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Original Article

Volatile compounds from *Phyla scaberrima* (Juss. ex Pers.) Moldenke and *Dysphania ambrosioides* (L.) Mosyakin & Clemants as possible SARS-CoV-2 protease inhibitors: Identification and *in-silico* study

[Compuestos volátiles de *Phyla scaberrima* (Juss. ex Pers.) Moldenke y *Dysphania ambrosioides* (L.) Mosyakin & Clemants como posibles inhibidores de proteasas de SARS-CoV-2: Identificación y estudio *in-silico*]

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Abstract

Context: COVID-19 is a pandemic that has affected the entire population, characterized by multisystemic involvement. With around 130 million cases of infection and more than 2.5 million deaths globally. However, the development of a low-efficacy treatment has led to the study of natural products as possible therapeutic alternatives against SARS-CoV-2.

Aims: To identify volatile compounds present in two plants in the Colombian Pacific and carry out *in-silico* studies to search for promising inhibitory molecules against SARS-CoV-2 proteases.

Methods: This research carried out the identification of metabolites of two plants identified in the Colombian Pacific, called *P. scaberrima* (Juss. ex Pers.) Moldenke *y D. ambrosioide*s (L.) Mosyakin & Clemants. Ethanolic extracts were obtained by rotary-evaporation and determinated by GC-MS. Subsequently, in-silico studies were carried out by molecular docking against Mpro and PLpro using Autodock-vina 1.1. Also, a prediction of ADMET properties using SwissADME and GUSAR-Online server was performed.

Results: Thus, 15 volatile compounds with similarities greater than 85% were identified from both extracts, mostly sesquiterpenic and monoterpenic compounds. The compounds that showed the highest affinity against Mpro were α-amorphene and phytol for PLpro. Likewise, these were contrasted with co-crystallized molecules such as boceprevir and VIR2-251 as control structures. Finally, the predictions of ADMET properties showed values consistent with the literature.

Conclusions: Therefore, the follow-up of *in-silico* studies with these plants from Colombian pacific are considered as possible tools in the search for active molecules against proteases linked to virus.

Keywords: *Dysphania ambrosioides*; GC-MS/MS*;* molecular docking; *Phyla scaberrima*; SARS-CoV-2.

Resumen

Contexto: COVID-19 es una pandemia que ha afectado a toda la población mundial, caracterizada por compromisos multisistémicos. Con alrededor de 130 millones de casos de infección y más de 2,5 millones de muertes en todo el mundo. Sin embargo, el desarrollo de un tratamiento de baja eficacia ha llevado al estudio de productos naturales como posibles alternativas terapéuticas frente al SARS-CoV-2.

Objetivos: Identificar compuestos volátiles presentes en dos plantas del Pacifico colombiano y realizar estudios *in-silico* para la búsqueda de promisorias moléculas inhibidoras contra proteasas de SARS-CoV-2.

Métodos: En esta investigación se realizó la identificación de metabolitos de dos plantas identificadas en el Pacífico colombiano, llamadas *P. scaberrima* (Juss. ex Pers.) Moldenke *y D. ambrosioides* (L.) Mosyakin & Clemants Se obtuvieron extractos etanólicos, preconcentrados con evaporación rotatoria y se determinaron por GC-MS. Posteriormente, se realizaron estudios *in-silico* mediante acoplamiento molecular contra Mpro y PLpro utilizando Autodock-vina. Además, prediciendo las propiedades de ADMET mediante SwissADME y GUSAR-Online.

Resultados: Se identificaron 15 compuestos volátiles con similitudes superiores al 85% de ambos extractos, en su mayoría compuestos sesquiterpénicos y monoterpénicos. Los compuestos que mostraron la mayor afinidad contra Mpro fue α-amorfeno y fitol para PLpro. Asimismo, se contrastaron con moléculas co-cristalizadas como boceprevir y VIR2-251 como estructuras control. Finalmente, en las predicciones de propiedades ADMET mostraron valores consistentes con la literatura.

Conclusiones: Se consideró el seguimiento de estudios *in-silico* con estas plantas del Pacífico colombiano como posibles herramientas en la búsqueda de moléculas activas frente a proteasas ligadas al virus.

Palabras Clave: acoplamiento molecular*; Dysphania ambrosioides*; GC-MS/MS; *Phyla scaberrima;* SARS-CoV-2.

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INTRODUCTION

The coronavirus (COVID-19) pandemic has had catastrophic human health implications worldwide. This disease, which originated in Wuhan (China), is characterized by pneumonia associated with acute severe respiratory syndrome, fever, cough and myalgia, being an extremely contagious and potentially fatal disease that can cause mild to severe respiratory tract infections (Esakandari et al., 2020). According to the World Health Organization (WHO) data, the COVID-19 pandemic is a global public health emergency with approximately 2.5% mortality percentage. On 01 April of 2021, there were a total of 128 540 982 cases and 2 808 308 deaths confirmed (WHO, 2020).

Advances in disease management have been insufficient, with patients recieving treatments according to their symptoms and diagnosis. Although there are several vaccines from different pharmaceutical companies, it is difficult to reach all inhabitants effectively; in addition, infections worldwide are increasing. The absence of a specific treatment has generated the use of medicinal plants with antiviral properties as an alternative for the treatment of COVID-19 in many regions of the world, including the department of Chocó (Colombia). In fact, in many regions of the world, most researchers are relying on the ethnopharmacology of known medicinal plants to treat COVID-19 (Rajabian and Hosseinzadeh, 2020). The global trend is focused on antivirals derived from plants due to their low toxicity and less possibility of developing resistance (Ghildiyal et al., 2020). *Phyla scaberrima* (Juss. ex Pers.) Moldenke (family *Verbenaceae*) and *Dysphania ambrosioides* (L.) Mosyakin & Clemants (family *Amaranthaceae*) are native in Colombian Pacific, with important antiviral ethnopharmacological properties. The aqueous extracts of these plants are used as infusions, shots or baths to treat the symptoms of COVID-19 by the inhabitants of the department of Chocó. In fact, many compounds extracted from plants have known antiviral activity. Volatile compounds such as monoterpenes, sesquiterpenes and diterpenes are among the secondary metabolites having this activity (Adorjan and Buchbauer, 2010; Da Silva et al., 2014; Vimalanathan and Hudson, 2014; Shakeri et al., 2017).

Some of those investigations were based on molecular docking studies to determine the activity of volatile compounds against COVID-19 (Da Silva et al., 2020; Sharma and Kaur, 2020; Panikar et al., 2021). Molecular docking is essential for discovering new drugs and is a computational technique used to determine the molecular affinity between two molecules, such as ligand-protein and protein-protein (Alrasheid et al., 2021). In the search for promising molecules as an alternative treatment, it is necessary to use a molecular screening through ligand-receptor docking.

Thus, the objective of this study was to identify the volatile compounds by gas chromatography coupled to mass spectrometry (GC/MS) in the medicinal plants *P. dulcis* and *C. ambrosioides*; then it was performed a screening of the identified compounds for molecular docking studies and interactions of these compounds, in order to determine the most promising molecules for the treatment of COVID-19 by inhibiting the main protease (Mpro) and papain-like protease (PLpro), and comparing the results with proposed drugs such as boceprevir and peptide inhibitor VIR251. Finally, a prediction of pharmacokinetic properties and toxicity of metabolites was done.

MATERIAL AND METHODS

Identification of plant material

The plant material (leaves, aerial parts and whole plant in some cases) was pressed and transferred to the herbarium of the University Technological of Chocó Diego Luis Córdoba for taxonomic identification, presenting registration number for *Phyla scaberrima* (Juss. ex Pers.) Moldenke (15123) and *Dysphania ambrosioides* (L.) Mosyakin & Clemants (15151).

Preparation of the extract

The plants were obtained in the marketplace of the city of Quibdó (5°41´12"N, 76°, 49´74"W), and then brought to the laboratory of the research group in Biosystematics (Technological University of Chocó). Later, the plants were washed and disinfected with a 2% NaClO solution. They were air-dried at room temperature for seven days. Then they were submitted to a temperature of 40°C, in an oven with circulating air (Memmert 854 Schwabach, Germany), always verifying humidity loss (three days approximately). The cortex was ground to a fine powder and then weighed.

The crushed and weighed plant material were then subjected to cold maceration for three days in 96% ethanol (Merck) using a ratio of 1:4 plant material:solvent for 72 h. Successive concentrations of this extract were made at reduced pressure in a digital rotaevaporator (model R-124, vacuum controller V-800, Buchi) at 40°C, in order to obtain an ethanolic extract, which was stored at 4°C until the phytochemical analysis was performed.

Analysis by gas chromatography coupled to mass spectrometer (GC-MS)

Plant material (100 g) was sent to the Laboratory of Instrumental Analysis of the National University of Colombia (Medellín Headquarters, Medellín, Colombia). The extraction of the volatile compounds of the vapor phase of the sample was performed through the technique of solid-phase microextraction (SPME), with monitoring in the vapor phase (headspace), using a fused silica fiber coated with polydimethylsiloxane-divinyl benzene of 65 µm thickness (PDMSDVB-65 µm, Sigma) (Ortiz-Rojas and Chaves-Bedoya, 2017).

