

Haplopappus platylepis resin for pest control

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3 **“*Haplopappus platylepis* (Asteraceae) resin: an adhesive trap for pest control of**
4 **crawling arthropods, with antimicrobial potential”**

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24

26 **Abstract**

27 The use of plant secondary metabolites has been incorporated as key part of integrated
28 pest management and as an alternative to the use of pesticides. This may even be more
29 relevant regarding domiciliary pest insects, capable of vectoring pathogens to humans. In
30 these environments control its more difficult due to its possible effect on non-target
31 organisms and human health. Here we evaluated the use of the resinous exudate of
32 Chile's endemic bush *Haplopappus platylepis* (Asteraceae) as a sticky trap for crawling
33 pest insects. We used *Blatta orientalis* Linneus (oriental cockroach), a cosmopolitan
34 synanthropic pest, as test organism. We compared effectiveness on cockroach-trapping of
35 *H. platylepis*' resin versus a commercially available sticky trap, and analyzed these two
36 sticky substances using UHPLC-DAD-MS and GC-MS. We found that *H. platylepis*
37 resin was as effective as the commercial adhesive on trapping *B. orientalis*. Plant
38 resinous exudate was composed by a mixture of flavonoids, labdane diterpenoids and
39 unsaturated fatty acids oxylipins, which are known for their antimicrobial and antioxidant
40 properties. In contrast, the commercial sticky trap was rich in 1-bromohexadecane and 2-
41 chlorocyclohexanol, which have been described as allergens and as potentially toxic to
42 humans. Considering these findings, we suggest the use of the resinous extract of *H.*
43 *platylepis* as an effective adhesive trapping method against pest cockroaches and possibly
44 other crawling synanthropic arthropods cohabiting with humans. We highlight the
45 importance of novel, non-toxic and eco-friendly products as strategies to be applied in the
46 management of insect pests.

47 **Keywords:** synanthropic pest, integrated pest management, labdane terpenoids
48 antimicrobial properties.

49 **Introduction**

50 Synthetic insecticides are controversial as they may represent a potential risk for human
51 health and non-target organisms, beside its contribution to air and soil pollution [1–3].
52 Furthermore, controlling effects on pests can be rapidly ameliorated due to the evolution
53 of resistance on target organisms [4–6]. This is especially concerning in the case of
54 synanthropic arthropods related to vector-borne and zoonotic diseases inhabiting
55 household, food storage facilities and hospitals [7,8]. These pests are hard to control due
56 to their proximity to human-used spaces, restricting even more the use of various
57 chemical control methods [9,10].

58 This is the case of several crawling pest arthropods including arachnids such as ticks
59 [11,12], and insects belonging to: Hemiptera, like bedbugs [13] and triatomines [14] and
60 Blattodea: such as pest cockroaches [15,16]. Synanthropic cockroaches [17] such as
61 *Periplaneta americana* (Blattidae), *Blattella germanica* (Ectobiidae) and *Blatta orientalis*
62 (Blattidae) have evolved associated to human-modified environments and usually act as
63 vectors of allergens and diverse pathogenous microorganisms responsible for human
64 diseases [18–21]. Thus, these insects represent a serious threat for human health [22].

65
66 The use of insecticides for the control of these insects has been extremely
67 difficult, as cockroaches may become resistant to commonly-used chemical compounds
68 [6]. Moreover, many insecticides at sublethal doses, are repellent to cockroaches and they
69 are capable to avoid its contact [23]. In addition, some studies have shown that the use of
70 pesticide against cockroach infestation paradoxically increases the level of the cockroach
71 allergens Bla g 1 and Bla g 2, and possibly other allergens [24,25]. For example, adults of

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72 *B. germanica* exposed to sub-lethal doses of the pesticide boric acid increase the
73 production of the major allergen of Bla g 2 [25], which can lead to significant health
74 problems, including asthma, eczemas skin reactions and allergic rhinitis [26].
75 Furthermore, it has been demonstrated the evolution of antibiotic resistance in pathogenic
76 strains carried by *P. americana* and *B. germanica* collected from domiciliary and
77 intensive care hospital facilities [27–30].

78 Therefore, in order to avoid the development of resistances either in the animal or
79 their microbial counterparts, control strategies must combine the suppression of both
80 crawling arthropod vectors and its associated pathogens. This approach must also
81 consider current concerns on the safe use of pesticides for controlling difficult insect
82 pests, especially regarding inhabited and food storing places [31,32]. In this work we
83 studied the chemical composition of the resinous exudate of a Chilean endemic shrub
84 *Haplopappus platylepis* Phil. (Asteraceae), focusing with particular interest on the
85 presence of antimicrobial potential compounds. Coupled with this, we studied if adhesive
86 extracts of this secretion can be used for the control of pest crawling arthropods, testing
87 its adhesive function against the cosmopolitan pest cockroach *Blatta orientalis* Linnaeus,
88 1758 (Blattodea: Blattidae).

89

90 The use of plant-derived substances, capable of repelling and/or killing
91 synanthropic pests, has been shown in several studies as an effective alternative to
92 insecticides [33–35]. Among these, plant resins have demonstrated to be effective not
93 only against several arthropods [36], but also in the combat against pathogenic
94 microorganisms [37,38]. Moreover, the use of sticky traps could represent a more

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95 restrictible pesticide format in comparison with air-borne product, where spray drift
96 unwanted consequences on human health have been reported [39].

97 In addition, adhesive traps can be displayed in refuge areas where airborne products can
98 not easily reach [40], and reduce pest insects mechanically by catching them [41].

99 Moreover, these collected insects allow pest density monitoring [42]. This latter is a
100 guide during decision-making for the most appropriate control measurement [43].

101 Considering the above-mentioned information, adhesive plant secretions such as resinous
102 extractions may arise as suitable candidate for safe pest control of house pest and
103 zoonotic vector insect [44].

