

1 **Anti-SARS-CoV-2 potential of *Cissampelos pareira* L. identified by Connectivity map-**
2 **based analysis and in vitro studies**

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31 *Abstract:*

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33 **Background:** Viral infections have a history of abrupt and severe eruptions through the
34 years in the form of pandemics. And yet, definitive therapies or preventive measures are
35 not present.

36 **Purpose:** Herbal medicines have been a source of various antiviral compounds. An
37 accelerated repurposing potential of antiviral herbs can provide usable drugs and identify
38 druggable targets. In this study, we dissect the anti-coronavirus activity of *Cissampelos*
39 *pareira* L (*Cipa*). using an integrative approach.

40 **Methods:** We analysed the signature similarities between predicted antiviral agents and
41 *Cipa* using the connectivity map (<https://clue.io/>). Next, we tested the anti-SARS-COV-2
42 activity of *Cipa in vitro*. A three-way comparative analysis of *Cipa* transcriptome, COVID-19
43 BALF transcriptome and CMAP signatures of small compounds was also performed.

44 **Results:** Several predicted antivirals showed a high positive connectivity score with *Cipa*
45 such as apcudin, emetine, homoharringtonine etc. We also observed 98% inhibition of
46 SARS-COV-2 replication in infected Vero cell cultures with the whole extract. Some of its
47 prominent pure constituents e.g pareirarine, cissamine, magnoflorine exhibited 40-80%
48 inhibition. Comparison of genes between BALF and *Cipa* showed an enrichment of
49 biological processes like transcription regulation and response to lipids, to be
50 downregulated in *Cipa* while being upregulated in COVID-19. CMAP also showed that
51 Triciribine, torin-1 and VU-0365114-2 had positive connectivity with BALF 1 and 2, and
52 negative connectivity with *Cipa*.

53

54 **Keywords:** *Cissampelos pareira* L., SARS-CoV-2, BALF, antiviral, whole plant extract,
55 Connectivity map

56

57 **Abbreviations:**

58 CMAP: Connectivity Map

59 BALF: Bronchoalveolar Lavage fluid

60 ESR1: Estrogen Receptor 1

61 ACE2: Angiotensin-converting enzyme 2

62 SARS: Severe Acute Respiratory Syndrome

63 MERS: Middle East respiratory syndrome

64 HDAC: Histone deacetylase

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67 *Introduction:*

68 SARS-CoV-2, the severe acquired respiratory syndrome agent coronavirus 2, has taken
69 many lives in the past year and is continuing to create an unsafe environment. Along with
70 numerous mutations, fast transmission and a wide range of symptoms, lack of a definite
71 therapeutic intervention has made this virus all the more deadly. Many studies have been
72 conducted in order to recognize small compound therapeutics effective against SARS-CoV-
73 2. Interestingly, some estrogen receptor modulators and protein synthesis inhibitors
74 having potential antiviral against SARS-CoV2 have also been identified (1). It has been
75 shown that *ESR1* as a drug target can modulate certain coronavirus associated genes. A
76 group recently demonstrated the downregulation of *ACE2* by estrogen (2).

77

78 *Cissampelos pareira* L. is a commonly used hormone modulator which is used to treat
79 reproductive disorders and fever. It has also been found to inhibit three serotypes of
80 dengue (3) and its effect on various hormones has also been evidenced (4). In a previous
81 study we have observed that *Cipa* can act as both, a protein synthesis inhibitor and an
82 estrogen receptor inhibitor (5). Many of the drugs positively connected with *Cipa* have
83 been reported to be a potential antiviral agent. Since there were several overlaps between
84 the therapeutics predicted to be effective against SARS-CoV-2 and our formulation, we
85 decided explore the repurposing potential of *Cipa* for this current pandemic.