The chromatographic analysis was performed in a 6890 Series Plus gas chromatograph, coupled to 5973N MSD with turbo pump (Agilent Technologies, USA). The column used for the analysis was DB-5MS [5%-phenyl-poly (methylsilane), 30 m×0.32 mm×0.5 µm, Agilent]; injection was performed with the SPME device. The chromatographic condition employed helium as the carrier gas in mode Splitless, with an injection volume of 1 µL, injector and detector temperature of 125°C. Finally, the furnace sequence was established in three steps: 1) Initial temperature of 70°C, maintained during 15 min; 2) Increase of temperature by 9.0°C/min until 200°C; 3) Increase of temperature by 5.0°C/min until 300°C, maintained during 15 min. Total time of 50 min. Identification of secondary metabolites was established based on their mass spectra (EI, 70 eV). The databases used were NIST98.l, NIST02.L and NIST5a.L from the laboratory mentioned above.

Preparation of ligands and receptors

A total of 15 molecules were selected (10 of *P. scaberrima* and 5 of *D. ambrosioides*). Subsequently, PubChem database (https://pubchem.ncbi.nlm.nih.gov/) was used to download the structural ligands, which were obtained in sdf format. Then, UCSF Chimera version 1.13 software (Pettersen et al., 2004) and Open Babel tools (O'Boyle et al., 2011) were used for structural correction, geometric optimization, hydrogen addition, charge arrangement and ionizable groups. Therefore, ligands were energetically minimized using the force-field mmff94; using conjugated gradients in 200 steps. On the other hand, the representative protein structures of the main protease (Mpro) were obtained from the Protein Data Bank database (https://www.rcsb.org/), identified with access code: 6WNP (co-crystalized with Boceprevir, 1.44 Å) and papain-like protease (PLpro), was registered with access code: 6WX4 (co-crystalized with peptide inhibitor VIR251, 1.66 Å). Similarly, proteins in PDB format were prepared by adding hydrogen atoms, eliminating the solvent (water), and removal of ligands using UCSF Chimera version 1.13 software packages (Pettersen et al., 2004).

Molecular docking

Molecular docking was performed through AutoDock-Vina using PyRx 0.8 software graphical interface (Dallakyan and Olson, 2015). A virtual screening was implemented to establish the molecules against MPro and PLpro identified in SARS-CoV-2. Proteins and ligands interacted in a center grid space of x = 7.566 Å, y = 24.955 Å, z = 23.740 Å with box size of x = 13.755 Å, y = 16.681 y z = 12.066 for Mpro and a center grid space of x = 9.617 Å, y = -25.936 Å, z = -38.929 Å with box size of x = 18.433 Å, y = 23.980 y z = 21.159 for PLpro. Equally, employing an exhaustiveness of 8. Then, it was simulated obtaining conformations classified according to affinity energy value and RMSD. The best conformation structures were obtained and converted to PDB format using PyMol software. The 2017 version of the BIOVIA Discovery Studio visualizer was used in the identification of interaction force and residues.

ADME properties and acute oral toxicity

ADME and drug-likeness prediction for metabolites identified in *P. scaberrima* and *D. ambrosioides* were developed by the SwissADME online server from the Swiss Institute of Bioinformatics (https://www.sib.swiss) (Daina et al., 2017). In order to do this, the canonical SMILES chain was entered into the server, obtaining ADME parameters such as inhibitory cytochrome P450 (CYP450) family, blood-brain barrier permeability (BBB), and binding to P-glycoproteins. On the other hand, pharmacological similarity prediction parameters were indicated according to the rule of Lipinski and bioavailability. Likewise, the metabolites were evaluated for acute oral toxicity using the GUSAR-Online server (Lagunin et al., 2011); inserting the canonical SMILE of molecules, followed by predictions of lethal dose 50 (LD_{50}) values for rats, and finally, classified in rodents based on the OECD Project (OECD, 2002).

RESULTS AND DISCUSSION

Traditionally *P. scaberrima* and *D. ambrosioides* have medicinal properties used to treat expectoration caused by cough, bronchitis and other respiratory complications (Compadre et al., 1986; Moreno-Murillo et al., 2010; Carvalho e Silva et al., 2016; Al-Badani et al., 2017). Some studies demarcate that *P. dulcis* demonstrates anti-inflammatory activity at doses of 400 mg/kg, with significant inhibition of carrageenan-induced edema and reduction of granuloma (Pé-

rez et al., 2005). On the other hand, some authors have suggested that the essential oils of *C. ambrosioides* have shown antiviral activity against the CV-B4 virus with an IC_{50} equal to 21.75 µg/mL and notable activity against *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Candida albicans* (Mokni et al., 2019).

The raw data with the retention time and the maximum intensity were acquired by GC-MS and were contrasted using the databases NIST02.L and NIST5a.L (Table 1; Figs. 1-4). A total of 15 compounds were identified among the volatile compounds of *P. scaberrima* and *D. ambrosioides* with a similarity score greater than 85%. Alike, 10 compounds for *P. scaberrima* and 6 molecules for *D. ambrosioides* were identified (Table 2). The majority of metabolites identified in *P. scaberrima* were classified as sesquiterpenes and those in *D. ambrosioides* were monoterpenoid compounds. Additionally, in the extracts from the two plants, phytol was determined and classified as acyclic diterpenes.

Furthermore, within the identified metabolites, it has been shown that carvacrol and β-caryophyllene have viral inhibitory activity, specifically against HSV-1, with IC_{50} values of 48.5 and 0.25 µg/mL, respectively (Astani et al., 2011; Da Silva et al., 2020). Likewise, it has been indicated that compounds such as thymol and carvacrol were shown to be active against strains of HSV-1, influenza type A (H1N1) virus, and SARS-CoV-2 strains isolated from patients (Asif et al., 2020; Seadawy et al., 2021). Additionally, there are some computational research focused on exploring the components of essential oils from these plants as elements for possible and notable COVID-19 treatment and possible clinical manifestations; thus, Da Silva et al. (2020) evaluated 171 molecules from essential oils with antiviral activity, with affinity energies between -71 and 115 KJ/mol, showing high affinity for compounds such as farnesene and derivatives. Similarly, compounds identified as copaene, δ-cadinene, carvacrol, (E)-caryophyllene, (+)-3-carene, phytol, and thymol also showed significant affinity against Mpro and other proteins associated with SARS-CoV-2.

The 15 molecules that were previously identified from the extracts of both plants, were evaluated through molecular docking against Mpro and PLpro (Figs. 5 and 6). Table 3 shows the highest affinity energies of these metabolites, which were recorded at values between -5.6, -5.4 and -5.3 Kcal/mol for δ-amorphene, β-cubebene, α-amorphene bound to Mpro, respectively. Additionally, Fig. 7A-D shows the molecular docking analyses that indicate the interaction of key residues of the MPro active site such as H41, M49 and M165, which were identified in the molecules including the inhibitory peptide. Differentially, it shows notable H-bond bonds with residues such as N142, G143, C145 and E166.

Table 1. Identification of metabolites by GC-MS.

PK	RT	Library/ID	CAS	Similarity
P. scaberrima				
1	21.44	Copaene	003856-25-5	99
2	21.57	1H-Pyrrole, 1-butyl-	000589-33-3	35
3	22.19	Caryophyllene	000087-44-5	99
4	22.59	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-, (Z,E)-	026560-14-5	64
5	22.65	(E,E)-7,11,15-Trimethyl-3-methylene-hexadeca-1,6,10,14-tetraene	070901-63-2	47
6	22.78	1,5-Dimethyl-1-vinyl-4-hexenyl butyrate	000078-36-4	35
7	22.92	1,6,10-Dodecatriene, 7,11-dimethyl-3-methylene-, (E)-	018794-84-8	91
8	23.22	1H-Cyclopenta[1,3]cyclopropa[1,2]benzene, octahydro-7-methyl-3-methylene-4-(1-methylethyl)-, [3aS-(3a.alpha.,3b.beta.,4.beta.,7.alpha.,7aS*)]-	013744-15-5	95
9	23.47	1,5-Heptadiene, 2,5-dimethyl-3-methylene-	074663-83-5	86
10	23.56	Naphthalene, 1,2,4a,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1.alpha.,4a.alpha.,8a.alpha.)-	031983-22-9	99
11	23.73	Cyclohexene, 1-methyl-4-(5-methyl-1-methylene-4-hexenyl)-, (S)-	000495-61-4	95
12	23.89	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	000483-76-1	96
13	24.00	Anthralic acid, 6-fluoro-	000434-76-4	9

Table 1. Identification of metabolites by GC-MS (continued…)

