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Revisión / Review

Larrea divaricata a native Argentine plant with potential activity against SARS-CoV-2?

[¿*Larrea divaricata* una planta nativa argentina con actividad potencial sobre SARS-CoV-2?]

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Section Review

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Abstract: *Larrea divaricata* Cav. is an autochthonous South American plant popularly used in inflammatory and infectious diseases with reported anti-inflammatory, immunomodulatory, antimicrobial and antioxidant activities. Covid-19 is an infection caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). This virus can cause pneumonia and even death in about 5% of the cases. The objective of the article was to demonstrate, through a literature review, that *L. divaricata* has sufficient attributes to be assayed against SARS-CoV-2. For this, the chemical composition, reported activities and docking studies were taken into account. This review demonstrated that the plant extracts are capable of inhibiting the proliferation of fungi, bacteria and viruses and that they exert anti-inflammatory and immunomodulatory actions in different "*in vitro*" and "*in vivo*" models. These results suggest that the plant is a good candidate to be studied for the prevention and/or treatment of SARS - CoV-2.

Keywords: Covid-19; SARS-CoV-2; *Larrea divaricata* Cav.; Immunomodulatory activity; Antiviral activity.

Resumen: *Larrea divaricata* Cav. es una planta autóctona Sudamericana, utilizada popularmente en enfermedades inflamatorias e infecciosas, con actividad anti-inflamatoria, inmunomoduladora, antimicrobiana y antioxidante reportada. El Covid-19 es una infección causada por una cepa de coronavirus, SARS-CoV-2 (coronavirus tipo 2 causante del síndrome respiratorio agudo severo). Este virus puede originar neumonía e incluso la muerte en alrededor del 5% de los casos. Nuestro objetivo fue demostrar, a través de una revisión bibliográfica, que esta planta tiene atributos suficientes para ser ensayada en estudios contra SARS-CoV-2. Se tuvo en cuenta la composición química, los antecedentes científicos y los estudios de acoplamiento molecular. Esta revisión permitió demostrar que extractos de la planta son capaces de inhibir la proliferación de hongos, bacterias y virus y que presentan acción anti-inflamatoria en diferentes modelos "*in vitro*" e "*in vivo*", lo que los hace candidatos a ser estudiados en la prevención y/o tratamiento de la infección contra SARS-CoV-2.

Palabras clave: Covid-19; SARS-CoV-2; *Larrea divaricata* Cav.; Actividad inmunomoduladora; Actividad antiviral.

INTRODUCTION

Larrea divaricata Cav. is an autochthonous South American plant widely distributed in Argentina and popularly used in inflammatory and infectious diseases. In folk medicine, *L. divaricata* is used for healing sores and wounds, rheumatism, bronchitis, feber, rheuma, inflammatory diseases, malaria, inflammation of the respiratory and intestinal tracts, gastric disorders, venereal diseases, and as tonic, corrective, antiseptic, expectorant, and emetic agent (Soraru & Bandoni 1978; Ratera & Ratera 1980; Veretoni, 1985; Del Vitto *et al.,* 1997). Numerous scientifically studies have proven the anti-inflammatory, immunomodulatory, antimicrobial and antioxidant activities of the extracts obtained from the plant.

Currently, millions of people worldwide are suffering from the emergent Covid-19. This infection is caused by a new strain of coronavirus, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). So far, 181722790 cases and 3942233 deaths have been reported, with an estimated mortality risk that reaches ~5% (WHO Coronavirus (COVID-19) Dashboard, July 2021). The clinical features of pneumonia cases of SARS-CoV-2 are fever, dry cough, dyspnea, and bilateral ground-glass opacity and consolidation of chest. Upper respiratory tract symptoms (sore throat and rhinorrhea) have also been reported (Huang *et al.,* 2020). The transmission mode of SARS-CoV-2 occurs through the inhalation of droplets containing viral particles, feces and contact with infected people (Tang *et al.,* 2020; Peng *et al.,* 2020). The mean incubation period is estimated to be 5 days (range of 4–7 days, 95% CI) (Li *et al.,* 2020). Numerous therapeutic strategies, such as supportive intervention and the use of immunomodulatory agents, antiviral agents, antibiotics, anti-malarial drugs, convalescent plasma infusion, have been adopted in the clinical setting (Li *et al.,* 2020) together with the development of vaccines. So far, no effective drugs have been discovered to treat the disease. Nevertheless, the potential activity of plant drugs and natural compounds against SARS-CoV-2 has been reported.

Therefore, the objective of the article was to demonstrate, through a literature review, that *L. divaricata* has plenty features to be assayed in future studies against SARS-CoV-2. To this end, the chemical composition and the antimicrobial, anti-inflammatory, antioxidant, and immunomodulatory activities of the extracts obtained from the plant and of its main chemical compounds will be presented. In addition, the main compounds' docking studies carried out to evaluate the interaction between ACE2 and the spike protein and 3CL proteases from SARCoV-2 and SARS-CoV will be shown.

The viral replication process and the mechanisms underlying inflammatory diseases will be briefly explained to identify the molecular targets involved in the biological effect of the plant's compounds.

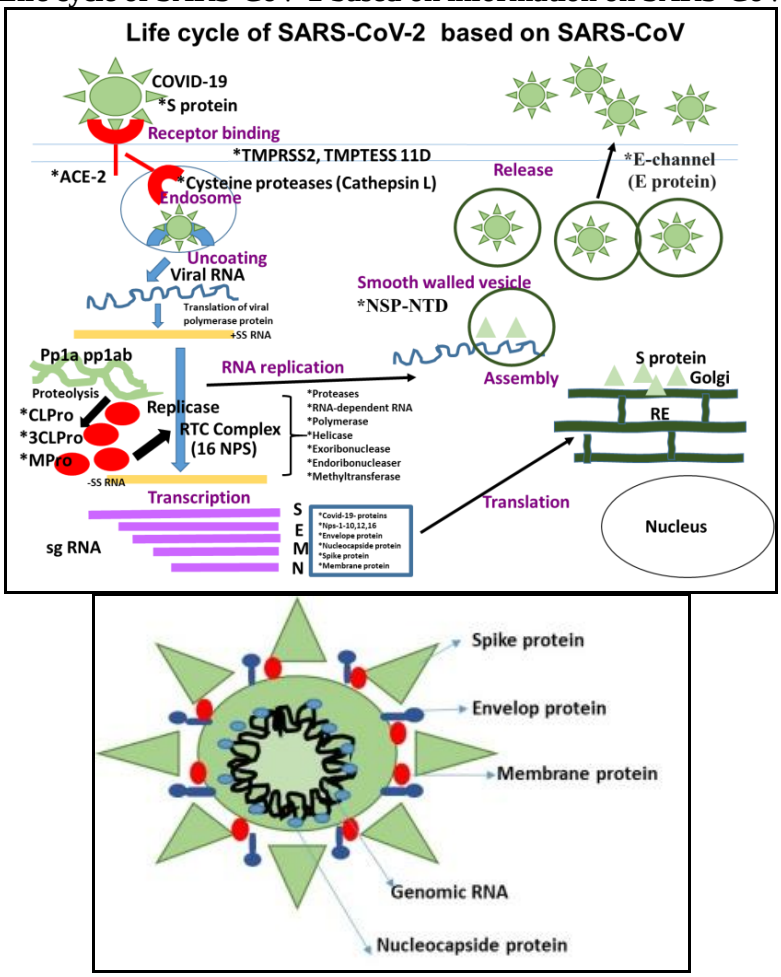
Life cycle of SARS-CoV-2 and possible sites of drug inhibition