104 *Haplopappus platylepis*, also known as “Devil’s Lollipop”, produces an adhesive
105 resinous secretion covering its leaves and forming a natural sticky trap over floral buds
106 [45]. This plant belongs to an asteraceous lineage presenting copious resin production
107 with known antibacterial and antifungal properties, widely distributed in north and central
108 Chile [38,46–48]. Previously, under field conditions, we showed that *H. platylepis*’ sticky
109 exudate was capable of trapping several groups of insects that were fatally adhered
110 during its blooming season [45]. In this study, we evaluated the potential use of *H.*
111 *platylepis* inflorescence’s sticky exudate as an alternative adhesive trap for pest crawling
112 insects. For these propose we tested it, in laboratory bioassays, on a common global
113 household pest: the oriental cockroach *B. orientalis*. We compared its effectiveness on
114 adhering pest cockroaches in relation to a commercial adhesive trap (Eco-opción®). In
115 addition, we analyzed and compared the chemical composition of the sticky exudate of
116 *H. platylepis* and the commercial adhesive trap using UHPLC-DAD-MS (ultra-high-
117 performance liquid chromatography-diode array detector- mass spectrometry) and GC-

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118 MS (gas chromatography-mass spectrometry). Finally, we reviewed for bioactivity of
119 compounds detected in both natural and commercial adhesives, in order to assess both
120 their potential toxicity and harmful effects for humans, as well as any additional
121 biological properties, especially focusing against pathogenic microorganisms.

122

123 **Materials and methods**

124 **Plant material and trap extractions**

125 Plant specimens of *Haplopappus platylepis* Phil. (Asteraceae) were determined following
126 Klingenberg's monography for *Haplopappus* genus [49]. Floral buds of devil's lollypop
127 were collected during March 2016 at Los Molles, Provincia de Petorca, V Region de
128 Valparaíso, Chile (32°14'07.0"S71°31'24"W) and at Punta Hueso, Pichidanguí, Provincia
129 de Choapa, IV Region de Coquimbo, Chile (32°10'27"S 71°31'21"W). Samples were
130 preserved until analysis at -10° C. Voucher specimens (SGO 166498) were deposited in
131 the Herbarium of the "Museo Nacional de Historia Natural" (MCCN), Santiago, Chile.

132 The sticky exudate of *H. platylepis* was obtained by dipping fresh plant material (300
133 g) in cold CH₂Cl₂ (8 L) for 48 h, following Urzúa 2004's method[50]. The resulting
134 extract was filtered through a cotton layer and concentrated to a sticky residue (36 g,
135 12%) Commercial adhesive trap used was Eco-Opción® (Anasac Corporation, Santiago,
136 Chile), sticky trap offered for the control of cursorial domiciliary pest such as ants,
137 cockroaches and spiders. Each unit brings four 29.6x23.3 cm cardboard sticky traps with
138 a total adhesive surface of 11x13 cm. The adhesive mixture from the cardboard was
139 removed with a spatula and followed above-mentioned procedure for extraction. Extracts

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140 of both natural (*H. platylepis* inflorescence's resin) and commercial sticky traps were
141 kept under 4°C for further chemical analyses (see below).

142

143 Insects

144 Oriental cockroaches used in this work were obtained from a population maintained in
145 our laboratory since year 2014. Further specimens used for this study were collected
146 from locations in San Miguel, Santiago, Metropolitan Region, Chile (33°29'54"S
147 70°38'42"W). For taxonomic identification a general key for cosmopolitan and pest
148 cockroaches present in Chile was used [51]. Insects were kept in captivity under
149 laboratory conditions (20°-25°C and 40%-50% humidity) in 120x50x15 cm plastic
150 rearing boxes, fed with dog food (MasterDog Adult ®) and water *ad libitum*, at Instituto
151 de Entomología, UMCE. *Blatta orientalis* from both sexes were used for sticky-trapping
152 bioassays (with body lengths among 5 to 25 mm, measured dorsally from head to last
153 abdominal segments).

154

155 Trapping bioassays

156 Two treatments and one control were defined for the experiment. Treatments
157 corresponded to cardboard surfaces (40x13cm) painted either with *H. platylepis* resinous
158 exudate or with the commercial trap's adhesive. For control, a cardboard surface
159 (40x13cm) with no adhesive mixture added was used. Each of these options was
160 presented individually in the experimental arena. For this, the cardboard section was
161 placed in the center of the horizontal space inside the arena, fixing its position with
162 double-contact tape (Fig. 1). For each replicate 10 individuals from different sizes

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163 (measured as explained above) were placed in the experimental arena habituation area
164 (Fig. 1), a subdivision of the box from where insect were released without contact them
165 directly. For each trial we lifted the opening section of the habituation area and gave light
166 pulses (10s) during three instances of the experiment: 0, 180 and 360s. At each of these
167 pulses cockroaches tended to leave the habituation area and run to the other extreme of
168 the box crossing the cardboard section. Total time of each test was 6min. After this
169 period, for each treatment and control the number of individuals found attached to the
170 cardboard was counted. Trapped insects were ultimately sacrificed by applying cold
171 temperature (-10 °C). For each of these alternatives we repeated this test 10 times. Before
172 using the experimental arena for each trial, this was cleaned with ethanol (95%), distilled
173 water and dried in order to remove any chemical cue. The response variable was the
174 proportion of insects trapped in each trial for each treatment. As data did not meet the
175 criterion of normality distribution (Hammer, 1999), it was analyzed with a non-
176 parametric analysis of variance Kruskal-Wallis followed by *post hoc* Mann Whitney test.
177 In order to determine if *H. platylepis* inflorescence's resin and the commercial sticky trap
178 are equally efficient trapping cockroaches of different sizes (seven ranges: from 5 to 7; 8
179 to 10; 11 to 13; 14 to 16; 17 a 19; 20 to 22 and 23 to 25mm), insect proportion per range,
180 captured in both traps, was compared. This was analyzed by using a Chi square test for
181 two proportions [53]. All analyses were done with the PAST Paleontological Statistic,
182 version 3.15.