86

87 *Material and Methods:*

88 **Transcriptome meta-analysis:**

89 We obtained the raw RNA sequencing data from SARS-CoV-2 patients Broncho-alveolar
90 lavage fluid (BALF) 1 and 2 from the recent publications and analyzed them inhouse
91 (supplementary information 1.1). The gene expression data for *Cissampelos pareira* L. was
92 taken from (5), which can be accessed at GSE156445.

93

94 **Functional enrichment and connectivity map of the differentially expressed genes.**

95 The differentially expressed genes were analyzed for functional enrichment using enrichr
96 (6) and for similar signatures using clue.io (7). The results were then compared with *Cipa*
97 to find intersections between gene ontologies, enriched gene sets, and connectivity map
98 perturbations between upregulated genes of BALF 1 and 2 and downregulated genes of
99 *Cipa* and between downregulated genes of BALF 1 and 2 and upregulated genes of *Cipa*.
100 Enrichments were also done for the genes whose knockdown signatures score >90
101 connectivity with *Cipa* and then compared with the upregulated processes and gene sets in
102 SARS-CoV-2.

103

104 **Collection of plant material and preparation of extract for *in vitro* inhibition assays:**

105 Whole plant of *Cissampelos pareira* L was collected from the Palampur, HP, India (alt. 1350
106 m). The identification of the plant material was done by a taxonomy expert in CSIR-IHBT,
107 Palampur and a voucher specimen (no. PLP16688) was deposited in the herbarium of
108 CSIR-IHBT, Palampur, HP-176,061, India. The plant has been obtained and isolated as per
109 CSIR, India guidelines (details for extract preparation in supplementary information 1.2).
110 The pure molecules from roots of the plant were isolated as reported recently (8).

111

112 **Cell culture, viral infection and drug treatment for inhibition of SARS-COV-2 by *Cipa*:**

113 The effect of PE50 and PER was tested against the SARS-CoV2 (ASTM, 2015) in a 96-well
114 tissue culture plates that was seeded with Vero Cells 24 h prior to infection with SARS-
115 CoV2 (Indian/a3i clade/2020 isolate) in BSL3 facility. After treatment, RNA was isolated
116 using MagMAX™ Viral/Pathogen Extraction Kit (Applied Biosystems, Thermofisher)
117 according to the manufacturer's instructions. The details of the experiment and RNA
118 isolation protocol are given in the supplementary information 1.3.

119

120 **TaqMan Real-time RT-PCR assay for Detection of SARS-CoV-2:**

121 The detection of genes specific to SARS-CoV2 was done using COVID-19 RT-qPCR Detection
122 Kit (Fosun 2019-nCoV qPCR, Shanghai Fosun Long March Medical Science Co. Ltd.)
123 according to the manufacturer's instructions (details in supplementary information 1.4).
124 The calculations for the relative viral RNA content and log reduced viral particles was
125 calculated using the linear regression equation obtained using the RNA extracted from the
126 known viral particles by RT-qPCR, using N, E and ORF1ab genes specific to SARS CoV2
127 virus. from the test sample (9).

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129

130 *Results:*

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132 ***Cissampelos pareira* L. shows high positive connectivity with small compounds 133 predicted to inhibit SARS-CoV-2.**

134

135 To assess which among the numerous small compounds predicted to have antiviral
136 potential against SARS-CoV2 in various studies might have similar signatures among the
137 same cell lines as *Cipa*, we queried the connectivity map. We observed 7 small compounds
138 to possess high signature similarity with *Cipa*. These include emetine (99.61), anisomycin
139 (99.58), cycloheximide (99.86), homoharringtonine (99.51), apcadin (86.32), ruxolitinib
140 (91.54), and sirolimus (95.45). These small compounds were predicted using different
141 methods in 4 different studies (Table 1).