PK	RT	Library/ID	CAS	Similarity
14	24.20	1,3,7-Octatriene, 3,7-dimethyl-	000502-99-	55
15	24.43	1,6,10-Dodecatrien-3-ol, 3,7,11-trimethyl-, [S-(Z)]-	000142-50-7	74
16	24.54	Ledene oxide-(II)	1000159-36-7	14
17	24.62	(2S,4R)-p-Mentha-[1(7),8]-diene 2-hydroperoxide	1000292-74-4	47
18	25.08	Cyclopentane, 1-ethyl-1-methyl-	016747-50-5	37
19	25.17	2,6-Octadienal, 2,6-dimethyl-8-(tetrahydro-2H-2-pyranyloxy)	1000196-64-1	72
20	25.23	1,5,9,11-Tridecatetraene, 12-methyl-, (E,E)-	062338-27-6	22
21	25.67	2(5H)-Furanone, 5-(2-methyl-2-propenyl)-	1000155-86-2	22
22	25.75	1,1-Dodecanediol, diacetate	056438-07-4	12
23	25.99	alpha.-Bisabolol	072691-24-8	91
24	26.27	Bicyclo[2.2.0]hexane-1-carboxaldehyde	201793-40-0	14
25	26.63	Histidine, 2-carboxy-	074419-66-2	53
26	26.97	4-Hydroxyhistamine	1000128-67-9	53
27	27.04	Histidine, 2-carboxy-	074419-66-2	40
28	27.40	2-Cyclohexen-1-one, 2-methyl-	001121-18-2	53
29	27.52	2-Cyclohexen-1-one, 2-methyl-	001121-18-2	47
30	27.61	2-Cyclohexen-1-one, 3-methyl-	001193-18-6	52
31	27.79	1-Aza-2-boracyclopentane, 2-ethyl-1-methyl-	1000149-42-8	45
32	27.86	Cyclohexane, [2-(pentyloxy)ethyl]-	054852-75-4	43
33	28.05	4-Cyclohepten-1-amine	053783-90-7	14
34	30.50	1,4-Butanediamine, N-(3-aminopropyl)-	000124-20-9	23
35	30.66	Phytol	000150-86-7	91
36	38.19	2,6,10,14,18,22-Tetracosahexaene, 2,6,10,15,19,23-hexamethyl-, (all-E)-	000111-02-4	10
37	41.04	Propanamide	000079-05-0	35
D. ambrosioides				
1	7.624	1-Penten-3-one, 4-methyl-	001606-47-9	32
2	7.722	3-Octyn-2-ol	041746-22-9	12
3	17.051	(+)-4-Carene	029050-33-7	91
4	17.224	3,5-Nonadien-7-yn-2-ol, (E,E)-	043142-43-4	35
5	17.412	1-Methylene-2b-hydroxymethyl-3,3-dimethyl-4b-(3-methylbut-2-enyl)-cyclohexane	1000144-10-6	38
6	18.037	3-Hexyne-2,5-diol, 2,5-dimethyl-	000142-30-3	72
7	18.398	Cyclopropane, 1,1-dichloro-2,2,3,3-tetramethyl-	003141-45-5	25
8	18.715	3-Hexyne-2,5-diol, 2,5-dimethyl-	000142-30-3	59
9	18.940	2,4-Hexadiene, 2,5-dimethyl-	000764-13-6	38
10	19.159	2-Piperidinone, N-[4-bromo-n-butyl]-	195194-80-0	25
11	19.264	Cyclohexene, 1-methoxy-	000931-57-7	50
12	19.362	Thymol	000089-83-8	87
13	19.558	Phenol, 2-methyl-5-(1-methylethyl)-	000499-75-2	93

Table 1. Identification of metabolites by GC-MS (continued...)

PK	RT	Library/ID	CAS	Similarity
14	19.723	Sorbic Acid	000110-44-1	52
15	20.122	2-Hydroxy-3-propyl-2-cyclopenten-1-one	025684-04-2	35
16	20.326	3-Piperidinol	006859-99-0	9
17	20.371	2-Ethylacrolein	000922-63-4	9
18	20.559	Hexanoic acid, anhydride	002051-49-2	43
19	20.838	3-Pyrrolidinol	040499-83-0	18
20	20.883	.alpha.-Pinene	000080-56-8	30
21	20.981	1,4-Dimethyl-5-oxabicyclo[2.1.0]pentane	002316-03-2	12
22	21.154	Guanidineacetic acid	000352-97-6	22
23	21.417	3-Isopropyl-4-methyl-1-pentyn-3-ol	005333-87-9	10
24	21.455	Bicyclo[3.1.0]hexan-3-ol, 4-methyl-1-(1-methylethyl)-	000513-23-5	10
25	21.568	2,3-Anhydro-d-galactosan	1000129-98-9	32
26	21.666	Hexanoic acid, 2-methylbutyl ester	002601-13-0	53
27	22.050	Pyrazine, methyl-	000109-08-0	12
28	22.110	4,8-Dioxatricyclo[5.1.0.0(3,5)]octane, 1-methyl-5-(1-methylethyl)-, (1.alpha.,3.alpha.,5.alpha.,7.alpha.)-	042569-58-4	22
29	22.336	Cyclobutanecarboxylic acid, propyl ester	1000280-40-0	53
30	22.517	Cyclohexene, 3,5,5-trimethyl-	000933-12-0	37
31	22.667	Propane, 2-(ethenyloxy)-	000926-65-8	14
32	22.818	cis-4-Decenal	021662-09-9	35
33	22.953	2-[5-(2-Hydroxy-propyl)-furan-2-yl]-propan-1-ol	1000187-25-5	37
34	23.059	3-Fluorobenzoic acid, 2-pentadecyl ester	1000280-60-7	38
35	23.126	4(1H)-Pteridinone, 2-amino-6-methyl-	000708-75-8	59
36	23.179	4,8-Dioxatricyclo[5.1.0.0(3,5)]octane, 1-methyl-5-(1-methylethyl)-, (1.alpha.,3.alpha.,5.alpha.,7.alpha.)-	042569-58-4	25
37	23.337	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-	015356-74-	93
38	23.729	4,8-Dioxatricyclo[5.1.0.0(3,5)]octane, 1-methyl-5-(1-methylethyl)-, (1.alpha.,3.alpha.,5.alpha.,7.alpha.)-	042569-58-4	12
39	23.917	2-Butenoic acid, 2-methyl-, 2-methylpropyl ester, (E)-	061692-84-0	47
40	24.007	.alpha.-Bromo-2,4-difluorotoluene	023915-07-3	47
41	24.482	Benzoic acid, 2-(1-oxopropyl)-	002360-45-4	50
42	24.564	Pentanoic acid, 9-decenyl ester	1000159-93-3	16
43	25.084	1-Heptadecanamine	004200-95-7	23
44	25.122	Silane, (bromomethyl)-	007570-21-0	27
45	25.182	(-)-cis-Myrtanyl acetate	1000157-78-2	10
46	25.325	2-Propenoic acid, 1,7,7-trimethylbicyclo[2.2.1]hept-2-yl ester, exo-	005888-33-5	14
47	25.423	Heptanal	000111-71-7	16
48	25.513	Octanal	000124-13-0	10
49	25.769	2,6-Octadien-1-ol, 3,7-dimethyl-, acetate, (Z)-	000141-12-8	43
50	25.822	1,5-Cyclooctadiene, 3,4-dimethyl-	021284-05-9	49
51	25.980	7-Octen-2-ol, 2-methyl-6-methylene-	000543-39-5	38

Table 1. Identification of metabolites by GC-MS (continued...)

PK	RT	Library/ID	CAS	Similarity
52	26.228	1,9-Nonanediol	003937-56-2	9
53	26.341	E-12-Tetradecenal	1000130-96-1	10
54	26.447	4-Methyl-pent-3-enylamine	013296-28-1	9
55	26.530	3-Piperidinol	006859-99-0	38
56	26.763	Dodecane, 1-chloro-	000112-52-7	9
57	26.891	1,7-Octadiene, 2-methyl-6-methylene-	001686-30-2	37
58	27.162	9-Oxabicyclo[6.1.0]nonane, cis-	004925-71-7	38
59	27.915	2-Undecanone, 6,10-dimethyl-	001604-34-8	86
60	28.020	Heptanal	000111-71-7	36
61	28.291	3-Decyn-2-ol	069668-93-5	40
62	29.488	Octadecanoic acid, ethyl ester	000111-61-5	72
63	30.678	Phytol	000150-86-7	91
64	31.642	4,8-Dioxatricyclo[5.1.0.0(3,5)]octane, 1-methyl-5-(1-methylethyl)-, (1.alpha.,3.alpha.,5.alpha.,7.alpha.)-	042569-58-4	9
65	32.206	Undecane, 2,4-dimethyl-	017312-80-0	25
66	32.425	Acetaldehyde	000075-07-0	3
67	32.605	Ethyne, fluoro-	002713-09-9	3

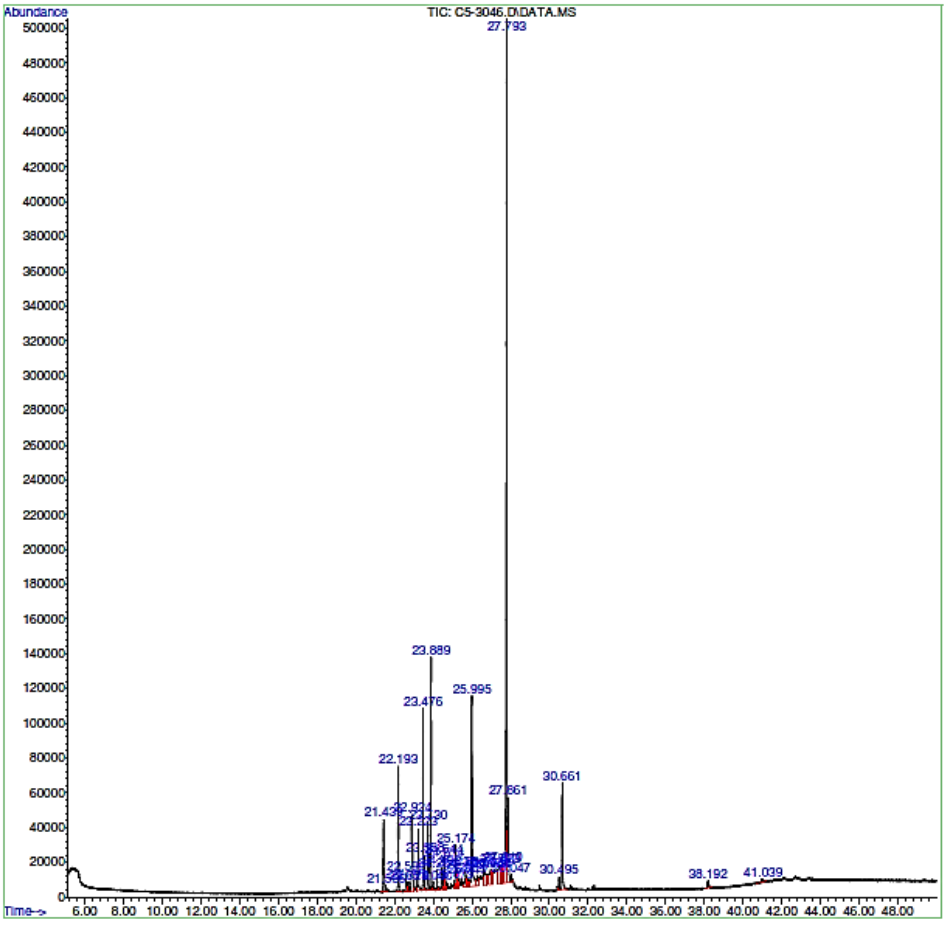

Figure 1. GC-MS chromatogram of compounds identified in extracts of *P. scaberrima*.