183

184 **Fig. 1. Bioassay setup A.** Experimental arena: a. Background pattern. b. Treatment Area,
185 c. Darkened walls, d: Hatch e: Habituation cubicle. f: Arena's door. **B.** Sticky trap made

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- 186 with *H. platylepis* resin (upper picture) and Eco-opción® adhesive (lower picture).
- 187 Trapped roaches are highlighted with arrows.

189 Chemicals

190 UHPLC-MS solvents, LC-MS formic acid and reagent grade chloroform were from
191 Merck (Santiago, Chile). Ultrapure water was obtained from a Millipore water
192 purification system (Milli-Q Merck Millipore, Chile). HPLC standards, (kaempferol,
193 quercetin, isorhamnetin, eriodictyol, luteolin, apigenin, naringenin, all standards with
194 purity higher than 95 % by HPLC) were purchased either from Sigma Aldrich (Saint
195 Louis, Mo, USA), ChromaDex (Santa Ana, CA, USA), or Extrasynthèse (Genay,
196 France).

197

198 UHPLC-DAD-MS analyses

199 Chemical resinous components were analyzed by using ultra-high-performance liquid
200 chromatography-diode array detector-tandem mass spectrometry (UHPLC-DAD-MS).
201 UHPLC-DAD-MS analysis was performed using a Thermo Scientific Dionex Ultimate
202 3000 UHPLC system hyphenated with a Thermo Q exactive focus machine as it was
203 reported by Simirgiotis et al. (2016). 5 mg of the resinous exudate were dissolved in 2
204 mL of methanol and filtered with a PTFE filter for a final injection of 10 μ L into the
205 instrument. Measurements were done as previously reported by Simirgiotis et al. (2016).
206 The generation of molecular formulas was performed using high resolution accurate mass
207 analysis (HRAM) and matching with the isotopic pattern. Lastly, analyses were
208 confirmed using MS/MS data and comparing the fragments found with the literature.

209

210 LC and MS parameters

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211 Liquid chromatography was performed using an UHPLC C18 column (Acclaim, 150 mm
212 × 4.6 mm ID, 2.5 μm, Thermo Fisher Scientific, Bremen, Germany) operated at 25 °C.
213 The detection wavelengths were 254, 280, 330 and 354 nm, and DAD was recorded from
214 200 to 800 nm for peak characterization. Mobile phases were 1 % formic aqueous
215 solution (A) and acetonitrile (B). The gradient program time (min, % B) was: (0.00, 5);
216 (5.00, 5); (10.00, 30); (15.00, 30); (20.00, 70); (25.00, 70); (35.00, 5) and 12 minutes for
217 column equilibration before each injection. The flow rate was 1.00 mL min⁻¹, and the
218 injection volume was 10 μL. Standards and the resin extract dissolved in methanol were
219 kept at 10°C during storage in the autosampler. The HESI II and Orbitrap spectrometer
220 parameters were optimized as previously reported [54].

221

222 GC-MS analyses

223 Chemical composition of the commercial adhesive trap was analyzed by gas
224 chromatography-mass spectrometry (GC-MS). GC-MS analysis was performed using a
225 Thermo Scientific Trace GC Ultra linked to an ISQ quadrupole mass spectrometric
226 detector with an integrated data system (Xcalibur 2.0, Thermo Fisher Scientific Inc.,
227 Waltham, MA, USA), equipped with a capillary column (Rtx-5 MS, film thickness 0.25
228 μm, 60 x 0.25 mm, Restek Corporation, Bellefonte, PA, USA) The operating conditions
229 were as follows: on-column injection; injector temperature, 250 °C; detector temperature,
230 280 °C; carrier gas, He at 1.25 mL/min; oven temperature program: 40 °C increase to 260
231 °C at 4 °C/min, and then 260 °C for 5 min. The mass spectra were obtained at an
232 ionization voltage of 70 eV. Recording conditions employed a scan time of 1.5 s and a
233 mass range of 40 to 400 amu. The identification of compounds in the chromatographic

234 profiles was achieved by comparison of their mass spectra with a library database
235 (NIST08, NIST, Gaithersburg, MD, USA) and by comparison of their calculated
236 retention indices with those reported in the literature [55] for the same type of column.

237

238 **Results**

239 **Trapping bioassays**

240 The proportion of insects found over the cardboards was statistically different among
241 treatments ($H(X^2) = 19.43$, $p < 0.001$, Kruskal-Wallis, Fig. 2A). *H. platylepis*
242 inflorescence's sticky exudate and the commercial sticky trap differed with statistical
243 significance from control clean cardboard (in both cases: U Mann-Whitney pairwise, $p <$
244 0.001). However, no differences were found in post hoc test for the total number of
245 insects attached on cardboards between the *H. platylepis*' resin and the commercial sticky
246 trap (U Mann-Whitney pairwise, $p = 0.691$). When the proportion of cockroaches trapped
247 by *H. platylepis*' sticky exudate and by the commercial sticky trap for each size range
248 was compared, no statistical differences were found between natural and commercial
249 sticky traps ($X^2 = 1.57$, $p = 0.211$) (Fig. 2B).