142

143

144 ***Cipa* whole extract and single molecule constituents can inhibit SARS-COV-2 in vitro**

145
146 Since metanalysis highlighted an inhibitory potential of *Cipa* against SARS-COV-2, we tested
147 the effect of whole plant and root extracts of *Cipa* in Vero cell culture assays infected with
148 SARS-CoV-2. The relative viral RNA (%) was calculated by considering the values averaged
149 from N (Nucleoprotein), ORF1ab (19 non-structural proteins, NSP1-16), and E (Envelope)
150 viral genes. The whole plant aqueous extract showed a definite antiviral activity, evidenced
151 by decreased relative viral RNA content with a reduction by 57% at 100µg/ml where the
152 viral particle number reduced from 10^{5.9} to 10^{5.6} (Figure 1A-C).

153
154 Next, we wanted to see whether the *Cipa* pure constituents can inhibit SARS-COV-2. The
155 total alkaloid content in the extract was 46.4 mg/g with cissamine (18.6 mg/g) being the
156 major one, followed by magnoflorine (12.9 mg/g). The pure molecules namely hayatinin
157 (US-50), salutaridine (US-DR-CP-2), cissamine (US-CP-3), pareirarine (US-CP-5),
158 magnoflorine (US-CP-7), aqueous whole plant extract (PE), 50% hydroalcoholic whole
159 plant extract (PE50) and 50% hydroalcoholic root extract (PER) were tested against SARS-
160 CoV2 at 200 µM concentration showed relative viral RNA (%) to 44, 58, 45, 16, 63, 24, 2
161 and 2 respectively in comparison with the virus control (Figure 1D).

162
163 ***Cipa* transcriptome oppositely regulates several biological pathways compared to**
164 **COVID-19 infected patient BALF transcriptome**

165
166 We looked for the differentially expressed genes which were common between *Cipa* and
167 BALF samples from two studies (10, 11), hereby referred to as BALF-1 and BALF-2
168 respectively. We observed that 39 genes were common between *Cipa* and BALF-1 and
169 BALF-2, out of which 29 showed an opposite expression (Table S1). Individually, 134 genes
170 were common between *Cipa* and BALF-1, and 174 genes common between *Cipa* and BALF-
171 2. Upon functional enrichment analysis we observed that the genes enriched for regulation
172 of vascular endothelial growth were upregulated in both BALF-1 and 2 datasets while
173 being downregulated by *Cipa*. Similarly, while regulation of gene expression was
174 upregulated by *Cipa* it was downregulated in BALF-1 and BALF-2 data sets (Figure 2A-C).

175
176 The connectivity map analysis of the gene signatures of BALF-1 and BALF-2 comparison
177 with *Cipa* results revealed a number of small compounds that had high positive scores with
178 *Cipa* and negative scores with either BALF transcriptomes. We observed several small
179 compounds having opposite signature similarities between *Cipa* and BALF. Triciribine,
180 torin-1 and VU-0365114-2 were three compounds with negative scores for *Cipa* and
181 positive scores for both BALF 1 and 2 (Figure 2D). Also, knockdown signatures of a number
182 of genes were found to exhibit opposing scores between *Cipa* and BALF transcriptomes.
183 Some of these include, MICALL1, CRK, FKBP1A, CBX4, and FGR10P (Figure 2E).

184

185 *Discussion:*

186

187 SARS-COV-2 has shown a very diverse set of clinical presentations in various populations
188 and among genders within the same population. It has been shown that estrogen can
189 regulate the expression of ACE-2 receptors (2). Since *Cipa* appears to have *ESR1*
190 modulatory effects (5), and has been shown to have antiviral potential (3), we
191 hypothesized it may have inhibitory effect on the novel coronavirus. CMAP analysis of *Cipa*
192 transcriptome signatures highlights several small compounds having been predicted to
193 have inhibitory activity against SARS-CoV-2. Among these, emetine, homoharringtonine,
194 and cycloheximide are known translation inhibitors. These have also been shown to inhibit
195 Zika and Ebola (12), SARS and MERS (13), and Newcastle disease virus (14). Apcidin, an
196 HDAC inhibitor has been predicted to inhibit SARS-CoV-2 in a recent study (15).

