           | Yes        |
|                              | ADP           |                      |         | 40                      | 56                              | 29           | *1         |
|                              | Collagen      |                      |         | 35                      | 65                              | 46           | Yes        |
|                              | ADP           |                      |         | 50                      | 71                              | 30           | *1         |
|                              | Collagen      |                      |         | 68                      | 56                              | -21          | *1         |
| *Lomatia ferruginea* (leaf)  | DCM : MeOH 1:1| 2.7                  | ADP     | 24                      | 55                              | 56           | Yes        |
|                              | Collagen      |                      |         | 9                       | 33                              | 73           | Yes        |
|                              | ADP           |                      |         | 43                      | 50                              | 14           | No         |
|                              | Collagen      |                      |         | 6                       | 55                              | 89           | *2         |
|                              | ADP           |                      |         | 57                      | 43                              | -33          | No         |
|                              | Collagen      |                      |         | 30                      | 50                              | 40           | *1         |
Table 2: Continued.

| Plant                        | Extract        | Extract Yield (% dw) | Agonist | % Aggregation (extract) | % Aggregation (vehicle control) | % Inhibition | Inhibition |
|------------------------------|----------------|----------------------|---------|-------------------------|---------------------------------|-------------|-----------|
| *Luma apiculata* (leaf)      | DCM: MeOH 1:1  | 4.5                  | ADP     | 51                      | 65                              | 22          | *1        |
|                              | MeOH           | 4.9                  | Collagen| 42                      | 65                              | 44          | *1        |
|                              | H₂O            | 9.1                  | ADP     | 19                      | 56                              | 66          | Yes       |
|                              |                |                      | Collagen| 21                      | 74                              | 72          | Yes       |
| *Pluchea absinthioides* (leaf)| DCM: MeOH 1:1  | 8.9                  | ADP     | 47                      | 57                              | 18          | *1        |
|                              | MeOH           | 2.9                  | Collagen| 17                      | 39                              | 56          | Yes       |
|                              | H₂O            | 4.4                  | ADP     | 43                      | 51                              | 16          | No        |
|                              |                |                      | Collagen| 9                       | 51                              | 82          | *2        |
| *Pseudopanax laetevirens* (leaf)| DCM: MeOH 1:1  | 7.3                  | ADP     | 45                      | 59                              | 24          | Yes       |
|                              | MeOH           | 6.2                  | Collagen| 23                      | 61                              | 62          | Yes       |
|                              | H₂O            | 13.3                 | ADP     | 50                      | 66                              | 24          | Yes       |
|                              |                |                      | Collagen| 8                       | 6                               | −33         | No        |
| *Satureja multiflora* (leaf + stem)| DCM: MeOH 1:1  | 7.2                  | ADP     | 44                      | 74                              | 41          | Yes       |
|                              | MeOH           | 7.1                  | Collagen| 31                      | 81                              | 62          | *1        |
|                              | H₂O            | 14.3                 | ADP     | 61                      | 64                              | 5           | No        |
|                              |                |                      | Collagen| 11                      | 43                              | 74          | *2        |

*1: Aggregation curve and output % does not correlate, and the result is doubtful.
*2: Aggregation curve is very flat, this is suspicious.

with 0.1 mg/mL yielding 38% (ADP agonist) and 90% (collagen agonist) inhibition of sheep blood aggregation. Taking into account that the 0.1 mg/mL was also significantly easier to dissolve in the testing buffers, it was decided to use this concentration throughout the screening. This would still give positive results for potent aggregation inhibitors.

2.6. Flow Cytometry. The DMSO:EtOH samples from *Anomyrtis luma* and *Luma apiculata* (1 mg/mL) diluted in HEPES-tyrode’s buffer containing BSA pH 7.4 was used for flow cytometry experiments. 0.5% EtOH in HEPES-tyrode’s buffer with BSA was used as vehicle control. Citrated human blood was incubated with DMSO:EtOH/HEPES-tyrode’s samples (final concentration of 0.1 mg/mL) or vehicle control at 37°C in 30 minutes.