250

251 **Fig. 2. Cockroach adhesion results.** A. Mean and 1SE for the proportion of *B. orientalis*
252 found over the cardboard (Y axis) painted with: *H. platylepis* resin (green), Eco-opción®
253 commercial adhesive (red) and control (clean cardboard, black) obtained from 10
254 replicates each (X axis). Different letters correspond to statistical differences after post
255 hoc test at $p < 0,05$. B. Proportion of cockroaches trapped (Y axis) by either *H. platylepis*
256 resin (light grey) or Eco-opción® commercial adhesive (dark grey) for each insect size

257 range (X axis). No statistical differences were found for each pair compared.

258

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261

262 Chemical analyses

263 The data-dependent scan experiment was very useful for the identification of unknown
264 compounds since it provides high resolution and accurate mass product ion spectra from
265 precursor ions that are unknown beforehand within a single run. Combining data-
266 dependent scans and MSⁿ experiments, phytochemicals were tentatively identified in *H.*
267 *platylepis* including simple phenolic acids flavones, flavanones, fatty acids, and labdane
268 diterpenoids. UHPLC Q-orbitrap mass spectrometry analysis of *H. platylepis* sticky
269 exudate showed the presence of twenty seven metabolites in the chromatograms (Fig. 3)
270 including: 7 flavonoids (peaks **5, 6, 8-10, 15** and **16**), 3 phenolic acids (peaks **1-3**), 8 fatty
271 acids (Peaks **4, 7, 13, 14, 18, 21, 22** and **25**), and 9 labdane terpenoids (peaks **11, 12, 17,**
272 **19,20, 23, 24, 26,** and **27**). The detailed identification is explained below (Table 1, Figs. 4
273 and 1S).

274 **Fig. 3: UHPLC chromatograms** A. TIC (total ion current, negative mode) and B. UV at
275 280 nm, of *H. platylepis* resin.

276 **Fig. 4: Proposed biogenetic relationships between labdane diterpenoids.**

277

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278 **Table 1:** High resolution UHPLC PDA-Q-orbitrap identification of metabolites in

279 *Haplopappus platylepis* resin.

Peak #	Retention time (min)	UV max (nm)	Tentative identification	Elemental composition [M-H]	Theoretical mass (m/z)	Measured mass (m/z)	Accuracy (δppm)	MS ⁿ ions (δppm)
1	11.43	-	12-Hydroxyjasmonate	C ₁₂ H ₁₇ O ₄ ⁻	225.11276	225.11313	4.27	
2	12.93	-	Dihydroxyphaseic acid	C ₁₅ H ₂₁ O ₅ ⁻	281.13953	281.13945	-0.28	
3	13.71	325	Ferulic acid	C ₁₀ H ₉ O ₄ ⁻	193.05063	193.05040	-1.19	
4	18.76	285	Trihydroxyoctadecaenoic acid	C ₁₈ H ₃₃ O ₅ ⁻	329.23335	329.23367	0.97	
5	19.05	255, 354	7,3'-dimethoxyquercetin	C ₁₇ H ₁₃ O ₇ ⁻	329.06668	329.06702	1.03	
6	19.26	287	Hesperetin	C ₁₆ H ₁₃ O ₆ ⁻	301.07176	301.07199	0.76	160.84154, 135.04446
7	19.38	285	Trihydroxyoctadecadienoic acid	C ₁₈ H ₃₁ O ₅ ⁻	327.21770	327.21799	0.89	
8	19.56	287	5,3',5'-trihydroxy-3,7,4'-trimethoxyflavanone	C ₁₇ H ₁₅ O ₇ ⁻	331.08261	331.08233	1.22	
9	20.02	255-354	5,3'-dihydroxy-3,7,4'-trimethoxyflavone	C ₁₈ H ₁₅ O ₈ ⁻	343.08233	343.08267	1.25	313.03580 (C ₁₆ H ₉ O ₇ ⁻ , [M-OCH ₃ -CH ₃])
10	20.04	255-354	7, 3', 5'-trimethoxymyricetin	C ₁₈ H ₁₅ O ₈ ⁻	359.07724	359.07748	0.58	285.04031 (C ₁₅ H ₉ O ₆ ⁻ , kaempferol)
11	20.07	289	Dehydropinifolic acid	C ₂₀ H ₃₃ O ₄ ⁻	337.23843	337.23886	1.28	
12	20.10	289	Pinifolic acid (labd-8(20)-en-15,18-dioic acid)	C ₂₀ H ₃₁ O ₄ ⁻	335.22278	335.22287	0.98	
13	21.13	305	Trihydroxyheneicosahexaenoic acid	C ₂₁ H ₂₉ O ₅ ⁻	361.20205	361.20242	1.02	
14	21.36	303	dihydroxyeicosapentaenoic acid	C ₂₀ H ₂₉ O ₄ ⁻	333.20713	333.20740	0.90	273.18622 (C ₁₈ H ₂₅ O ₂ ⁻); [M ⁻ - (CO ₂ - CH ₃ - H)]
15	21.71	255-354	3,7-dimethoxyquercetin	C ₁₇ H ₁₃ O ₇ ⁻	329.06668	329.06705	1.12	
16	21.96	255-354	3,5, dihydroxy-3',4',7'-trimethoxyflavone	C ₁₈ H ₁₅ O ₈ ⁻	343.08233	343.08273	1.17	313.03467 (C ₁₆ H ₉ O ₇ ⁻ , (M ⁻ -OCH ₃ - CH ₃))