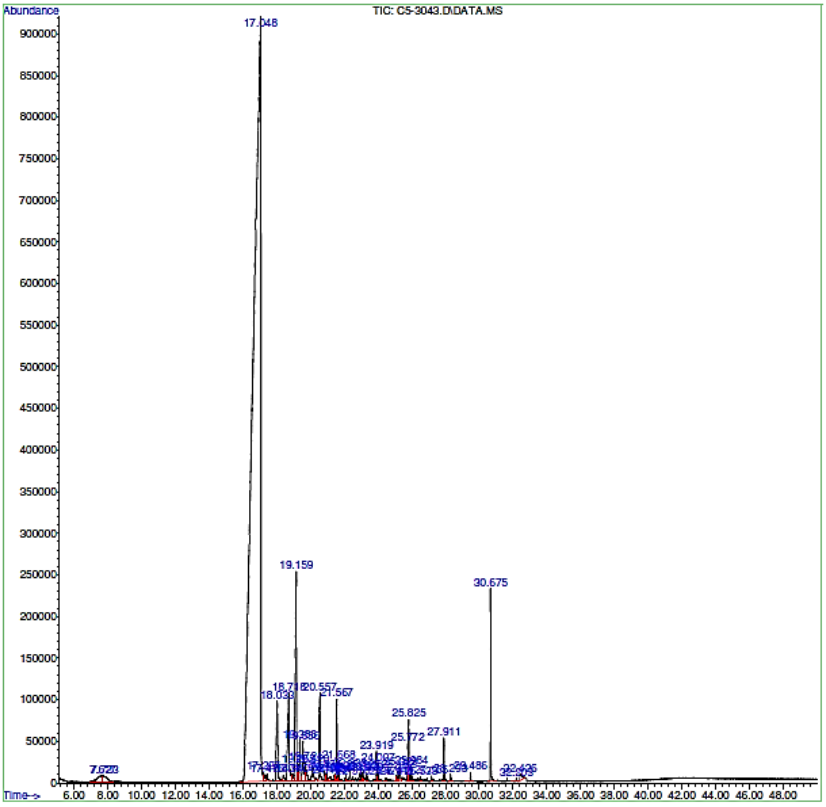

Figure 2. GC-MS chromatogram of compounds identified in extracts of *D. ambrosioides*.

In molecular docking studies against Mpro, the presence of key active site residues such as H41, M49, C145, and M165 has been evidenced (Anson et al., 2020; Zhang et al., 2020). Also, in the analysis of the identified structures there are mainly hydrophobic interactions with most of these aforementioned residues, which were related to the viral protease inhibitor. Likewise, δ and β-amorphene interact with these residues through the structural portions of propan-2-yl by the aromatic nucleus hexahydronaphthalene and β-cubebene through alkyl interactions with the structure ^{1}Hcyclopenta-(1,3)-cyclopropa(1,2)-benzene. Additionally, when comparing the molecular docking results with the control structure of the boceprevir inhibitor, the control structure mainly interacts through a Sγ atom of the Cys145 forming a nucleophilic setting and a C-S bond with the cetocarbon of the drug (Fu et al., 2020). However, in the case of the co-crystallized inhibitor in some interactions, hydrogen bonds were identified with N142, G143 and E166, which demonstrate that residues such as G143 and H164 generate hydrogen bond interactions with the amide structure and E166 with the alternate portion (Fu et al., 2020; Kneller et al., 2020).

For the results of binding energy with PLpro, it was shown that phytol, copaene and β-bisabolene presented values of -6.0, -5.8 and -5.7 Kcal/mol, respectively. Similarly, these results were contrasted with the co-crystallized structures for each protein obtaining binding energies of -7.7 and 10.5 Kcal/mol for boceprevir (Mpro inhibitor) and VIR251 (peptide inhibitor), respectively. On the other hand, in Fig. 8A-D, the interaction analysis for PLpro evidenced the presence of hydrophobic interactions with common residues such as P248 and Y268, as well as phytol and VIR251 showed hydrogen bonds with Y264, Y268 and G271.

Regarding to VIR251 with PLpro, it was evidenced that most of the interactions with this inhibitor are mediated by hydrogen bonds with G271 and G163 residues, which are denoted in positions P1-P3 of the structure (Anson et al., 2020). Additionally, residues Y264 and Y268 have been related too. A similar behavior was observed with phytol, showing hydrogen bonds interactions with Y268, G271 and Y264 through the hydroxyl group of the structure; however, in the Y273, P247 and P248 residues of the pocket, they established hydrophobic interactions with methyl groups in the C11 and C15 position of the molecule. Similarly, residues Y264 and P248 where shown to bind to alkyl interactions with methyl substituents and the tricyclic structure of copaene, as well as the cyclohexene structural portion of β-bisabolene.

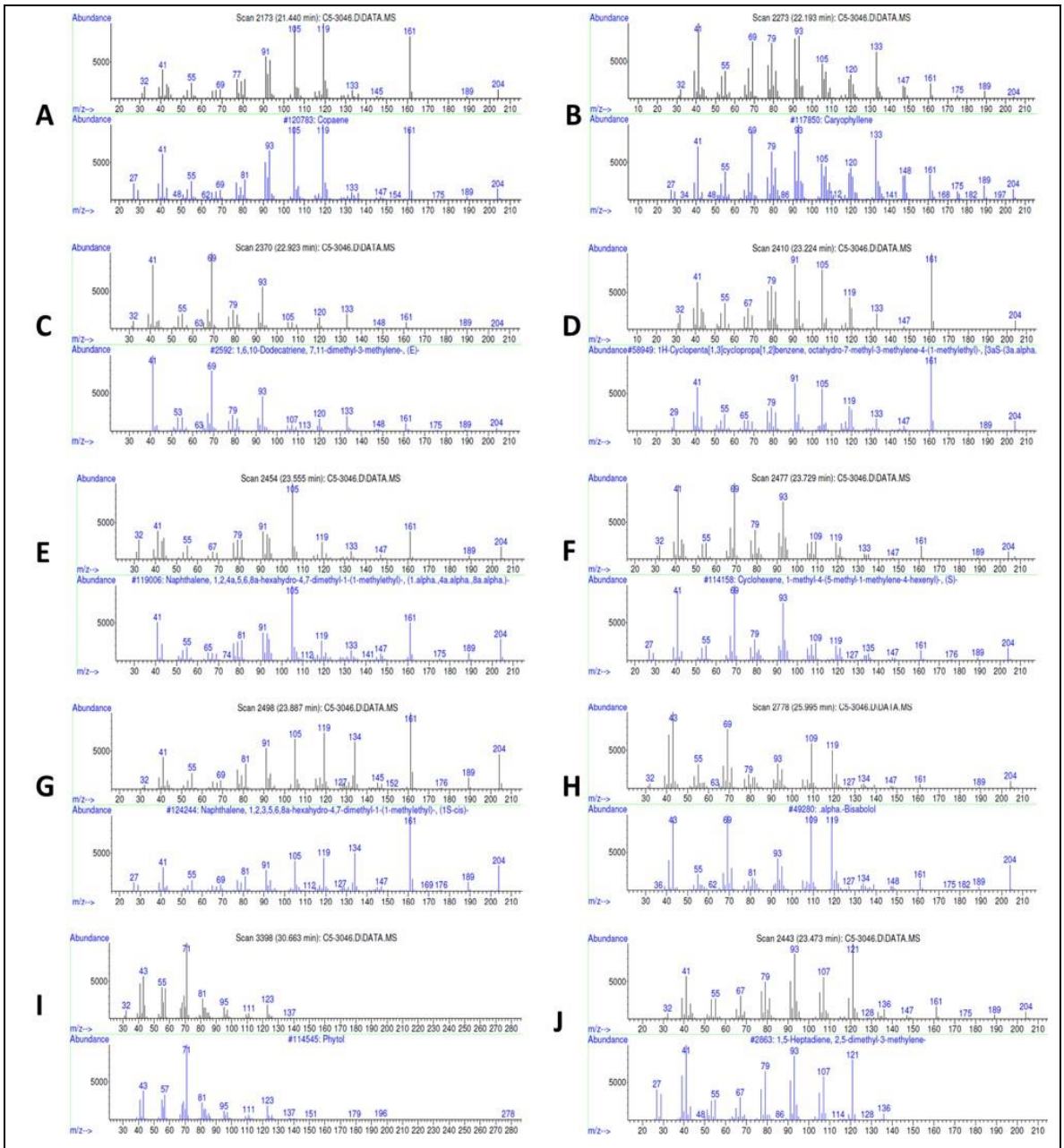

Figure 3. MS spectrum of compounds identified in *P. scaberrima.*

(A) Copaene; **(B)** caryophyllene; **(C)** β-farnesene; **(D)** β-cubebene; **(E)** α-amorphene; **(F)** β-bisabolene; **(G)** δ-amorphene; **(H)** α-bisabolol; **(I)** phytol; **(J)** 1,5-heptadiene, 2,5-dimethyl-3-methylene-.