Samples were assayed within 15 minutes from venipuncture. Microcentrifuge tubes were prepared containing a mixture of either HEPES-Tyrode’s buffer, phycoerythrin (PE) conjugated anti-CD62P (Santa Cruz Biotechnology, Santa Cruz, Calif, USA), fluorescein isothiocyanate (FITC) conjugated PAC-1 (Becton Dickinson, San Jose, Calif, USA), or HEPES-Tyrode’s buffer, PE-Cy5-conjugated anti-CD42b (Becton Dickinson), fluorescein isothiocyanate (FITC) conjugated PAC-1 (Becton Dickinson, San Jose, Calif, USA) and eptifibatide. To both mixes platelet agonist was added for the detection of platelet surface P-selectin and activated GPIIb/IIIa. Pilot experiments using several different agonist concentrations were performed to identify agonist concentrations giving maximal and submaximal platelet activation. Final concentrations of agonists in the reaction mixture were 1 or 5 μM of thrombin receptor activating peptide (TRAP, Sigma-Aldrich, Brondby, Denmark), 0.5 or 20 μM of ADP (Bio/Data Co., Horsham, Pa, USA), or no agonist (HEPES-Tyrode’s buffer). All ADP and TRAP dilutions were made as batches and stored at −20°C along with vehicle control for the controls to minimize dilution variation. Antibody mixtures were prepared as batches and kept at 4°C. After incubation, P-selectin and activated GPIIb/IIIa samples were fixed by 1% formaldehyde in HEPES-saline. Samples were analyzed in an FACS Calibur (Becton Dickinson) flow cytometer. Platelets were identified by light scatter properties and expression of CD42b. All samples were tested in triplicates.

3. Results

3.1. Platelet Aggregation in Sheep Blood. After conducting the initial experiments, a total of 33 extracts, from 12
different plants were screened in ADP (5.0 μM) and collagen (5.0 μg/mL) induced aggregations in the aggregometer. Table 2 shows the average reading of duplicates and whether or not the plant extracts were able to inhibit ADP and collagen induced aggregation. All extract was compared towards the vehicle control and if the observed aggregation was 20% lower for extract test than for the vehicle is was concluded that the extract inhibited aggregation. Additionally, all extracts was tested with two different inducers, this further support the validity of the inhibition results.

Plant samples that in a convincing way were able to inhibit the aggregation in sheep blood were subsequently tested in a similar experiment with human blood. The below seven plants in the respective extracts were the ones chosen to be tested again.

The DCM : MeOH 1:1 extracts of *Anomyrtus luma*, *Blechnum chilense*, *Cestrum parqui*, *Lomatia ferruginea*, and *Pseudopanax laetevirens* were active as were the MeOH extracts of *A. luma*, *Anthoxanthum utriculatum*, *B. chilense*, *C. parqui*, *Luma apiculata*, and *P. laetevirens*, and the water extracts of *A. luma*, *C. parqui*, and *L. apiculata*. These samples were all chosen to be tested in human blood.

3.2. Platelet Aggregation in Human Blood. Seven of the species tested in sheep blood was tested in human blood. The activity is listed in Table 3. The four plants *A. luma* (DCM : MeOH 1:1 extract), *B. chilense* (MeOH extract), *C. parqui* (DCM : MeOH 1:1 extract), and *L. apiculata* (H2O extract) showed inhibitory effect in both sheep and human blood in both ADP- and collagen induced aggregations (see Tables 2 and 3). Furthermore, the H2O extract from *A. luma* showed inhibition in the collagen induced aggregation.

3.3. Flow Cytometry. In order to confirm the obtained results from the aggregation experiments the *A. luma* DCM : MeOH 1:1 extract and the *L. apiculata* H2O extract were tested for inhibition of platelet surface activation markers flow cytometry.

Table 4 shows the tested extracts and the percent inhibition of PAC1 MFI and CD62P (P-selectin) MFI by addition of ADP (0.5 μM and 20 μM) and the human specific inducer TRAP (1.0 μM and 5.0 μM). PAC1 and CD62P are both markers of platelet activation, and in order to be assigned an inhibitory effect the extracts should inhibit both activation markers using both agonists at all concentrations.

The extracts from *L. apiculata* and *A. luma* showed clearly inhibitory effect of both PAC1 MFI and CD62P MFI in the tested ADP concentrations as well as with the addition of 1.0 μM TRAP, whereas only a slight inhibitory effect is observed when 5.0 μM TRAP was added. TRAP was used in the flow cytometry assays since it is a human specific platelet inducer.