197

198 *In vitro* experiments for viral inhibition of SARS-COV-2, reveal that all whole plant extracts
199 of *Cipa* (aqueous and alcoholic) could inhibit the virus at least up to 60%. Hydroalcoholic
200 whole plant extract showed an inhibition of 98%. The single molecule constituents of *Cipa*
201 could also inhibit the viral particles, with pareirarine showing the highest inhibition of
202 80%. This showed that *Cipa* does have the potential to inhibit SARS-CoV-2 virus *in vitro*.
203 Interestingly, the highest inhibition is shown by the whole plant hydroalcoholic extract
204 which comprises of various small compound constituents. This suggests a synergistic effect
205 of the constituents towards viral inhibition.

206

207 We also found that the signatures of the transcriptomic changes in *Cipa* treated MCF7 cells
208 and BALF from patients' lungs, have interesting overlaps. Among the connected small
209 compounds tricitiribine, torin-1 and VU-0365114-2, tricitiribine has been shown to inhibit
210 Human Immunodeficiency virus sera types 1 and 2 (16). Another study has shown that VU-
211 0365114-2, which is a muscarinic acetylcholine receptor M5 inhibitor, has repurposing
212 potential against SARS-CoV-2 (17). While mTOR inhibitor torin-1 may modulate immune
213 activity and enhance antiviral response, even against SARS-COV-2 (18).

214

215 *Conclusion*

216 In summary, we report here a framework applicable for repurposing of herbal
217 formulations using an integrated multi-pronged approach using transcriptome-based
218 connectivity mapping, *in vitro* validation and conjoint analysis with disease signatures. We
219 demonstrate the potential repurposing of *Cissampelos pareira* L for sars-cov-2 using this
220 approach.

221

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233 **Conflict of Interest Statement:**

234 The authors declare no conflict of statement

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239 repurposing. *Nature* (2020) <https://doi.org/10.1038/s41586-020-2286-9>.
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307 *Table and Figures:*

308 Table 1. Connectivity scores of small compounds with repurposing potential against SARS-
 309 CoV-2 as predicted by various studies.

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Compounds	Score	Reference
Emetine	99.61	(Dyall et al., 2014)(13)
Chloroquine	-45.51	
mefloquine	-3.93	
Amodiaquine	-13.11	
Gemcitabine	1.59	
Tamoxifen	-23.6	
Toremifene	-17.23	
Terconazole	-14.17	
Anisomycin	99.58	
cycloheximide	99.86	
Homoharringtonine	99.51	
Fluspirilene	-35.73	
Thiothixene	-1.66	
Fluphenazine	17.51	
Chlorpromazine	-77.85	
Triflupromazine	-0.35	
Clomipramine	-11.65	
Imatinib	-1.34	
Dasatinib	-75.92	
Vidarabine	-2.82	(Micholas and Jeremy C., 2020)(19)
eriodictyol	-13.82	
phenformin	1.23	
Apicidin	86.32	(Gordon et al., 2020)(1)
Haloperidol	-0.74	
Entacapone	6.41	
Metformin	4.37	
H-89	-82.31	
Ribavirin	7.17	
Midostaurin	48.43	
Ruxolitinib	91.54	
Daunorubicin	-73.76	
Captopril	13.92	
Chloramphenicol	-21.3	

Linezolid	17.29	(Y. Zhou et al., 2020)(20)
Irbesartan	1.09	
Equilin	22.26	
Mesalazine	7.09	
Mercaptopurine	6.02	
Paroxetine	-1.84	
Sirolimus	95.45	
Carvedilol	-13.58	
Dactinomycin	-39.25	
Eplerenone	10.74	
Oxymetholone	-2.34	