The infection starts with the binding of the virus through its spike protein to the ACE2, a receptor located on the cell surface (Yang & Shen, 2020; Hoffmann *et al.,* 2020; Wrapp *et al.,* 2020). ACE2 is expressed on epithelial cells of the lung and intestine, and to a lesser extent, in the kidney, heart, adipose, and both male and female reproductive tissues (Zhang *et al.,* 2020; Lamers *et al.,* 2020; Chen *et al.,* 2020a; Jing *et al.,* 2020; Subramanian *et al.,* 2020). ACE2 plays a fundamental role in the pathophysiology of Covid-19, thus becoming an important site to be inhibited by drugs. The binding to the receptor is followed by the activation of the spike protein through proteolytic cleavage by a host protease (Hoffmann *et al.,* 2018) which allows the virion to enter via endocytosis. In this process, some proteases play a crucial role; such is the case of the cysteine protease cathepsin (Bosch *et al.,* 2008; Millet *et al.,* 2014) and Trypsin-like serine proteases like the transmembrane serine protease 2TMPRSS2 and TMPTESS11D. Therefore, these proteases are also interesting targets to be inhibited by drugs. Afterwards, viral genomic RNA is released into the cytoplasm and is directly translated into pp1a and pp1ab, which are polyproteins that will undergo enzymatic proteolysis to generate the 16 proteins (NSPs) of the RTC complex. The enzymatic proteolisis is conducted by viral proteases such as the papain-like protease (PLPro), the 3-chymotrypsin-like protease (3-CLPro) and the main protease (MPro) which are also interesting targets for drugs (Nakagawa *et al.,* 2016). The RTC complex replicates and synthesizes a set of (sgRNA) that encode the main structural proteins S, M, E and N and accessory proteins (Rokni *et al.,* 2020; Ahn *et al.,* 2020; Chen *et al.,* 2020b; Han *et al.,* 2020). All these proteins, together with the nucleocapsid, are then assembled in the Golgi complex to form the new viral particles and thus, finally, to be released from the infected cell by exocytosis. The latter process then

constitutes another target to be inhibited (Figure No. 1).

In summary, the potential coronavirus drug molecular targets can be divided into several categories based on the specific pathways involved: (1) enzymes or functional proteins that play a role on viral RNA synthesis and replication, (2) viral structural proteins involved in the binding to human cell receptors or in the virus self-assembly process, (3) proteins acting as virulence factors. The target proteins are: Nsp1, Nsp3 (Nsp3b, Nsp3c, PLpro, and Nsp3e), the Nsp7-Nsp8 complex, Nsp9e, Nsp10, Nsp14e, Nsp16, 3CLpro, E-channel (E protein), ORF7a, SpikeACE2, C-terminal RNA binding domain (CRBD), N-terminal RNA binding domain (NRBD), helicase, RdRp, and TMPRSSS2 (Wu *et al.*, 2020).

Figure No. 1
Life cycle of SARS-CoV-2 based on information on SARS-CoV



Infection begins with the S protein binding to the host cell's ACE2 receptor. The virion enters via endocytosis and, later, viral genomic RNA is released into the cytoplasm and is directly translated into pp1a and pp1ab, which are polyproteins that will undergo enzymatic proteolysis to generate the 16 proteins (NSPs) of the RTC complex. The RTC complex replicates and synthesizes a set of (sgRNA) that encode the main structural proteins S, M, E and N; and accessory proteins. All these proteins, together with the nucleocapsid, are then assembled in the Golgi complex to form the new viral particles and thus, finally, to be released from the infected cell.*Proteins that maybe inhibited by drugs

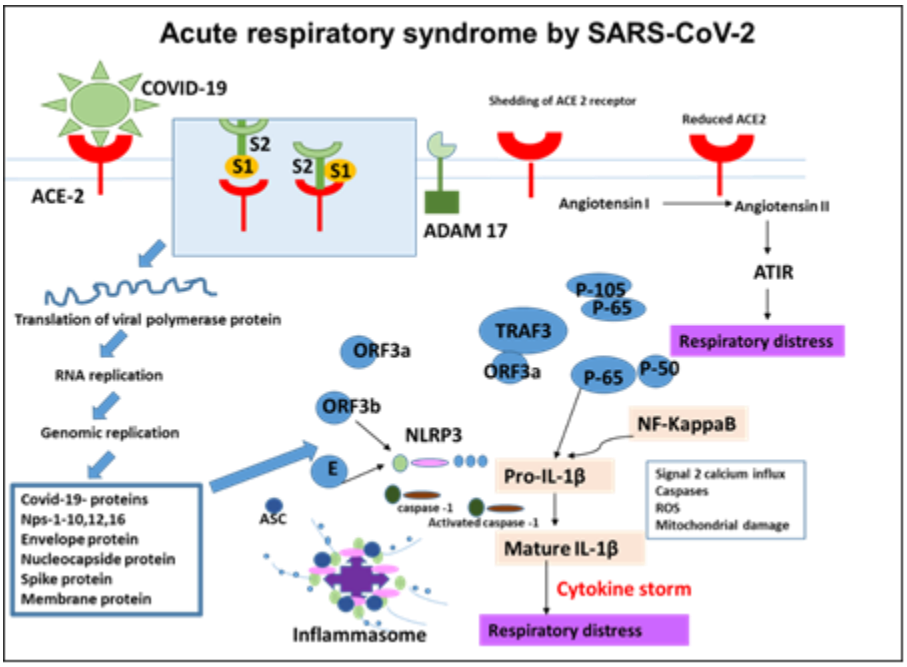
Mechanism of inflammation induced by SARS-CoV-2

The replicative cycle of SARS-CoV-2 causes a local immune response, cell death and tissue damage (Tabas & Ron, 2011). In some cases, a dysregulation of the immune response occurs, triggering a cytokine storm that leads to the influx of inflammatory mononuclear cells and consequently to lung

inflammation (Tay *et al.*, 2020). In this situation, NLRP3 inflammasome pattern recognition receptors (PRRs) play a key role by recognizing pathogen-associated molecular patterns (PAMP) and damage-associated molecular patterns (DAMP), as well as by recruiting other proteins involved in the NLRP3 inflammasome signaling pathway. The NLRP3 inflammasome, generating active forms of the cytokines interleukin 1 beta (IL-1β) and IL-18, induces inflammation and activation of oxidative stress, which in turn exacerbates cytokine storm and oxidative stress for microbial clearance. Yet, these events lead to septic shock (Zahid *et al.*, 2019; Zhao & Zhao, 2020).