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17	22.12	-	(epi) Pinifolic acid	C ₂₀ H ₃₁ O ₄ ⁻	335.22278	335.22287	0.54	317.21219 (C ₂₀ H ₂₉ O ₃ ⁻ ; [M ⁻ - H ₂ O]; 273.18652 (C ₁₈ H ₂₅ O ₂ ⁻)
18	22.87	302	Tetrahydroxytetraecohexaenoic acid	C ₂₄ H ₃₅ O ₆ ⁻	419.24423	419.24391	3.37	319.22806
19	22.92	289	18-hydroxy-8(17)en-15-labdanoic acid	C ₂₀ H ₃₃ O ₃ ⁻	321.24377	321.24377	0.00	
20	23.94	289	Dehydropinifolic acid isomer	C ₂₀ H ₃₃ O ₄ ⁻	337.23843	337.23886	1.28	
21	24.25	308	Hydroxyeicosapentaenoic acid	C ₂₀ H ₂₉ O ₃ ⁻	317.21222	317.21255	1.04	
22	22.87	303	Hydroxyeicosatetraenoic acid	C ₂₀ H ₃₁ O ₃ ⁻	319.22787	319.22821	1.07	
23	25.40	289	13-en-Pinifolic acid methyl ester	C ₂₁ H ₃₁ O ₄ ⁻	347.22278	347.22311	1.04	273.18616 (C ₁₈ H ₂₅ O ₂ ⁻); 239.26134 (C ₁₆ H ₃₁ O ⁻)
24	25.78	289	Pinifolic acid methyl ester	C ₂₁ H ₃₃ O ₄ ⁻	349.23843	349.23880	1.06	
25	25.88	306	Trihydroxydocosaheptaenoic acid	C ₂₂ H ₃₁ O ₅ ⁻	375.21770	375.21823	1.41	
26	25.99	289	18-acetyl-13,8 (17)dien-15-labdanoic acid	C ₂₂ H ₃₃ O ₄ ⁻	361.23843	361.23877	0.94	
27	26.56	289	18-acetyl-8(17)en-15-labdanoic acid	C ₂₂ H ₃₅ O ₄ ⁻	363.25408	363.25443	1.13	321.24319 (C ₁₈ H ₂₅ O ₂ ⁻ ; M ⁻ - H ₂ O)

280

281 Flavonoids

282 Peak **15** with a [M-H]⁻ ion at *m/z* 329.06705 was identified 3,7-dimethoxyquercetin
 283 (C₁₇H₁₃O₇⁻) and peak **5** with an ion at *m/z* 329.06702 as its isomer: 7,3'-
 284 dimethoxyquercetin (Table 1). Peak **9** with a [M-H]⁻ ion at *m/z* 343.08276 was identified
 285 as the trimethoxylated flavonoid 5,3'-dihydroxy-3,7,4'-trimethoxyflavone (C₁₈H₁₅O₈⁻),
 286 while peak **10** with a [M-H]⁻ ion at *m/z* 359.07745 as 7,3',5'-trimethoxymyricetin
 287 (C₁₈H₁₅O₈⁻). Peak **16** with a pseudomolecular ion at *m/z* 343.08273 was identified as 3,5-
 288 dihydroxy-3',4',7-trimethoxyflavone (C₁₈H₁₅O₈⁻). The flavanone hesperetin, peak **6**, have

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289 been previously reported as main component in extracts of several *Nolana* species by
290 some of us (Simirgiotis, et al., 2015) and its HR-MS ($C_{16}H_{13}O_6^-$) and UV data matched
291 the one obtained in our chromatograms (m/z : 301.07176). Another flavanone, peak **8** with
292 a $[M-H]^-$ ion at m/z 331.08261 was identified as 5,3',5'-trihydroxy-3,7,4'-
293 trimethoxyflavanone ($C_{17}H_{15}O_7^-$).

294

295 Phenolic acids

296 The examination of the chromatograms revealed the presence of 3 phenolic acids:
297 dihydroxyphaseic acid (peak **2**, ion at m/z 281.13945, $C_{15}H_{21}O_5^-$) [56], ferulic acid (peak
298 **1**, m/z 193.05040) and 12-hydroxy jasmonate (peak **3**, m/z 225.11313) [57].

299

300 Fatty acids

301 Several peaks were tentatively identified as the dietary antioxidant polyhydroxylated
302 unsaturated fatty acids known as oxylipins [58,59], antioxidant fatty acids. Peak **4** with a
303 $[M-H]^-$ ion at m/z 329.23367 was identified as trihydroxy-octadecenoic acid ($C_{18}H_{33}O_5^-$),
304 and peak **7** as its diene derivative ($C_{18}H_{31}O_5^-$), as previously reported by some of us from
305 Keule fruits [59]. Peak **13** with a pseudomolecular ion at m/z 361.20242 was identified as
306 trihydroxyheneicosahexaenoic acid ($C_{21}H_{29}O_5^-$). Peak **14** with a $[M-H]^-$ ion at m/z
307 333.20743 was identified as a dihydroxyeicosapentaenoic acid ($C_{20}H_{29}O_4^-$) while peak **18**
308 with a $[M-H]^-$ ion at m/z 419.24391 was identified as dihydroxytetracosatrienoic acid
309 ($C_{24}H_{35}O_6^-$) [58]. Peak **21** and **22** were identified as hydroxyeicosapentaenoic acid and
310 hydroxyeicosatetraenoic acid ($C_{20}H_{29}O_3^-$) and ($C_{20}H_{31}O_3^-$), respectively. Finally, peak **25**

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311 with a [M-H]⁻ ion at m/z 375.21823 was identified as trihydroxydocosaheptaenoic acid
312 (C₂₂H₃₁O₅⁻).