Predictions of ADME properties show that only five compounds could have viable gastrointestinal absorption, six compounds with the possibility of permeable to BBB, only phytol indicates the possibility of a P-gp substrate, six compounds are related to possible inhibition of the isoform 1A2, as well as it is indicated to comply with the Lipinski rule and an adequate bioavailability score. Additionally, they report an *in-silico* prediction classification of acute oral toxicity in rats between class IV and V (Table 4).

On the other hand, the estimation of ADME properties and toxicity of the metabolites indicated that compounds α-bisabolol, thymol, carvacrol, and dihydroactinidiolide showed high gastrointestinal absorption. Some reports show that an oral administration of 50 mg/kg of thymol presented rapid absorption and slow elimination; likewise, carvacrol and α-bisabolol have denoted a gradual intestinal absorption, which can favor a uniform distribution to tissues and organs (Corpas-López et al., 2015; Javed et al., 2020). Additionally, it has been reported that only about 30% of these molecules can remain in the gastrointestinal tract. Likewise, the permeability through the blood-brain barrier shows that compounds such as caryophyllene, carvacrol, and thymol have the ability to

penetrate membranes due to their lipophilicity and tendency to accumulate at the brain (Zotti et al., 2013; Baldissera et al., 2018), which is corroborated at an experimental level in mouse models where it has been shown that after oral administration of thymol, it can permeate the BBB through the significant observation of the passage of Evans blue staining with respect to the control group (Baldissera et al., 2018).

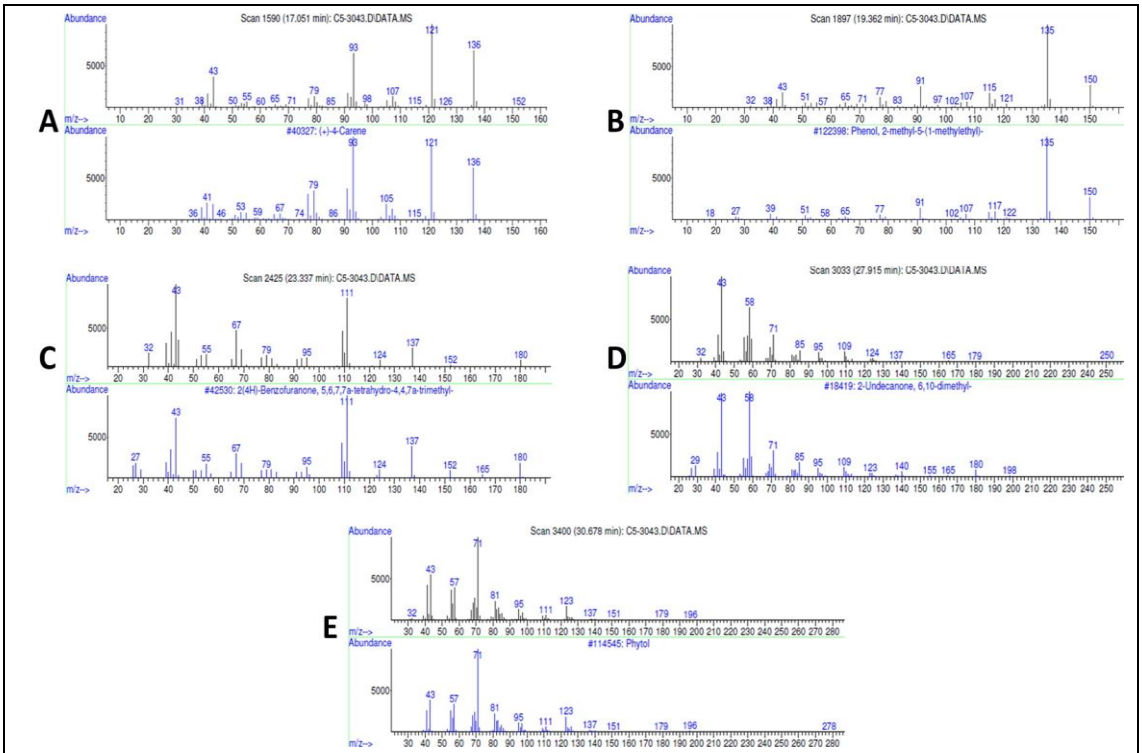

Figure 4. MS spectrum of compounds identified in *D. ambrosioides*.
(A) (+)-4-carene; **(B)** carvacrol; **(C)** dihydroactinidiolide; **(D)** 6,10-dimethylundecan-2-one; **(E)** phytol.

Table 2. Principal metabolites identified on gas chromatography-mass spectrometry (GC-MS) in extracts of *P. scaberrima* and *D. ambrosioides.*

PK	RT	Library/ID	Type	PubChem compound CID	CAS	Similarity
P. scaberrima						
1	21.44	Copaene	Tricyclic Sesquiterpene	19725	003856-olta25-5	99
2	22.19	Caryophyllene	Bicyclic sesquiterpene	5281515	000087-44-5	99
3	22.92	β-Farnesene	Acyclic sesquiterpenes alkene	5281517	018794-84-8	91
4	23.22	β-Cubebene	Tricyclic sesquiterpene	6432083	013744-15-5	95
5	23.47	1,5-Heptadiene, 2,5-dimethyl-3-methylene-	ND	535159	074663-83-5	86
6	23.56	α-Amorphene	Sesquiterpene	101708	031983-22-9	99
7	23.73	β-Bisabolene	Sesquiterpene	10104370	000495-61-4	95
8	23.89	δ-Amorphene	Sesquiterpene	10223	000483-76-1	96
9	25.99	α-Bisabolol	Monocyclic sesquiterpene	1549992	072691-24-8	91
10	30.66	Phytol	Acyclic diterpene alcohol	5280435	000150-86-7	91
D. ambrosioides						
1	17.05	(+)-4-Carene	Bicyclic monoterpenoids	530422	029050-33-7	91
2	19.36	Thymol	Monoterpenoid phenol	6989	000089-83-8	87
3	19.56	Carvacrol	Monoterpenoid phenol	10364	000499-75-2	93
4	23.34	Dihydroactinidiolide	Benzofurans	27209	015356-74-	93
5	27.91	6,10-Dimethylundecan-2-one	ND	95495	001604-34-8	86
6	30.68	Phytol	Acyclic diterpene alcohol	5280435	000150-86-7	91

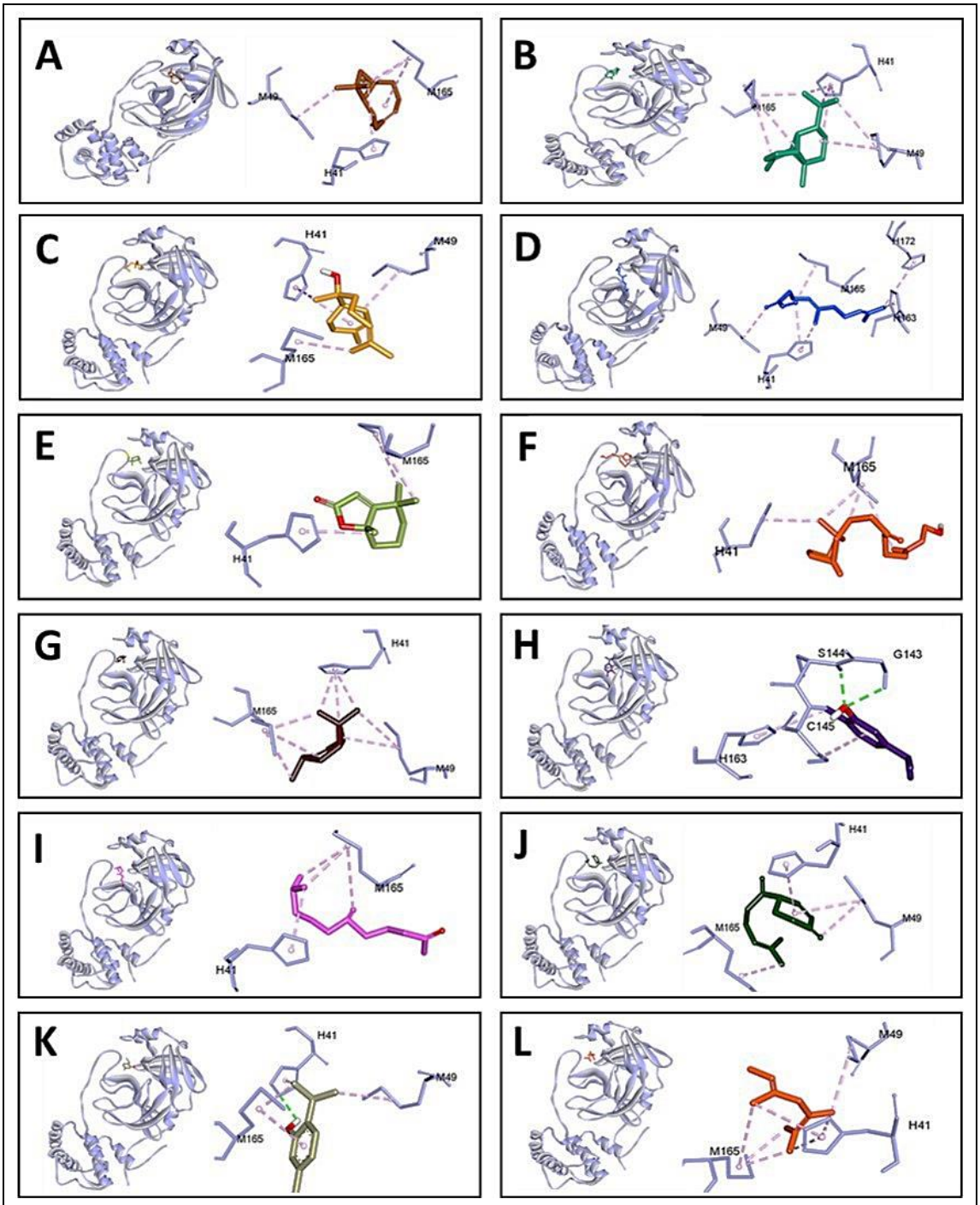

Figure 5. Interactions of metabolites and inhibitor against Mpro.