The downregulation of the ACE2 receptor implies a reduction in the levels of Ang 1-7, a lower degree of activation of the Mas 1 oncogene receptor (Mas receptor) and an increase in Ang II levels with activation of the AT1 receptor. All these events contribute to development of lung inflammation and tissue fibrosis observed in patients with severe covid-19. ACE2 maintains the proper function of the heart and kidneys, and the negative regulation of ACE2 induced by SARS-CoV-2 can negatively affect this protective characteristic and contribute to the damage caused by the infection of these organs (Datta *et al.*, 2020) (Figure No. 2).

Figure No. 2
Possible mechanism of SARS-CoV-2-induced inflammation



The S1 and S2 subunits of spike protein are cleaved. This event is followed by the shedding of ACE-2 by ADAM 17, resulting in an increased amount of angiotensin II, which leads to respiratory distress. Upon binding to ACE2, the virus fuses with the cytoplasmic membrane, enters the cell and translation and replication of viral proteins occur. ORF3a, ORF8b, E proteins and the NF-κB pathway activates the inflammasome pathway through several mechanisms, leading to the increase in the levels of proinflammatory cytokines. This results in a cytokine storm, further resulting in respiratory distress

MATERIALS AND METHODS

PubMed and the Science Direct data bases were used to conduct the bibliographic search on pharmacological and *in silico* studies performed with *L. divaricata* compounds, as well as on the antimicrobial, immunomodulatory, anti-inflammatory and antioxidant activities. Some of our own laboratory results (no published anywhere) about the composition of *L. divaricata´*s aqueous extract were also added.

RESULTS AND DISCUSSION

Larrea divaricata Cav.

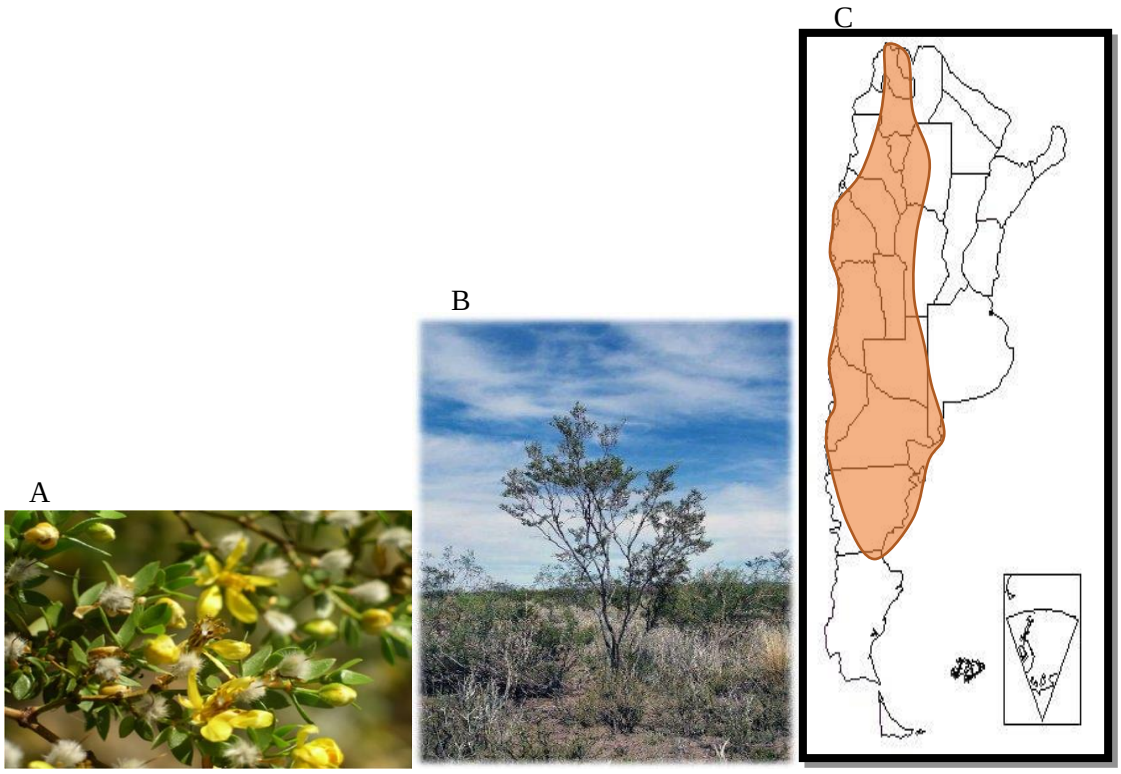
Popular uses

Larrea divaricata Cav. (Zygophyllaceae), whose common name **"**jarilla hembra", "jarilla", "jarilla del

cerro", and "jarilla de la sierra" is an autochthonous South American plant widely distributed in the north-western, center, and south-eastern regions of Argentina (Discole *et al.*, 1940). It is an evergreen shrub 3 to 6 feet (0.9 to 1.8 m) tall; the botanical and geographical distribution is shown in Figure No. 3. In folk medicine, *L. divaricata* is used under the form of infusion and decoction both internally and externally. It is employed for the treatment of healing sores and wounds, rheumatism, bronchitis, feber, rheuma, inflammatory diseases, malaria, inflammation of the respiratory and intestinal tracts, gastric disorders, venereal diseases, and as tonic, corrective, antiseptic, expectorant, and emetic agent (Soraru & Bandoni, 1978; Ratera & Ratera, 1980; Veretoni, 1985). It is also used as a rubefacient and anti-inflammatory agent (Del Vitto *et al.*, 1997). In Santiago del Estero, Argentina, aqueous extracts of the plant are used as mouthwashes to calm toothaches and as antitussive (Marzocca, 1997). In the Northwest of Argentina, the root is used for the treatment of rheumatism, syphilis, gout, externally to treat fungal infection and hyperhidrosis on the feet (Pérez de Nucci, 1988). The bark and the leaves are used externally, in decoction to treat rheumatism and to disinfect wounds and snakebites (Soraru & Bandoni, 1978).