313

314 Labdane terpenoids

315 Labdane terpenoids corresponded to derivatives of pinifolic acid (labd-8(20)-en-15,18-
316 dioic acid, peak **12**, C₂₀H₃₆O₃) [60] most of them reported for the first time in this
317 species. Thus, peak **11** with a [M-H]⁻ ion at m/z 337.23886 was identified as its
318 hydrogenated derivative of dehydropinifolic acid (C₂₀H₃₃O₄⁻) and peak **17** with a [M-H]⁻
319 ion at m/z 335.22296 as an isomer of pinifolic acid (C₂₀H₃₁O₄⁻), probably the epimer at C-
320 4 of the latter. Peak **24** was identified as pinifolic acid methyl ester (C₂₁H₃₃O₄⁻) and peak
321 **23** as its derivative 13-en-pinifolic acid methyl ester (C₂₁H₃₁O₄⁻). Peak **20** with a [M-H]⁻
322 ion at m/z 337,23886 was identified as pinifolic acid derivative (C₂₀H₃₃O₄⁻). Three
323 compounds were identified as labdanoic acid derivatives [61]. Thus, peak **19** with a [M-
324 H]⁻ ion at m/z 321.24377 was identified as 18-hydroxy-8(17)en-15-labdanoic acid
325 (C₂₀H₃₃O₃⁻), Peak **27** with a [M-H]⁻ ion at m/z 363.25449 was identified as 18-acetyl-
326 8(17)en-15-labdanoic acid (C₂₂H₃₅O₄⁻) and peak **26** as its diene derivative (C₂₂H₃₃O₄⁻)
327 (Fig. 4).

328

329 Components identified in the commercial sticky trap

330 GC-MS identified only two compound in the commercial sticky trap as: 1-
331 bromohexadecane and 2-chlorocyclohexanol.

332

333 **Discussion**

334 The aim of this study was to compare the effectiveness of a natural sticky trap against a
335 commercial one in capturing cockroaches by adhesion. In addition, the chemical
336 composition of both traps was analyzed in order to estimate potential harmful effects for
337 humans as well as potential antimicrobial chemical compounds. Our results provide
338 evidence that the natural sticky trap of *H. platylepis* was as effective as the commercial
339 one on trapping pest cockroaches. Considerable differences, however, were found in the
340 chemical composition between the natural and the commercial trap. Whereas the former
341 was rich in plant-derived antimicrobial compounds, the latter was rich in halogenated
342 compounds, whose potential toxic effects for humans have been previously reported.

343 The *H. platylepis* sticky exudate seems to offer multiple benefits in relation to its
344 use for controlling synanthropic pest crawling insect, such as cockroaches. First, because
345 of its stickiness, it resulted as effective as the commercial trap for capturing cursorial
346 insects, and second, due to its chemical composition rich in antibacterial compounds [62],
347 it shows a further potential for controlling pest arthropod-borne transmitted pathogens.
348 As far as we know, most of the compounds identified for *H. platylepis* resin are reported
349 for the first time in this species. Antibacterial properties of *H. platylepis* sticky exudate
350 can be associated with the phytochemical families detected in the mixture [62]. For
351 instance, flavonoids have shown a wide-spectrum of inhibitory activity against a variety
352 of human pathogens, including antibiotic-resistant Gram-positive and Gram-negative
353 bacteria, viruses and fungus [62–66]. Labdane diterpenoids are also well known as
354 antimicrobials [67,68]. It has been proved that the presence of a carboxylic acid in the C-
355 15 position, which acted as a hydrogen-bond donor (HBD), is essential for the
356 antibacterial activity of *ent*-labdanes [64]. Furthermore, derivatives of pinifolic acid,

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357 which were characterized in the *H. platylepis* sticky exudate, showed this main structural
358 characteristic of labdanes. In addition, pinifolic acid has been previously reported as an
359 effective compound in the treatment of leishmaniasis [69], a global insect-borne disease
360 related to trypanosomes [70]. Long-chain polyunsaturated fatty acids, which were also
361 abundant in *H. platylepis* resin, including oxylipins, have been widely tested for its
362 antimicrobial activity [71–75]. Therefore, further functions of chemical compounds
363 found in *H. platylepis*' resinous exudate expand the potential value of this plant-derived
364 adhesive to act as a control against various vectoring-disease scenarios.

365 Synanthropic crawling arthropods are usual carriers of several human pathogens
366 [76]. In the case of *B. orientalis*, it has been described to bear several human pathogenic
367 bacteria genera such as *Mycobacteria*, *Klebsiella*, *Staphylococcus*, *Escherichia* and
368 *Enterobacter* [77,78]. Therefore, the occurrence of compounds with anti-microbial
369 functions in the sticky exudate of *H. platylepis* may synergistically contribute as an
370 integrative pest control method, not only directly affecting the insect pests but also its
371 associated pathogenic microorganisms. The commercial sticky trap, in contrast, is poor in
372 its chemical composition and lacks antimicrobial compounds. 1-Bromohexadecane (**1**)
373 and 2-chlorocyclohexanol (**2**) were the only two compounds identified on the commercial
374 trap. Both are known as halogenated compounds. Based on Globally Harmonized System
375 of Classification and Labeling of Chemicals (GHS), both are characterized as irritant for
376 humans, due to the fact that these compounds induce skin corrosion (category 2),
377 respiratory tract irritation (category 3) as well as severe eye irritation (category 2A)
378 (European Chemical Agency- ECHA, 2017). This chemical profile suggests that this
379 commercial trap would not be innocuous for human health; nevertheless, it is

380 commercially offered as an eco-friendly option. Our results highly suggest that *H.*
381 *platylepis* sticky exudate may be a suitable alternative for controlling synanthropic
382 crawling insects, including cockroaches, at low cost and with additional benefits such as
383 potential antimicrobial properties. These virtues of *H. platylepis* sticky exudate trap fit
384 the current needs and trends in pest control, where several methodologies must be
385 integrated in order to generate novel alternatives in consideration of human and
386 environmental health [79]. Further research is needed in order to test this adhesive resin
387 in other formats for insect trapping as well as to evaluate its effectiveness against other
388 pest insects. For instance, resinous materials have been considered among the updated
389 alternatives for controlling domiciliary termites [44].