(A) Caryophyllene; **(B)** copaene; **(C)** α-bisabolol; **(D)** β-farnesene; **(E)** dihydroactinidiolide; **(F)** phytol; **(G)** (+)-4-carene; **(H)** carvacrol; **(I)** 6,10-dimethylundecan-2-one; **(J)** β-bisabolene; **(K)** thymol; **(L)** 1,5-heptadiene, 2,5-dimethyl-3-methylene.

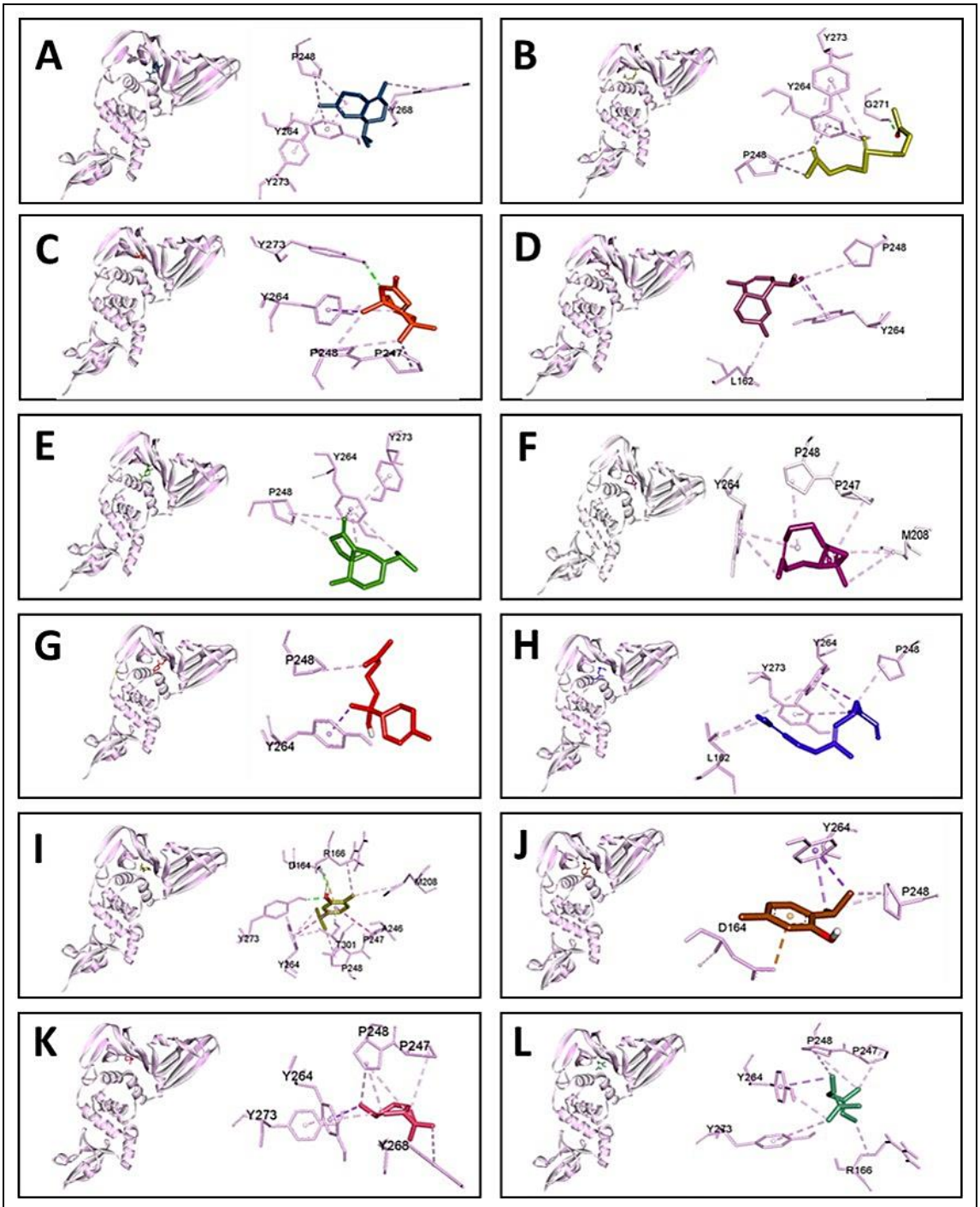

Figure 6. Interactions of metabolites and inhibitor against PLpro.

(A) δ-Amorphene; **(B)** 6,10-dimethylundecan-2-one; **(C)** dihydroactinidiolide; **(D)** α-amorphene; **(E)** β-cubebene; **(F)** β-caryophyllene; **(G)** α-bisabolol; **(H)** β-farnesene; **(I)** carvacrol; **(J)** thymol; **(K)** (+)-4-carene; **(L)** 1,5-heptadiene, 2,5-dimethyl-3-methylene.

Table 3. Metabolites of higher score of *P. scaberrima* and *D. ambrosioides* against Mpro and PLpro.

Protein	Ligands	Docking score (Kcal/mol)	Interacting residues	
			Hydrogen bond	Hydrophobic interactions and others
M_{PRO}	δ-Amorphene	-5.6	-	H41, M49, M165
	β-Cubebene	-5.4	-	H41, M49, M165
	α-Amorphene	-5.3	-	H41, M49, C145, M165
	β-Caryophyllene	-5,3	-	H41, M49, M165
	Copaene	-5,1	-	H41, M49, M165
	α-Bisabolol	-4,9	-	H41, M49, M165
	β-Farnesene	-4,8	-	H41, M49, H163, M165, H172
	Dihydroactinidiolide	-4,7	-	H41, M165
	Phytol	-4,7	-	H41, M165
	(+)-4-Carene	-4,4	-	H41, M49, M165
	Carvacrol	-4,4	G143, S144	C145, H163
	6,10-Dimethylundecan-2-one	-4,4	-	H41, M165
	β-Bisabolene	-4,4	-	H41, M49, M165
	Thymol	-4,4	H41	M49, M165
	1,5-Heptadiene, 2,5-dimethyl-3-methylene-	-3,8	-	H41, M49, M165
	Boceprevir	-7.7	N142, G143, C145, E166	H41, M49, M165
PL_{PRO}	Phytol	-6.0	Y264, Y268, G271	P247, P248, Y273
	Copaene	-5.8	-	P248, Y264
	β-Bisabolene	-5.7	-	L162, P248, Y264
	δ-Amorphene	-5,7	-	P248, Y264, Y268, Y273
	6,10-Dimethylundecan-2-one	-5,7	G271	P248, Y264, Y273
	Dihydroactinidiolide	-5,7	Y273	P247, P248, Y264
	α-Amorphene	-5,6	-	L162, P248, Y264
	β-Cubebene	-5,6	-	P248, Y264, Y273
	β-Caryophyllene	-5,5	-	M208, P247, P248, Y264
	α-Bisabolol	-5,2	-	P248, Y264
	β-Farnesene	-5,2	-	L162, P248, Y264, Y273
	Carvacrol	-4,9	D164, Y273, T301	R166, M208, A246, P248, Y264
	Thymol	-4,7	-	D164, P248, Y264
	(+)-4-Carene	-4,6		P247, P248, Y264, Y268, Y273
	1,5-Heptadiene, 2,5-dimethyl-3-methylene-	-4,3		R166, P247, P248, Y264, Y273
	VIR251 (Peptide inhibitor)	-10.5	G163, D164, Y264, Y268, G271	P248