The use of other plants of *Larrea* genus is described in the treatment of arthritis and cancer (Tyler, 1994), tuberculosis (Tyler & Foster, 1999; Rodriguez-Fragoso *et al.*, 2008) and common cold (Tyler & Foster, 1999).

Figure No. 3
***Larrea divaricata* Cav.: flowers and leaves (A), shrub (B), geographical distribution in Argentina (C)**



It is a shrub 0.9-1.8 m tall that has small resinous dark green leaves and yellow flowers. The photograph corresponds to a "jarilla" specimen growing in Valle Grande, Km 25, route 173, San Rafael, Mendoza, Argentina (www.bosqueeuco.com)

REPORTED EFFECTS

Antimicrobial effects

The antimicrobial effect of *L. divaricata* has been reported for bacteria, fungi, mycobacteria and viruses. The antiviral activity of different *L. divaricata* aqueous (Aq), ethanolic (EtOH) and dichloromethane (CH_2Cl_2) extracts have been demonstrated on Junín virus (Arenaviridae family), which is the etiologic agent of the Argentinean Hemorrhagic Fever (AHF). AHF is an endemo-

epidemic zoonotic illness that is transmitted to man by rodents, e.g., *Calomys musculinus*. The Aq and CH_2Cl_2 extracts exerted a higher inhibition percent of the viral replication, when the virus was previously incubated with the extracts at room temperature for 1 h than when it was incubated at 36ºC and then inoculated in cell monolayers and kept at 36ºC for 1 h. Meanwhile, the EtOH extract was active at both incubations temperatures. In this study, neither the mechanism nor the active compounds are studied (Konigheim *et al.,* 2006).

The major reports of *L. divaricata* extracts are on its antibacterial activity. The antimicrobial effect of the aqueous extract of *L. divaricata* against penicillin G-resistant *Staphyloccocus aureus* and *Salmonella typhi* was first reported by Pérez & Anesini (1994) and Anesini & Pérez (1993).

One study was performed with the aim to investigate the antimicrobial, anti-biofilm, and anti-cell adherence activities of native plants collected in northwestern Argentina. An ethanol extract of *Larrea divaricata* exerted anti-*Staphylococcus* spp. and anti-*Bacillus* spp. biofilm formation activity, with percentages of anti-biofilm activity ranging from 55% to 62% for *Staphylococcus* spp., and 66% for *Bacillus* spp. Moreover, *L. divaricata* exerted a moderate antimicrobial activity against *Bacillus* sp. with a minimum inhibitory concentration (MIC) of 250 µg/mL; the effect against *Staphylococcus* sp. was higher with MIC: 31.25 µg/mL. The *L. divaricata* extract showed a remarkable activity against the genus *Staphylococcus*, with a sub-inhibitory concentration (SIC) value equal to that of gentamicin (15.62 µg/mL), which was used as the reference antibiotic. With respect to the anti cell adherence, the effect of *L. divaricata* extracts against *Bacillus* sp. and against *Staphylococcus* sp. was lower than their anti-biofilm activity, indicating that its ability to inhibit biofilm formation occurs during a step subsequent to cell adherence (Romero *et al.,* 2016).

Recently, the antimicrobial and nematicide activity of a cold CH_2Cl_2 extract of *L. divaricata* has been demonstrated. Its resin has a strong antimicrobial activity against methicillin-sensitive *Staphylococcus aureus* ATCC 25923 (MSSA) and methicillin-resistant *S. aureus* ATCC 43300 (MRSA) (minimum inhibitory concentrations, MIC values of 16-32 µg resin/mL). Additionally, the combination of *L. divaricata* and *L. nitida* could constitute a potential strategy to reduce the antibiotic doses employed, thus being a potential alternative to reduce bacterial resistance. On the other hand, *L. divaricata* resins have a low nematicidal activity toward J2 *Meloidogyne incognita*, an important nematode infecting horticultural crops. The authors demonstrated a relationship between the nematicide effect and the polyphenols content of the plant (Gómez *et al.,* 2021).

It has also been demonstrated that a cold extract, an infusion, a decoction and a digest obtained by simulated digestion of *L. divaricata* have inhibitory activity against clarithromycin- and metronidazole-sensitive and resistant *Helicobacter pylori* strains with MIC values for the infusion and digested extracts ranging from 0.04 to 0.08 mg/L, as compared to the MIC values obtained with the cold extract and the decoction (0.04-0.1 mg/L) (Stege *et al.,* 2006).

Moreover, the plant presents anti-*Mycobacterium* activity. The CH_2Cl_2/MeOH (1:1) extract caused a 100% growth inhibition of *M. tuberculosis* at a concentration of 50 µg/mL (Rivero-Cruz *et al.,* 2005; Gomez-Cansino *et al.,* 2017).

Antifungal activity has also been reported for this plant. For example, the ethanol extract has antifungal activity on filamentous fungi such as *Penicillum notatum*, causing an inhibition of 47%, 68% and 89% at 0.4, 0.6 and 0.8 mg/mL dry matter, respectively. The extracts are also capable of inhibiting the growth of *Saccharomyces carlsbergensis* and *Rhodotorula* spp., on which the highest growth inhibition activity was observed (Quiroga *et al.,* 2001).

Furthermore, high growth inhibitory effects were also reported for *Phytophthora nicotianae, P. citrophthora* and *P. palmivora,* which are known to produce a severe damage to olive trees known as "dry branch" (Boiteux *et al.,* 2014).

More recently, the antifungal activity of a methanol extract of *L. divaricata*, either alone or as microcapsules or tablets, was demonstrated *in vitro* against 10 yeast strains obtained from vaginal exudates of patients with yeast infection. The study was performed on three strains of *Saccharomyces cerevisiae*, three strains of *Candida albicans*, three strains of *C. glabrata* and one strain of *C. tropicalis*. Some of the *S. cerevisiae, C. albicans* and non-albicans strains were azole-susceptible (85%) and some were resistant (15%). Since it presented MIC values < 0.5 mg/mL, the extract was considered a strong antifungal drug for all *Candida* strains (Moreno *et al.,* 2018).