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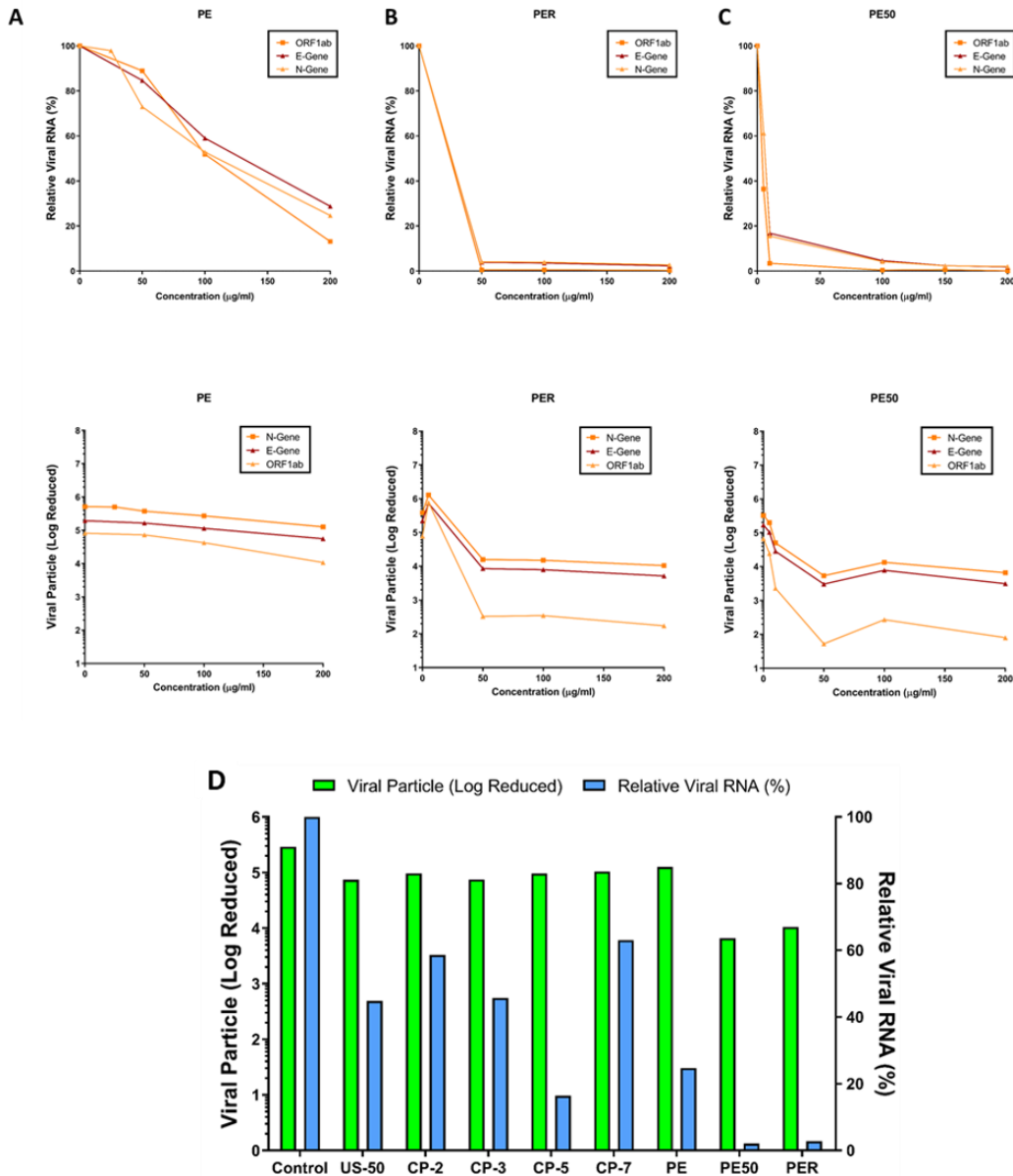
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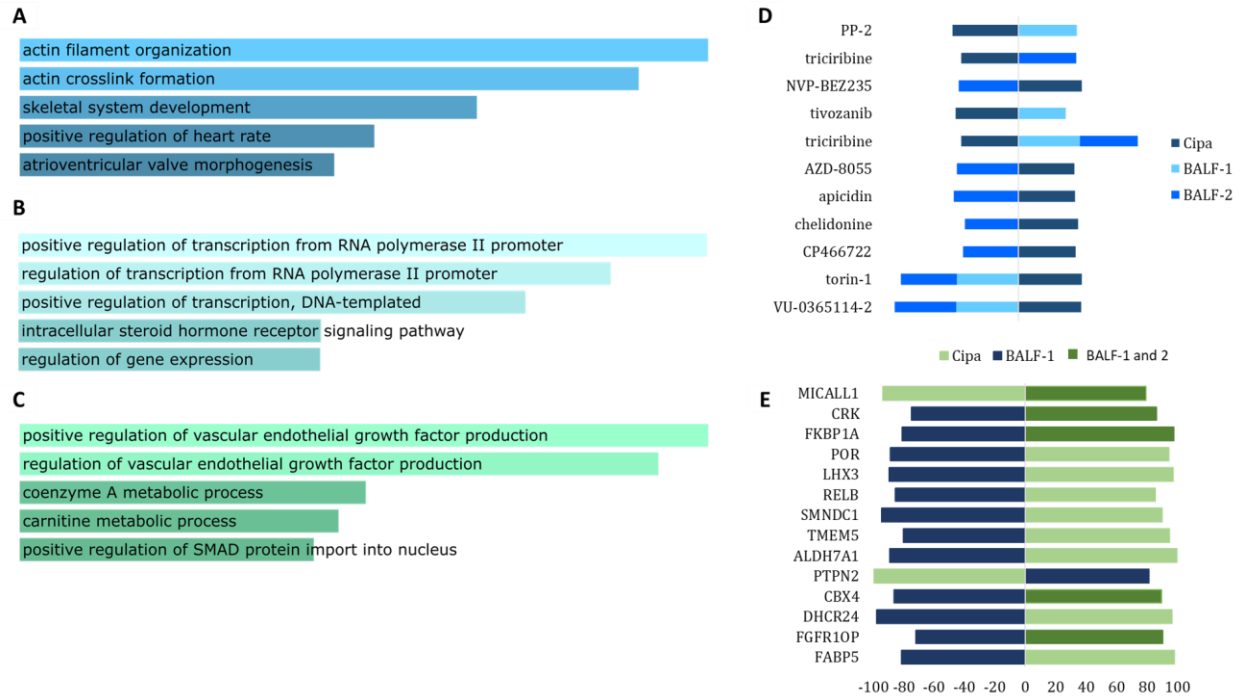


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335 Figure 1: Inhibition of SARS-CoV-2 in vitro by Cipa whole extract and its constituents:
 336 Relative viral RNA % and Log reduction in viral particles in vero cells upon treatment at 50,
 337 100, 150 and 200µg of A) whole plant aqueous extract (PE), B) root extract (PER) and C)
 338 hydro-alcoholic extracts (PE50) of Cipa. D) Sars-cov-2 viral titers inhibition by Cipa
 339 constituents CP-2 Salutaridine, CP-3 Cissamine, CP-5 pareirarine, CP-7 Magnoflorine, PE
 340 aqueous extract, PE50 50% hydroalcoholic extract and PER root extract.

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344 Figure 2: Comparative analysis of Cipa with BALF-1 and BALF-2 transcriptome: Functional
 345 enrichment of genes common between A) Cipa and BALF-1, B) Cipa and BALF-2 and C)
 346 Cipa, BALF-1 and BALF-2. D) Small compound signatures common between all three, the
 347 space on the left of the vertical axis indicates negative signatures while the one on the right
 348 indicates positive signatures. E) Genetic signatures common between all three, the space on
 349 the left of the vertical axis indicates negative signatures while the one on the right indicates
 350 positive signatures.

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