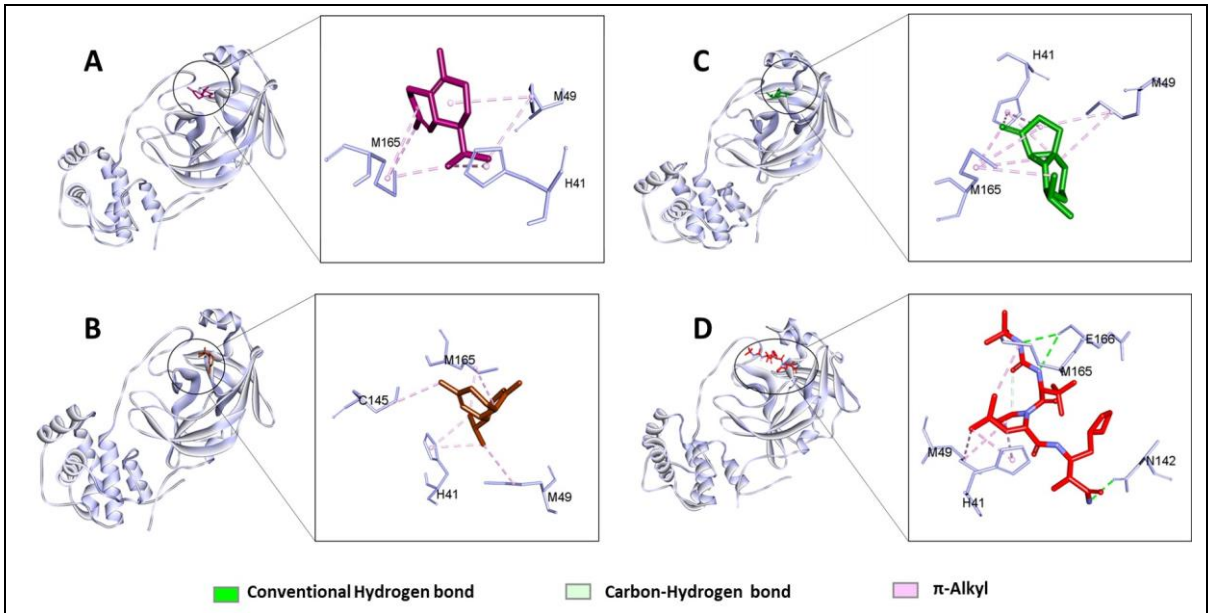

Figure 7. Molecular interaction of metabolites identified in *P. scaberrima* and *D. ambrosioides* against binding site of Mpro.
(A) δ-Amorphene; **(B)** β-cubebene; **(C)** α-amorphene; **(D)** boceprevir (inhibitor of Mpro).

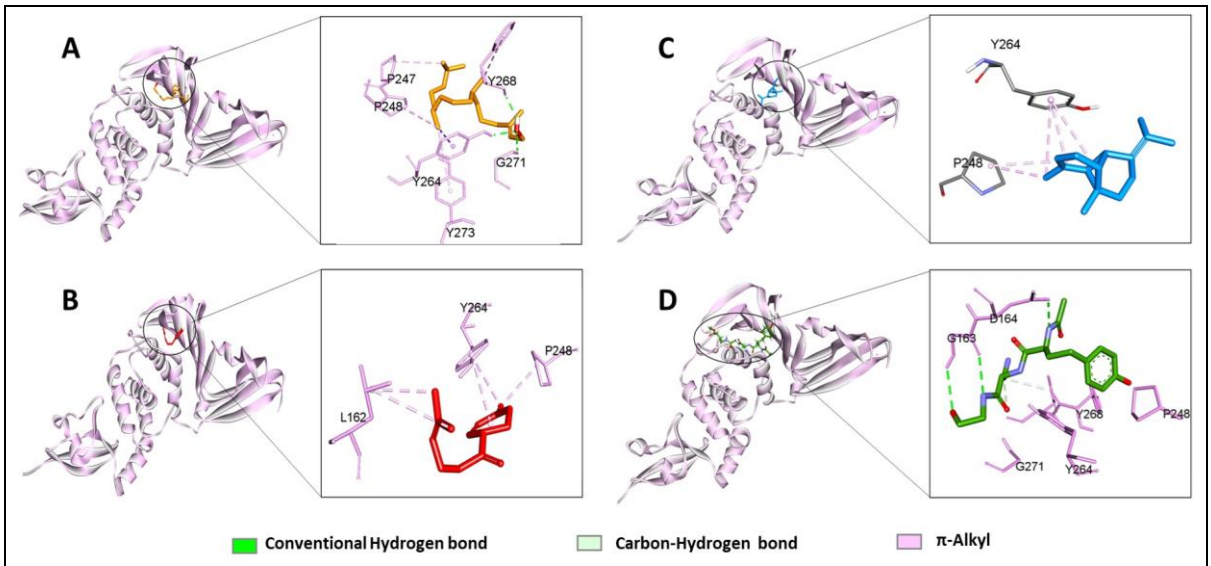

Figure 8. Molecular interaction of metabolites identified in *P. scaberrima* and *D. ambrosioides* against binding site of PLpro.
(A) Phytol; **(B)** copaene; **(C)** β-bisabolene; **(D)** VIR251.

About the metabolism of the studied plants and metabolites, reports demonstrated the presence of products such as 14-hydroxy-(+)-epi-α-bisabolol due to the activation of enzymatic systems such as (+)-epi-α-bisabolol synthase and cytochrome P450 reductase of NADPH, proving 1A1 and 2D6 CYP activation (Sarrade-Loucheur et al., 2020).

The toxicity of the metabolites indicated values between 1020 and 6559 mg/kg, classified as low risk of toxicity according to the OECD (2002). Some studies carried out show that the administration of extracts through oral infusions of *P. dulcis* does not show acute toxicity effects in mice at values between 1 to 5 g/kg (Granados-Dieseldorff et al., 2013). In the case of *C. ambrosioides*, values between 0.5 and 5 g/kg have been reported, in which no notable toxic effects have been observed after the oral administration of extracts (Pereira et al., 2010; Da Silva et al., 2014).

Table 4. ADME prediction, acute oral toxicity and drug-likeness of metabolites identified in *P. scaberrima* and *D. ambrosioides.*

Compounds	ADME							Toxicity	Similarity	
	GI abs.	BBB	P-gp	Log Kp (cm/s)	CYP			Acute oral rats (mg/kg - OECD)*	Lipinski	Bioavailability score
					1A2	2D6	3A4			
Copaene	Low	Yes	No	-4.37	Yes	No	No	3082 (V)	Yes	0.55
β-Caryophyllene	Low	Yes	No	-4.44	No	No	No	2321 (V)	Yes	0.55
β-Farnesene	Low	No	No	-3.27	Yes	No	No	3970 (V)	Yes	0.55
β-Cubebene	Low	Yes	No	-4.20	Yes	No	No	1020 (IV)	Yes	0.55
1,5-Heptadiene, 2,5-dimethyl-3-methylene-	Low	Yes	No	-3.82	No	No	No	1894 (IV)	Yes	0.55
α-Amorphene	Low	No	No	-4.65	No	No	No	3201 (V)	Yes	0.55
β-Bisabolene	Low	No	No	-2.98	No	No	No	3443 (V)	Yes	0.55
δ-Amorphene	Low	No	No	-4.85	No	No	No	2090 (V)	Yes	0.55
α-Bisabolol	High	Yes	No	-4.97	No	No	No	3963 (IV)	Yes	0.55
(+)-4-Carene	Low	Yes	No	-4.82	No	No	No	2193 (V)	Yes	0.55
Thymol	High	Yes	No	-4.87	Yes	No	No	1303 (IV)	Yes	0.55
Carvacrol	High	Yes	No	-4.74	Yes	No	No	1124 (IV)	Yes	0.55
Dihydroactinidiolide	High	Yes	No	-5.87	No	No	No	2803 (V)	Yes	0.55
6,10-Dimethylundecan-2-one	Low	Yes	No	-4.29	No	No	No	5392 (NT)	Yes	0.55
Phytol	Low	No	Yes	-2.29	No	No	No	6559 (NT)	Yes	0.55

BBB: Blood–brain barrier; GI abs.: Gastrointestinal absorption; NT: Non-toxic; P-gp: P-glycoprotein. *I-II: Fatal if swallowed; III: Toxic if wallowed; IV: Harmful if swallowed; V: May be harmful if swallowed. NT: No toxic or NR. (OECD, 2002; Strickland et al., 2019).

CONCLUSION

Through ethnobotanical studies, plants from the Colombian Pacific have been studied with potential biological value in the search for bioactives against emerging diseases such as COVID-19. In this way, the characterization of extracts of volatile compounds from two plants widely used in the region, such as *P. scaberrima* and *D. ambrosioides*, has been approached, in which 15 volatile compounds were identified through GC-MS with a high similarity value. Likewise, the compounds were evaluated by molecular docking against crystallized structures of Mpro and PLpro of SARS-CoV-2, obtaining higher affinity records for δ-amorphene, β-cubebene and α-amorphene against Mpro and phytol, copaene and β-bisabolene for PLpro. Finally, the pharmacokinetics and toxicity predictions were made for all compounds, showing some relevant data regarding the similarities reported in the literature for the extracts and metabolites.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

Adorjan B, Buchbauer G (2010) Biological properties of essential oils: An updated review. Flavour Fragr J 25(6): 407–426.

Al-Badani RN, da Silva JKR, Mansi I, Muharam BA, Setzer WN, Awadh Ali NA (2017) Chemical composition and biological activity of *Lavandula pubescens* essential oil from Yemen. J Essent Oil-Bearing Plants 20(2): 509–515.

Alrasheid AA, Babiker MY, Awad TA (2021) Evaluation of certain medicinal plants compounds as new potential inhibitors of novel corona virus (COVID-19) using molecular docking analysis. Silico Pharmacol 9(1): 10.

Anson B, Chapman M, Lendy E, Pshenychnyi S, D'Aquila R, Satchell K, Mesecar A (2020) Broad-spectrum inhibition of coronavirus main and papain-like proteases by HCV drugs. Res Sq [Preprint]. https://doi.org/10.21203/rs.3.rs-26344/v1

Asif M, Saleem M, Saadullah M, Yaseen HS, Al Zarzour R (2020) COVID-19 and therapy with essential oils having

antiviral, anti-inflammatory, and immunomodulatory properties. Inflammopharmacology 28(5): 1153–1161.