The lactic acid: glucose: water (LGH) extract of *L. divaricata* and other species of *Larrea*

demonstrated a significant antimicrobial activity against *C. albicans*, and this activity was higher than that exerted by the individual bioactive constituents. Notably, the mixture of *L. cuneifolia* and *L. divaricata* extracts, prepared in topical formulations, revealed a synergistic antifungal effect, thus highlighting their potential for the treatment of candidiasis (Espino *et al.*, 2019).

In another study, the association of extracts obtained from *Zuccagnia punctata* and *L. divaricata* showed inhibitory activity on *S. cerevisiae* and *Candida* spp. strains. Polyphenols such as Nordihydroguaiaretic acid (NDGA) and 2,4 dihydrochalcone were found to be involved in the effects observed. The authors propose the use of the combination in vaginal infections (Moreno *et al*,. 2020).

Immunomodulatory effects: Pro- and anti-inflammatory effects

In folk medicine, *L. divaricata* is widely used as anti-inflammatory (Del Vitto *et al.*, 1997); nevertheless, scientific studies have demonstrated a dual pro-inflammatory and anti-inflammatory behavior that is dependent on the type and extract concentration. Pedernera *et al*. (2006) studied the anti-inflammatory effect by two methods: the cotton pellet-induced granuloma and the carrageenan-induced arthritis models. The authors also demonstrated the anti-ulcerogenic activity of a methanol extract. In all cases, the extract proved to be more active in the acute phase of inflammation.

The anti-inflammatory activity of an aqueous extract of *L. divaricata* was also demonstrated in the carrageenan-induced inflammation test and the ear edema test in C3H mice. A dose-dependent anti-inflammatory effect was found when the extract was administered at 10 mg/kg, 50 mg/kg and 100 mg/kg orally. Moreover, the extract modulated the secretion of pro-inflammatory cytokines such as TNF-α and IL-6 and the anti-inflammatory cytokine IL-10 (Peralta *et al.*, 2015).

L. divaricata and *L. nitida* were recently reported to be capable of inhibiting lipoxygenase (LOX) *in vitro* at a low concentration. This effect could explain the anti-inflammatory effect of the plant described previously (Moreno *et al.*, 2020).

The capacity to modulate the immune response has also been reported. Two aqueous extracts, decoction and infusion, from *L. divaricata*, at both low and high concentrations, can modulate the activity of peritoneal macrophages (MΦ), inducing the phagocytosis of zymosan and *Candida albicans*, the production of NO and the activation of iNOS expression. However, high extract concentrations induce apoptosis (Davicino et al., 2006). The same authors corroborated that high concentrations of the decoction of the leaves produce the activation of MΦ but also apoptosis related to an increase of phagocytosis of zymosan, lysosomal enzymatic activity, NO production, TNF-α release, and expression of CD14, TLR4, and CR3. The decoction is also capable of increasing the binding of zymosan-FITC and superoxide anion (O_2^-) production in relation to the increase of dectin-1 expression in MΦ (Davicino *et al.*, 2007; Davicino *et al.*, 2015). These effects would play a crucial role in the induction of innate defense mechanisms in infections with intracellular pathogens such as mycobacteria. When the same study was performed *in vivo* and the decoction was administered at a dose of 0.5 mg/kg, the effects observed were the same as those observed previously *in vitro* in MΦ isolated from treated animals (Davicino *et al.*, 2007).

In another study it was demonstrated that a fraction obtained from an NDGA-free aqueous extract of *L. divaricata* was capable of exerting an immunomodulatory effect *in vitro* by inducing the release of $O_2^-$and H_2O_2, but decreasing the release of NO in normal MΦ (Martino *et al.*, 2010). In a mice systemic infection model with *C. albicans*, the same fraction induced phagolisosomal O_2^- release, cytosolic proteins synthesis, and was able to revert the decrease of NO levels induced by *Candida* spp. In this model, apoptosis was induced in both normal and infected cells. The latter phenomenon was confirmed by the presence of DNA fragmentation. Taken together, all these effects would lead to a long-term activation of MΦ (Martino *et al.*, 2012).

It has been demonstrated that proteins from the crude aqueous extract of *L. divaricata* administered with adjuvants to mice can induce a good specific immune response, showing cross-reaction with proteins of different Gram-negative bacteria such as *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. In this sense, the IgG and IgA antibodies elicited by immunization with *L. divaricata* proteins were able to opsonize these bacteria and favor phagocytosis (de Anaya *et al.*, 2009; Sasso *et al.*, 2012; Canale *et al.*, 2018). The anti-*L divaricata* proteins antiserum can inhibit enzymatic activities of *P. aeruginosa* such as the hemolytic activity of soluble cell proteins, the protease activity and

extracellular proteins by 100%, 55%-70% and 44% and 95%, respectively (Sasso *et al.*, 2012).

The immunogenic protein fractions of *L. divaricata* (30 kDa-50 kDa), are like the extracellular adhesin proteins of *P. aeruginosa*, such as elastase B and protease IV, which are involved in cellmatrix adhesion. Therefore, the antibodies obtained by immunization with these proteins are able to recognize conformational epitopes of *P. aeruginosa* extracellular proteins (Ferramola *et al.*, 2020). These findings could be relevant for the development of alternative therapies for patients suffering from nosocomial infections with *P. aeruginosa*.

Antioxidant effects

The aqueous extract of *L. divaricata* presents a well-known antioxidant activity. Anesini *et al.* (2004) demonstrated that an aqueous extract of *L. divaricata* induces a dose-dependent secretion of peroxidase by stimulation of adrenoreceptors in the submandibular glands of female rats and also increases the total peroxidase activity. NDGA is not related to this activity, even though it is capable of inducing total peroxidase activity but at a lesser extent than the crude extract.

It has also been demonstrated that the aqueous extract presents peroxidase (Px)-, catalase (CAT)- and superoxide dismutase (SOD)-like activities. Unlike the SOD-like activity, the CAT activity is related to the presence of NDGA. The extract also inhibits lipid peroxidation and has free radical scavenger capacity. Both activities are not related to the presence of NDGA. The fact that the crude extract is capable of modulating the peroxidase activity could be of great importance in the oral cavity, since peroxidase plays a role in the defense of the oral cavity through the scavenging of ROS and through the modulation of inflammatory processes induced by microorganisms. The extract was then proposed to be used in pharmaceutical preparations (mouth rinse, dental cream, gels and powders) as an oral antioxidant to prevent cancer, periodontitis and other oral diseases in humans (Turner *et al.*, 2018).

The aqueous extract of *L. divaricata* can modulate the antioxidant status in submandibular glands of both normal and STZ-induced diabetic rats. The extract reverted the oxidative stress induced by STZ, decreasing the lipid-peroxidation, as well as protein carboxylation and the production of ROS induced by STZ (Peralta *et al.*, 2013; Peralta *et al.*, 2019).