4. Discussion

The four plants *Anomyrtus luma*, *Blechnum chilense*, *Cestrum parqui*, and *Luma apiculata* showed inhibitory effect in both sheep and human blood in both ADP and collagen induced aggregations. Of these *L. apiculata* (H2O extract) and *A. luma* (DCM : MeOH 1:1 extract) was the most prominent candidates for further examinations. The two extracts were examined using platelet specific markers PAC1 and CD62P and the human-specific inducer TRAP and ADP in a flow cytometry assay. PAC1 and CD62P (P-selectin) does not bind to resting platelets but only to activated platelets [19]. These studies showed clear platelet inhibitory effect on platelet surface activation markers by the two markers as shown in Table 4. The effect observed in the flow cytometry confirms the results seen in the aggregometer.

The ethanol extract of the leaves of *A. luma* has been shown to contain 1-phenylpentan-3-one (4.6/8.5%) and 1-phenylhexan-3-one (3.5/12.3%) as well as β-caryophyllene oxide (10.7/6.6%) and linalool (59.3/11.3%) [20], of these the β-caryophyllene oxide has been shown to spontaneously aggregate blood platelets [21] at 100 μg/mL concentrations. This effect contradicts the observed effect of the extract, where aggregations was inhibited and suggest a strong inhibition of the organic extracts of *A. luma* since β-caryophyllene oxide would have been extracted with both DCM : MeOH 1:1 and to some extend also MeOH. The presence of β-caryophyllene oxide could be part of the explanation on why no inhibition was observed for the MeOH extract using ADP as an inducer. Further studies are needed to determine the active constituents in *A. luma*.

The MeOH extracts of *B. chilensis* have previously been shown to have antimicrobial effects [3]. *L. apiculata* have previously been shown to have xanthine oxidase inhibitory activity (30% inhibition at 50 μg/mL EtOH : H2O 7 : 3 extract) [22], and antiviral activity on herpes (IC50 = 100 μg/mL, EtOH extract) [23]. But none of these studies provides information to what could be active constituent, and no phytochemical data was found for these two species. COX inhibitory activity indirectly inhibiting P-selectin expression on human platelets [24]. It has been demonstrated that caffedyme from cocoa, have COX inhibitory activity, with 43% inhibition of COX-1 at 0.01 μM, and that caffedyme suppress P-selectin (CD62P) expression on platelets by 33% at a concentration of 0.05 μM [24]. The inhibition of COX enzymes may be a main contributing factor to suppressing P-selectin expression [24], which could be the effect observed with extracts of *L. apiculata* and *A. luma*. In order to confirm or invalidate this theory, COX inhibitory effect of *L. apiculata* and *A. luma* would have to be examined. Several plant extracts have already been tested for their COX activity [25] and this would need to evaluated along with determination of the active constituents.

It has previously been shown, that a MeOH : H2O 1:1 extract from *C. parqui* was able to inhibit aggregation of human blood platelets induced by ADP [26]. This confirms that some extracts from *C. parqui* are able to inhibit ADP induced platelet aggregations. However, the same was not observed in an AA-induced platelet aggregation, which implies that, the extracts anti-inflammatory activity did not implicate the inhibition of the cyclooxygenase pathway, that has been seen in other studies [27]. A suggestion is that the extract inhibited a site upstream of AA metabolism, since the case might be that ADP has triggered the release of AA in the
Table 3: Seven plant species that showed inhibitory effect in sheep blood were subsequently tested in human blood. The agonists are ADP (5.0 μM) and collagen (2.0 μg/mL) and the obtained aggregations are shown in percent. Extracts are tested in 0.1 mg/mL end concentrations. The aggregation is shown for both extract and vehicle control. If the extract aggregation is 20% lower than that of the vehicle control inhibition is observed.