Astani A, Reichling J, Schnitzler P (2011) Screening for antiviral activities of isolated compounds from essential oils. Evid Based Complement Altern Med 2011: 253643.

Baldissera MD, Souza CF, De Matos AFIM, Doleski PH, Baldisserotto B, Da Silva AS, Monteiro SG (2018) Blood-brain barrier breakdown, memory impairment and neurotoxicity caused in mice submitted to orally treatment with thymol. Environ Toxicol Pharmacol 62: 114–119.

Carvalho e Silva MAG, Carneiro LP, Castelo Branco MFG, Barros EML, Lemos SI, de Barros TL, Marques RB (2016) Anti-Inflammatory effect of mastruz (*Chenopodium ambrosioides*) extract in respiratory distress syndrome. Int J Pharm Sci Invent 5(5): 34–39.

Compadre CM, Robbins EF, Kinghorn AD (1986) The intensely sweet herb, *Lippia dulcis* Trev.: Historical uses, field inquiries, and constituents. J Ethnopharmacol 15(1): 89–106.

Corpas-López V, Morillas-Márquez F, Navarro-Moll MC, Merino-Espinosa G, Díaz-Sáez V, Martín-Sánchez J (2015) (-)-α-Bisabolol, a promising oral compound for the treatment of visceral leishmaniasis. J Nat Prod 78(6): 1202–1207.

Da Silva JKR, Figueiredo PLB, Byler KG, Setzer WN (2020) Essential oils as antiviral agents. Potential of essential oils to treat sars−cov−2 infection: An in-silico investigation. Int J Mol Sci 21(10): 3426.

Da Silva MGC, Amorim RNL, Câmara CC, Fontenele Neto JD, Soto-Blanco B (2014) Acute and sub-chronic toxicity of aqueous extracts of chenopodium ambrosioides leaves in rats. J Med Food 17(9): 979–984.

Daina A, Michielin O, Zoete V (2017) SwissADME: A free web tool to evaluate pharmacokinetics , drug- likeness and medicinal chemistry friendliness of small molecules. Sci Rep 7: 42717.

Dallakyan S, Olson AJ (2015) Small-molecule library screening by docking with PyRx. Methods Mol Biol 1263: 243–250.

Esakandari H, Nabi-Afjadi M, Fakkari-Afjadi J, Farahmandian N, Miresmaeili SM, Bahreini E (2020) A comprehensive review of COVID-19 characteristics. Biol Proced Online 22: 19.

Fu L, Ye F, Feng Y, Yu F, Wang Q, Wu Y, Zhao C, Sun H, Huang B, Niu P, Song H, Shi Y, Li X, Tan W, Qi J, Gao GF (2020) Both boceprevir and GC376 efficaciously inhibit SARS-CoV-2 by targeting its main protease. Nat Commun 11(1): 4417.

Ghildiyal R, Prakash V, Chaudhary VK, Gupta V, Gabrani R (2020) Phytochemicals as antiviral agents: Recent updates. In: Swamy M. (eds), Plant-derived Bioactives. Singapore: Springer, pp. 279–295.

Granados-Dieseldorff N, Paredes M, Ordóñez M, Martínez V (2013) Phyla dulcis (Trevir.) Moldenke: Descripción de características anatómicas diagnósticas de la droga cruda. Rev Cient Fac Cienc Quím Farm 23(1): 70–80.

Javed H, Meeran MFN, Azimullah S, Eddin LB, Dwivedi VD, Jha NK, Ojha S (2020) α-Bisabolol, a dietary bioactive phytochemical attenuates dopaminergic neurodegeneration through modulation of oxidative stress, neuroinflammation and apoptosis in rotenone-induced rat model of Parkinson's disease. Biomolecules 10(10): 1421.

Kneller DW, Galanie S, Phillips G, O'Neill HM, Coates L, Kovalevsky A (2020) Malleability of the SARS-CoV-2 3CL Mpro active-site cavity facilitates binding of clinical antivirals. Structure 28(12): 1313-1320.e3.

Lagunin A, Zakharov A, Filimonov D, Poroikov V (2011) QSAR modelling of rat acute toxicity on the basis of PASS prediction. Mol Inform 30(2–3): 241–250.

Mokni R El, Youssef FS, Jmii H, Khmiri A, Bouazzi S, Jlassi I, Jaidane H, Dhaouadi H, Ashour ML, Hammami S (2019) The essential oil of Tunisian *Dysphania ambrosioides* and its antimicrobial and antiviral properties. J Essent Oil-Bearing Plants 22(1): 282–294.

Moreno-Murillo B, Quijano-Célis C, Romero R A, Pinod JA (2010) Essential oil from leaves of *Lippia dulcis* grown in Colombia. Nat Prod Commun 5(4): 613–614.

O'Boyle NM, Banck M, James CA, Morley C, Vandermeersch T, Hutchison GR (2011) Open Babel: An open chemical toolbox. J Cheminform 3: 33.

OECD (2002) Test No. 423: Acute Oral toxicity - Acute Toxic Class Method, OECD Guidelines for the Testing of Chemicals, Section 4, Paris: OECD Publishing, https://doi.org/10.1787/9789264071001-en

Ortiz-Rojas LY, Chaves-Bedoya G (2017) Composición fitoquímica del extracto de raíz de *Ichthyothere terminalis* de dos regiones geográficas de Colombia. Rev Colomb Quím 46(3): 11–16.

Panikar S, Shoba G, Arun M, Sahayarayan JJ, Nanthini AUR, Chinnathambi A, Alharbi SA, Nasif O, Kim H-J (2021) Essential oils as an effective alternative for the treatment of COVID-19: Molecular interaction analysis of protease (Mpro) with pharmacokinetics and toxicological properties. J Infect Public Health 14(5): 601–610.

Pereira WS, Ribeiro BP, Sousa AIP, Serra ICPB, Mattar NS, Fortes TS, Reis AS, Silva LA, Barroqueiro ESB, Guerra RNM, Nascimento FRF (2010) Evaluation of the subchronic toxicity of oral treatment with *Chenopodium ambrosioides* in mice. J Ethnopharmacol 127(3): 602–605.

Pérez S, Meckes M, Pérez C, Susunaga A, Zavala MA (2005) Anti-inflammatory activity of *Lippia dulcis*. J Ethnopharmacol 102(1): 1–4.

Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC, Ferrin TE (2004) UCSF Chimera--a visualization system for exploratory research and analysis. J Comput Chem 25(13): 1605–1612.

Rajabian A, Hosseinzadeh H (2020) Dermatological effects of *Nigella sativa* and its constituent, thymoquinone: A review. In: Nuts and Seeds in Health and Disease Prevention. Elsevier Inc., pp. 329–355.

Sarrade-Loucheur A, Ro DK, Fauré R, Remaud-Siméon M, Truan G (2020) Synthetic derivatives of (+)- epi-α-

bisabolol are formed by mammalian cytochromes p450 expressed in a yeast reconstituted pathway. ACS Synth Biol 9(2): 368–380.

Seadawy MG, Gad AF, Shamel M, Elharty B, Mohamed MF, Elfiky AA, Ahmed A, Zekri ARN (2021) *In vitro*: Natural compounds (thymol, carvacrol, hesperidine, and thymoquinone) against Sars-Cov2 strain isolated from Egyptian patients. Biomed J Sci Tech Res 34(3): 26750–26757.

Shakeri A, Akhtari J, Soheili V, Taghizadeh SF, Sahebkar A, Shaddel R, Asili J (2017) Identification and biological activity of the volatile compounds of *Glycyrrhiza triphylla* Fisch. & C.A.Mey. Microb Pathog 109: 39–44.

Sharma AD, Kaur I (2020) Bioactive molecules from eucalyptus essential oil as potential inhibitors of COVID 19 corona virus infection by molecular docking studies. Kragujev J Sci 42: 29–43.

Strickland J, Paris MW, Allen D, Casey W (2019) Approaches to Reducing Animal Use for Acute Toxicity Testing: Retrospective Analyses of Pesticide Data. In: Kojima H., Seidle T., Spielmann H. (eds) Alternatives to Animal Testing. Singapore: Springer, pp. 1-128.

Vimalanathan S, Hudson J (2014) Anti-influenza virus activity of essential oils and vapors. Am J Essent Oils Nat Prod 2(1): 47–53.

WHO (2020) COVID-19 Weekly Epidemiological Update 78. World Heal. Organ. 1–3. https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---8-february-2022. [Consulted 22 November 2021].

Zhang L, Lin D, Sun X, Rox K, Hilgenfeld R (2020) X-ray structure of main protease of the novel coronavirus SARS-CoV-2 enables design of α-ketoamide inhibitors. bioRxiv [Preprint]. https://doi.org/10.1101/2020.02.17.952879

Zotti M, Colaianna M, Morgese MG, Tucci P, Schiavone S, Avato P, Trabace L (2013) Carvacrol: From ancient flavoring to neuromodulatory agent. Molecules 18(6): 6161–6172.

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Contribution	Contreras-Puentes N	Salas-Moreno MH	Mosquera-Chaverra L	Córdoba-Tovar L	Alvíz-Amador AA
Concepts or ideas	x	x			
Design	x	x			
Definition of intellectual content	x	x	x	x	x
Literature search	x	x			x
Experimental studies		x		x	
Data acquisition	x	x	x	x	x
Data analysis	x	x	x	x	x
Statistical analysis	x	x			
Manuscript preparation	x	x			x
Manuscript editing			x	x	
Manuscript review	x	x	x	x	x

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