In another study, the antioxidant activity of *L. divaricata* and *L. cuneifolia* collected in the province of Catamarca, Argentina, was corroborated by different methods such as the ferric reducing antioxidant power (FRAP), the Trolox equivalent antioxidant capacity (TEAC) and the 2,2-diphenyl-1-picrylhydrazyl scavenger activity (DPPH) *in vitro* assays. These assays demonstrated that *L. cuneifolia* extracts had the highest antioxidant activity, followed by *L. divaricata* (Lorenzo *et al.*, 2020).

The antioxidant activity of the association of methanolic extracts of *L. divaricata* and *Z. puntacta* was also determined by different methods. The association showed a good potency in the 2,2-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diamonium salt (ABTS) assay and in the azo-compound 2,2'-azobis(2-amidinopropane dihydro-chloride (AAPH) assay. In the hydroxyl radical and H_2O_2 scavenging assays, the scavenging potency of the mixtures was similar to that of individual extracts and no significant differences were observed between each extracts and the mixtures (Moreno *et al.*, 2020) The aqueous extract of *L. divaricata* also presents β-carotene bleaching capacity and metal chelating capacity (Carabajal *et al.*, 2020).

More recently, when Chi-CMCSiO2 composites were loaded with a *L. divaricata* aqueous extract, a high incorporation rate and a release of 100% was achieved in almost 4 days. The released extract retained the free radical scavenging capacity. These results would support the use of the extract in this new formulation for the treatment of periodontal diseases (Alvarez-Echazú *et al.*, 2018).

Finally, the antioxidant activity of the aqueous extract of *L. divaricata* could be involved in the anti-inflammatory effects exerted by the extract. The effects are summarized in Table No. 1.

Table No. 1
Summary of reported effects of *L. divaricata* Cav

Activities	***Reported activity***	***References***
Antimicrobial	**Antiviral activity** Arenaviridae family	Konigheim *et al.*, 2006
	Antibacterial activity	Perez & Anesini, 1994

	Staphylococcus aureus *Salmonella typhi* *Helicobacter pylori (clarithromycin and metronidazole susceptible and resistant strains)*	Anesini & Perez, 1993
	Anti-biofilm activity *Staphylococcus* spp. *Bacillus* spp.	Romero *et al.*, 2016
	Anti-mycobacterial activity	Rivero–Cruz *et al.*, 2005
	Nematicidal activity: *Meloidogyne incognita*	Gómez *et al.*, 2021 Gómez Cansino *et al.*, 2017
	Antifungal activity *Penicillium notarum,* *Saccharomyces carlsbergensis* *Rhodotorula*s pp. Growth inhibitory effects *Phytophthora nicotianae,* *Phytophthora citrophthora* *Phytophthora palmivora* *Saccaromyces cerevisiae* *Candida albicans* *Candida glabrata* *Candida tropicalis*	Quiroga *et al.*, 2001 Boiteux *et al.*, 2014 Moreno *et al.*, 2018; Boiteux *et al.*, 2014;
Immunomodulatory	**Anti-inflammatory effect** Modulation of the pro-inflammatory cytokines responses: TNF-α, IL-6 and the anti-inflammatory cytokines IL-10 Inhibition of LOX	Del Vitto *et al.*, 1997 Pedernera *et al.*, 2006 Peralta *et al.*, 2015 Moreno *et al.*, 2020 Davicino *et al.*, 2006 Davicino *et al.*, 2007a Davicino *et al*., 2007b Davicino *et al*., 2015
	Modulation of the immune system	Martino *et al.*, 2010 Martino *et al.*, 2012
	Immunogenicity of the protein fraction	Ferramola *et al.*, 2020
Antioxidant	**Induces peroxidase secretion and stimulates the peroxidase activity**	Anesini *et al.*, 2004
	Peroxidase, catalase and superoxide dismutase-like activities **Inhibitory action on lipid peroxidation** **Free radical scavenger activity**	Turner *et al.*, 2011 Alvarez-Echazú *et al.*, 2018
	FRAP, TEAC and DPPH activities	Lorenzo *et al.*, 2020
	ABTS and AAPH bleaching activities **β-carotene bleaching capacity** **Metal-chelating capacity**	Carabajal *et al.*, 2020

Could the aqueous extract of Larrea divaricata be a good agent to use against COVID-19?

To respond this question, the toxicity study made with the aqueous extract, its chemical composition and the effect of the mayority compounds found in the extract are presented bellow.

Toxicity studies of the aqueous extract

Toxicity studies indicated that the crude aqueous extracts of *L. divaricata* leaves were non-toxic, with high LD_{50} values in both female and male mice. The extract tested in mice did not produce signs of toxicity or behavioral changes when administered orally, as assessed by routine histological and clinical evaluation. For example, LD_{50} values in Swiss Albino mice were 10000 mg/kg (8196 mg/kg-12200 mg/kg) for male mice, and 4000 mg/kg (3030 mg/kg-5276 mg/kg) for female mice (Anesini *et al.,* 1997). According to Kennedy et al. (1986), those substances presenting LD_{50} values that are about 5.0 g/kg administered by the oral route may be considered non-toxic.

In another study, an infusion of *L. divaricata* administered orally in a dose of 0.76 g/kg to male Albino Swiss mice for 16 days proved to be safe, since it did not modify parameters such as drink intake, organ weight/body weight (OW/BW) ratio, organ histology, bronchoalveolar fluid (BALF) characteristics and did not modify the elevated plus-maze test. Moreover, a non-anxiolytic-like activity was observed with the extract (Bigliani *et al.,* 2010).

More recently, the sub-chronic toxicity and the local toxicity of an aqueous extract of the plant have been determined in mice and rabbits. To test sub-chronic toxicity, the extract was administered to mice orally at a dose of 250 mg/kg during 28 days; while the local toxicity was tested on rabbits by applying a dose of 500 mg/animal on the skin, and a dose of 100 mg/animal on the eyes. To determine sub-chronic toxicity, biochemical and histopathological studies were carried out on the organs together with the evaluation of the effects on the central nervous system (CNS). The toxicity on CNS was evaluated by the forced swimming test and the tail suspension test, while local toxicity was assessed by the local toxicity primary acute-irritation dermal test and the ocular irritation test. When the sub-chronic toxicity was evaluated, with the exception of the eosinophil counts, hematological and biochemical parameters remained within the reference range for control animals and no statistically significant differences were observed between groups. Moreover, the extract modified neither the levels of alanine aminotransferase (ALT), nor cholesterol, nor creatinine, which are good indicators of liver and kidney functions. No histological damage was observed in these organs either. Moreover, the extract did not cause CNS toxicity as regards depressant or stimulant activities. Finally, the application of the extract on the skin or on eyes of rabbits produced neither edema nor erythema, indicating the absence of local irritation (Peralta *et al.,* 2013). The studies presented above demonstrate the low toxicity of the extract when administered as phytomedicine preparations.