390

391 **Conclusions**

392 Results here demonstrated that devil's lollypop resin is a natural source of terpenoids and
393 flavonoids with potential applications as insecticide and antibacterial. Using UHPLC-
394 DAD-MS we have identified 27 secondary metabolites in *H. platylepis*' resin. Most of
395 which, as far as we know, are reported here for the first time. Many of these compounds
396 are flavones, flavanones, phenolic acids, fatty acids, and labdane terpenoids. This
397 chemical knowledge may be helpful for further research on *H. platylepis* and its
398 applications in biomedicine and pest and pathogens control industry. In conclusion, this
399 plant is a rich source of phenolic and clerodane compounds with insecticide and
400 antibacterial activity that may be used as an effective biocontrol agent against zoonotic
401 crawling insects and their associate microorganisms .

402

403 **Supporting Information**

404 **Fig. A.1:** Full HR-MS spectra and structures of compounds 3 (a), 9 (b), 10 (c), 12 (d), 14
405 (e), 22 (f), 23 (g), 26 (h) and 27 (i).

406

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417

418

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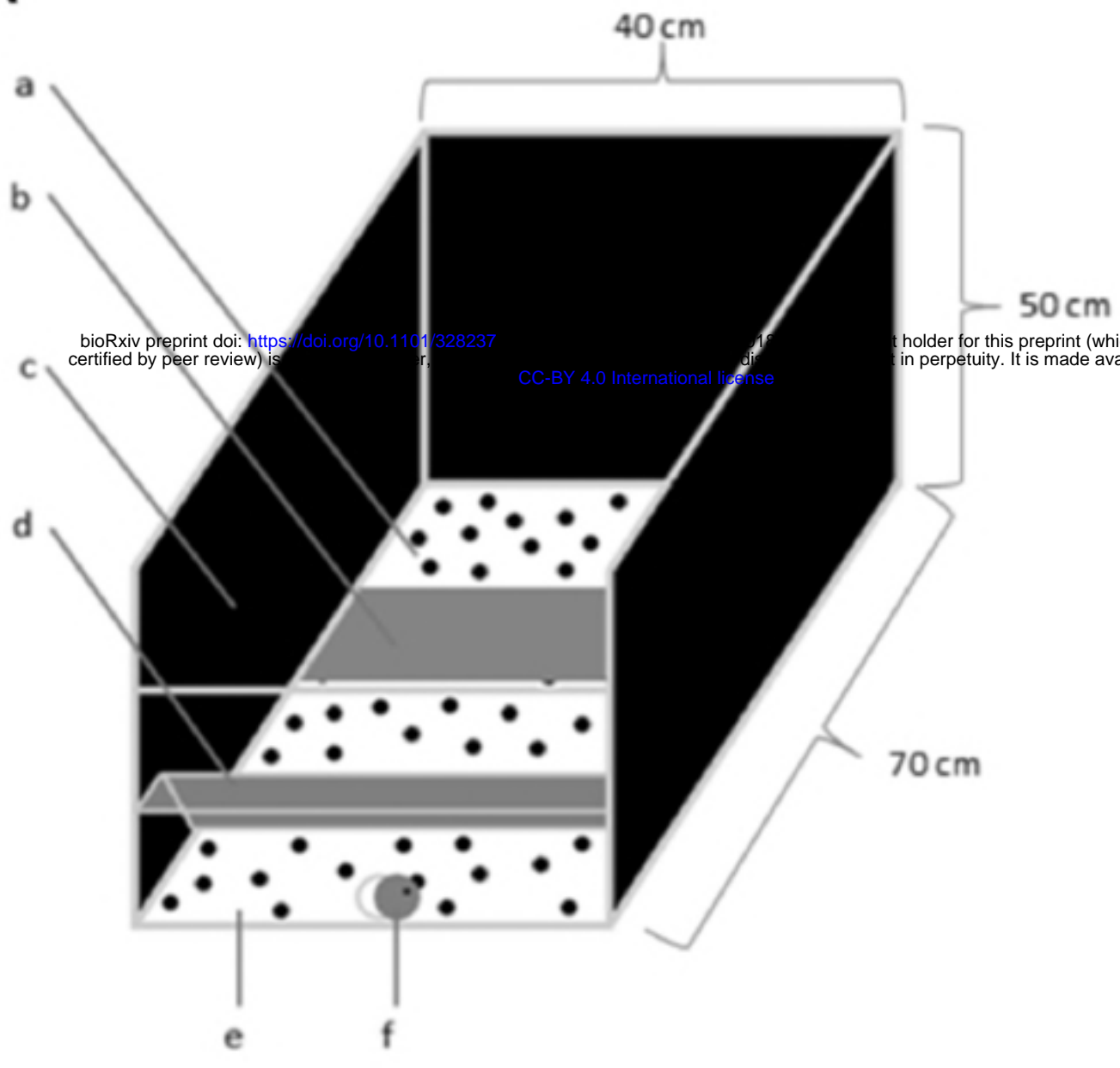
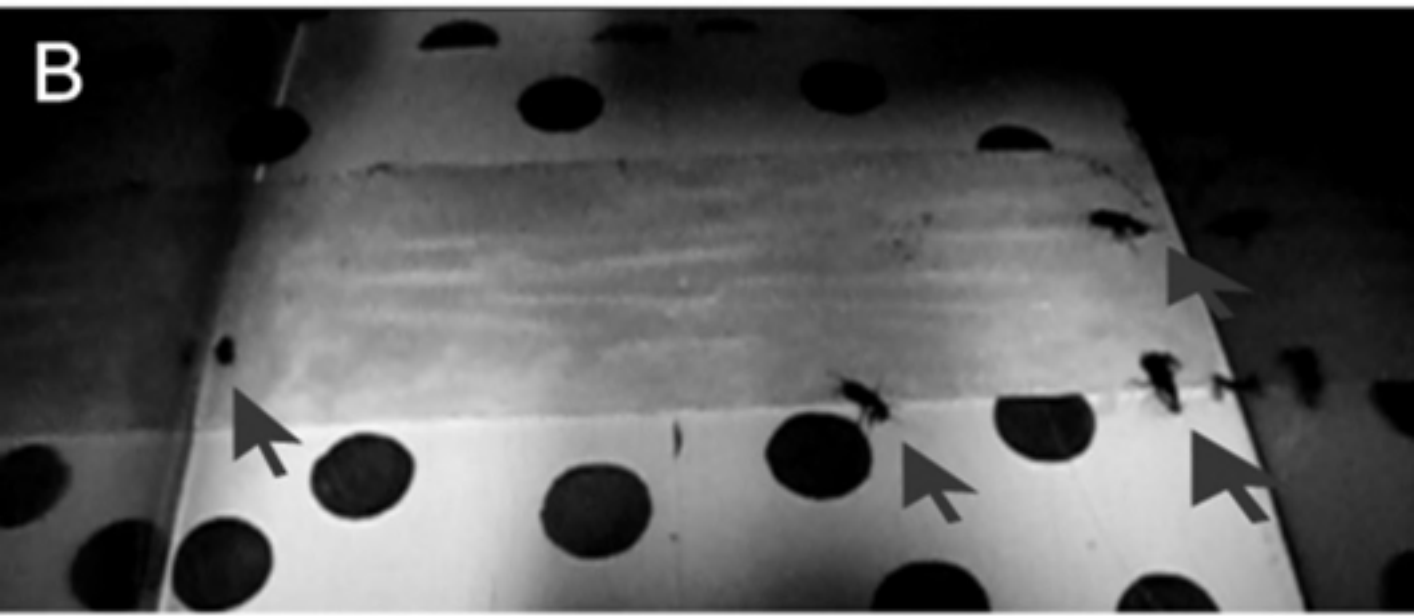
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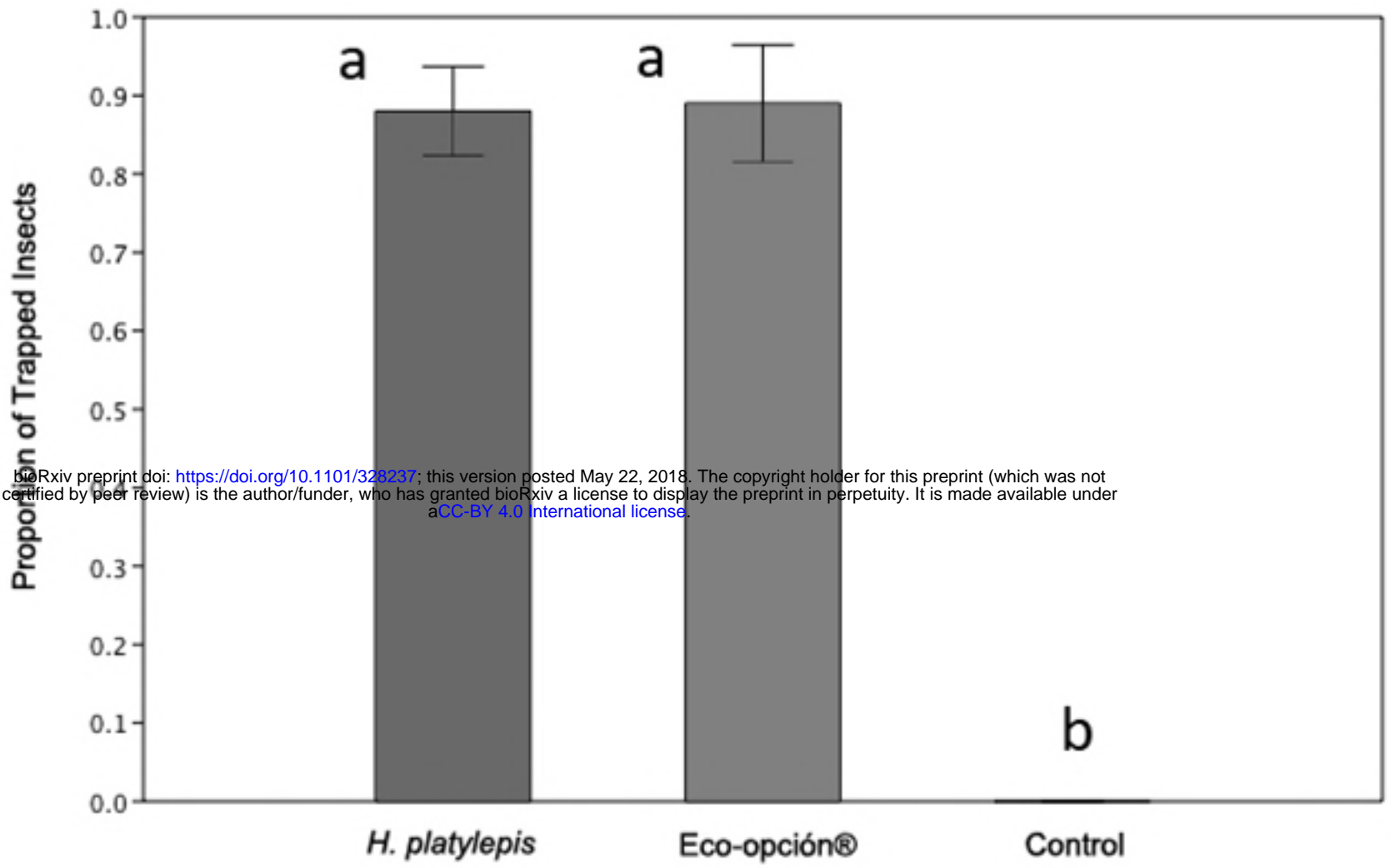
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