| Plant Extract | Plant | Agonist | % Aggregation (extract) | % Aggregation (vehicle control) | % Inhibition | Inhibition |
|---------------|-------|---------|-------------------------|---------------------------------|--------------|------------|
| DCM : MeOH 1:1 | Amomyrtus luma (leaf) | ADP | 57 | 73 | 22 | Yes |
| | | Collagen | 62 | 87 | 29 | Yes |
| | MeOH | ADP | 66 | 70 | 6 | No |
| | | Collagen | 68 | 70 | 3 | No |
| | H₂O | ADP | 66 | 69 | 4 | No |
| | | Collagen | 60 | 76 | 21 | Yes |
| DCM : MeOH 1:1 | Anthoxanthum utriculatum (leaf) | ADP | 63 | 64 | 2 | No |
| | | Collagen | 63 | 68 | 7 | No |
| MeOH | DCM : MeOH 1:1 | ADP | 73 | 77 | 5 | No |
| | | Collagen | 74 | 73 | −1 | No |
| | | ADP | 60 | 83 | 28 | Yes |
| | | Collagen | 61 | 86 | 29 | Yes |
| DCM : MeOH 1:1 | Blechnum chilense (leaf) | ADP | 61 | 84 | 27 | Yes |
| | | Collagen | 67 | 84 | 20 | Yes |
| | | ADP | 70 | 70 | 0 | No |
| | | Collagen | 71 | 71 | 0 | No |
| | | ADP | 74 | 71 | −4 | No |
| | | Collagen | 64 | 68 | 6 | No |
| DCM : MeOH 1:1 | Cestrum parqui (leaf) | ADP | 72 | 72 | 0 | No |
| | | Collagen | 73 | 71 | −3 | No |
| MeOH | DCM : MeOH 1:1 | ADP | 70 | 67 | −4 | No |
| | | Collagen | 65 | 71 | 8 | No |
| | | ADP | 46 | 84 | 45 | Yes |
| | | Collagen | 54 | 85 | 36 | Yes |
| H₂O | Lomatia ferruginea (leaf) | ADP | 69 | 72 | 4 | No |
| | | Collagen | 66 | 72 | 8 | No |
| | | ADP | 69 | 72 | 4 | No |
| | | Collagen | 69 | 76 | 9 | No |
| DCM : MeOH 1:1 | Luma apiculata (leaf) | ADP | 34.4 | 20.1 | 38.9 | 28.1 |
| | | Collagen | 54.1 | 27.4 | 45.2 | 32.1 |
| MeOH | Pseudopanax laetevirens (leaf) | ADP | 30.1 | 17.8 | 12.4 | 7.6 |
| | | Collagen | 78.1 | 52.4 | 27.4 | 32.1 |

Table 4: Flow cytometry results from L. apiculata H₂O extract, and A. luma DCM : MeOH 1 : 1 extract. Inhibitions of the platelet activation markers, PAC1 and CD62P are shown in percent compared with vehicle control. The agonist is ADP in 0.5 μM and 20.0 μM, and TRAP in 1.0 μM and 5.0 μM. Extracts are tested in 0.1 mg/mL end concentrations.

| Plant extract | % Inhibition of PAC1 MFI | % inhibition of CD62P MFI |
|---------------|--------------------------|--------------------------|
| ADP 0.5 μM    | ADP 20.0 μM | TRAP 1.0 μM | TRAP 5.0 μM | ADP 0.5 μM | ADP 20.0 μM | TRAP 1.0 μM | TRAP 5.0 μM |
| Luma apiculata (H₂O extract) | 34.4 | 20.1 | 74.1 | 8.0 | 38.9 | 28.1 | 80.7 | 3.5 |
| Amomyrtus luma (DCM : MeOH 1 : 1 extract) | 30.1 | 17.8 | 78.1 | 12.4 | 37.7 | 27.4 | 83.2 | 6.4 |

pathway [26]. These data could explain the data observed and the two datasets suggest that C. parqui contains several active constituents. The plant itself have long been known to cause poisoning in cows, and it has been shown that the toxicity is in the organic phase that contained low molecular weight phenols [28], among these flavonoids that as mentioned have been shown to have antiplatelet activity. With more than 150 publication on C. parqui and its pharmacology and several toxic and pharmacologically active terpenoids isolated from the plant [29] further studies are not of high priority.
5. Conclusion

In the present work, four Chilean plant species were shown to inhibit platelet aggregating induced by ADP and collagen in both sheep and human blood. The four species were Blechnum chilense (MeOH extract), Luma apiculata (H2O extract), Amomyrtus luma (DCM:MeOH 1:1 extract) and Cestrum parqui (DCM:MeOH 1:1 extract). The platelet aggregating inhibitory effects of A. luma (DCM:MeOH 1:1 extract) and L. apiculata (H2O extract) were furthermore confirmed by inhibition of platelet surface activation markers.

At present, there is still a great need for preventative and therapeutic, anticoagulant medicine, and plants and their fruits seem to constitute possible alternatives to drugs currently used. It is of great interest to explore this inhibition further for the three species B. chilense, L. apiculata, A. luma.

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