Chemical composition of the aqueous extract

The determination of the chemical composition of *L. divaricata* was done in different extracts; however, the aqueous extract seems to be the most relevant due to its innocuousness and the ease of preparation to be used in future phytomedicines.

Table No. 2
Chemical composition of the aqueous extract of *Larrea divaricata*

A-

Total GAE g %	***Total Quercitrin g %***
10.68 ± 0.43	2.46 ± 0.16

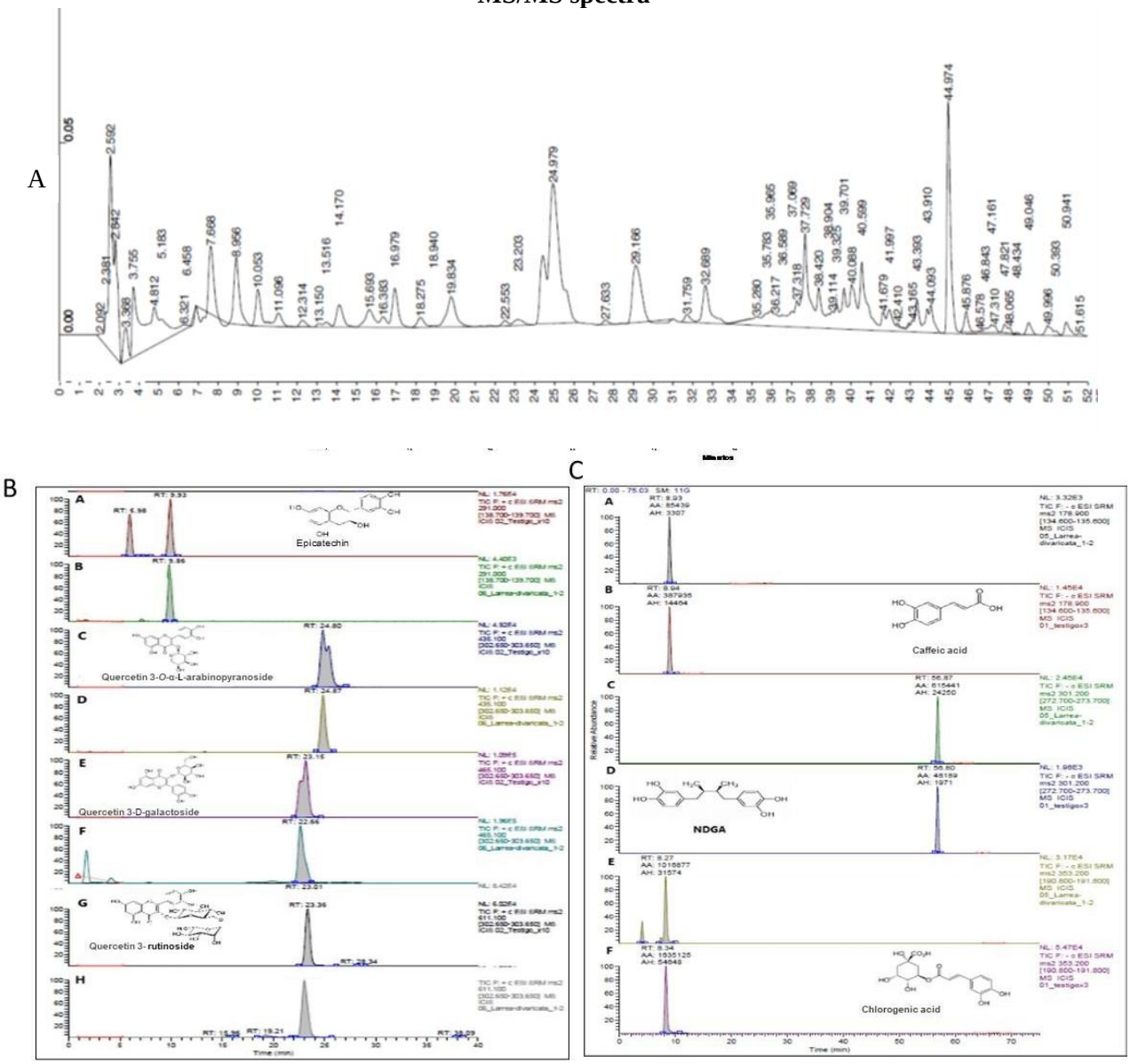
B-

Compound	**g%**
Caffeic acid	0.0075
Chlorogenic acid	0.0024
p-Hydroxybenzoic acid	0.0343
2-Vicenin	0.083
Nordihydroguariaretic acid (NDGA)	0.94
Quercetin-3-O-arabopyranoside	0.005
Quercetin-3-O-galactoside	0.085

Rutin	0.034
Epicatechin	0.0048

A. Results are expressed as GAE g% or quercitrin % and represent mean ± SEM of two independent experiments performed by triplicate. B. Data obtained from HPLC-UV and HPLC-MS/MS (Own laboratory data not published elsewhere)

Figure No. 4
Phytochemical composition of *Larrea divaricata* Cav. Aqueous extract. A. HPLC-UV B and C. HPLC MS/MS spectra



The presence of polyphenols such as flavonoids and caffeoylquinic acids was demonstrated by HPLC-MS/MS and HPLC-UV. The HPLC-UV chromatogram of the aqueous extract revealed the presence of NDGA (retention time: 44.97 min) and 4-hydroxybenzoic acid (4-HBA) (retention time: 10.05 min) together with a mixture of flavonoids (Figure No. 4A). To identify more compounds, an HPLC-MS/MS was done. Working in positive mode, the flavonoids rutin, epicatechin, quercetin-3-O-arabinopyranoside and quercetin-3-O-galactoside were identified (Figure No. 4B). In negative mode, the phenolic acids caffeic acid, chlorogenic acid and NDGA were identified (Figure No. 4C) (Own laboratory data not published elsewhere)

Pharmacological effects and potential effects of the main compounds found in L. divaricata aqueous extract against SARS-CoV-2

Docking experiments have been performed to screen for compounds that could potentially bind and inhibit key SARS-CoV-2 proteins. In these experiments the free binding energy between a ligand and a receptor is determined, with a low free binding energy indicating a stronger bond between the ligand and receptor (Forli *et al.,* 2016). The compounds identified by this method can be then tested on cell-based assays to assess their effectiveness and toxicity *in vitro* before being tested in animal and clinical trials. The effect of pure compounds is shown bellow.

Effects of NDGA

NDGA, also named masoprocol, is a molecule vastly studied that can be isolated from *Larrea* species. Depending on the concentration, NDGA may present either antioxidant or pro-oxidant activity. It is assumed that the effects of NDGA *in vivo* depends on the balance between its protective effect, which is related to its antioxidant capacity and the electrophilic interaction with several signaling proteins, and its toxic effect, which is related to an enhanced O_2^- formation and GSH depletion (Satoh *et al.,* 2013; Peralta *et al.,* 2018). Not only is NDGA a ROS scavenger but it can also modulate the functioning of endogenous antioxidant systems by the inhibition of the redox sensor KEAP1 (Kelch-like Erythroid Cell-derived Protein with Cap'n'collar Homology (ECH)-associated Protein) leading to nuclear factor erythroid-2-related factor2 (Nrf2) activation. Nrf2 controls the basal and induced expression of an array of antioxidant response element-dependent genes to regulate the physiological and pathophysiological outcomes of oxidant exposure.

NDGA is also known to be an inhibitor of lipoxygenases (LOXs) (Czapski *et al.,* 2012). Therefore, by exerting anti-inflammatory and antioxidant effects, NDGA induces a protective effect on renal function (Mundhe *et al.,* 2019) and neuroprotection (Czapski *et al.,* 2012; Xue *et al.,* 2013) and, by modulating plasma lipid levels, the compound protects the liver function (Zhang *et al.,* 2016).

With respect to the inhibitory capacity on SARS-CoV-2 proteases, Laskar & Choudhury, (2021), have shown in docking studies carried out with the SARS- CoV-2papain-like protease (PLpro) that NDGA has a low affinity for this enzyme. On another study, NDGA demonstrates, binding affinity to different conformations of PLpro SARSCoV-2 with high docking scores and interaction with Tyr268 and Gln269 key binding residues (Wu *et al*., 2020; Ismail *et al.,* 2021). Moreover, it was demonstrated that NDGA binds to the main SARS-CoV-2 protease with a binding affinity of -7.9 Kcal/mol but the isomers of the flavonoid quercetrin, analogs and derivatives were considered the best candidates (Wang *et al.,* 2020).

The antiviral activity of this compound is well-known. NDGA has been reported to inhibit the replication of dengue virus (Soto-Acosta *et al.,* 2014). In addition, NDGA also inhibits the replication of hepatitis C virus (HCV), a member of the Hepacivirus genus within the Flaviviridae family and Sherman virus (Orthobunyavirus) (Syed & Siddiqui, 2011; Martinez *et al.,* 2021). This compound also has activity against herpes simplex virus, human immunodeficiency virus, human papillomavirus, cowpox virus, vaccinia virus and Junín virus (Chen *el al.,* 1998; Craigo *et al*., 2000; Konigheim *et al*., 2005; Hwu *et al.,* 2008).

It has recently been demonstrated that NDGA inhibits the replication of the West Nile virus and Zika virus (Merino-Ramos *et al.,* 2017).

Effects of quercetin and derivatives

Quercetin, also known as 3,3′,4′,5,7-pentahydroxyflavone is a widely distributed plant flavonoid found in the leaves of several plants, seeds, and grains, in which it is conjugated with residual sugars to form quercetin glycosides (Li *et al.,* 2016). This compound has a wide range of activities such as antioxidant, anti-inflammatory, immunomodulatory and antiviral (Hollman *et al.,* 1995; Nair *et al.,* 2002; Robaszkiewicz *et al.,* 2007; Uchide & Toyoda, 2011). All these properties make this molecule a good candidate for the treatment of covid-19. It is known that quercetin, luteolin and epigallocatechin gallate (EGCG) present anti-inflammatory and antioxidant activities by reducing ROS production and inhibiting the TXNIP-NLRP3 inflammasome in endothelial cells, which leads to a decrease ofIL-1βsynthesis. Moreover, quercetin can prevent cell death by inhibiting apoptosis (Wu *et al.,* 2014). Another study shows that quercetin can induce the synthesis ofsirtuin1 (SIRT1), whose suppression is related to the development of acute pulmonary inflammation during sepsis, by controlling the NLRP3 inflammasome activation (Gao *et al.,* 2015).

The inhibition of SARS-CoV-2 replication could be achieved by interfering with the functions of the main viral proteinases Mpro or 3CLpro and the papain-like protease, as explained above. In this sense, Ryu *et al*. (2010) reported that flavonoids such as apigenin, luteolin, and quercetin show significant SARS-CoV 3CLpro inhibiting activity. The IC_{50} values of the 3CLpro inhibitors, apigenin, luteolin, and quercetin were 280.8 μM, 20.2 μM, and 23.8 μM. In another study, using biophysical techniques and binding simulation experiments, quercetin was identified as a reasonably potent inhibitor of SARS-CoV and SARS-CoV-2 3CLpro (Ki ~ 7 μM) (Nguyen *et al*., 2012; Abian *et al.*, 2020)

Moreover, docking studies determined that quercetin has a high affinity for other proteases, for example, the SARS CoV-2papain-like protease was selected as a drug target due to its viral polyprotein and host cell proteins processing activity that are crucial for viral replication. Using the FleX software, it has been demonstrated that quercetin has a stable bonding pattern, forming eight hydrogen bonds with a docking score of -24.9869 Kcal/mol and with good IC_{50} values. Similar results were found with the flavonoid rutin (rutinoside of quercertin), which docked with the target forming ten hydrogen bonds with a docking score of -27.0507 Kcal/mol (Laskar & Chodhury, 2021). Quercetin showed an IC_{50}value of 8.6 ± 3.2 μM against SARS-CoV PLpro (Park *et al*., 2017) and was shown to interact with both the viral S proteinACE2 receptor and with the S protein (Smith & Smith, 2020).

Furthermore, quercetin-3β-galactoside binds the SARS-CoV 3CL protease and inhibits its proteolytic activity with an IC_{50}value of 42.79 ± 4.95 μM (Chen *et al*., 2006). Quercetin can also block SARS-CoV entry into Vero E6 cells with a half-effective concentration (EC_{50}) of 83.4μM and with low cytotoxicity (CC_{50}=3.32 mM) (Yi *et al.*, 2004).

Quercetin also rebalances dysbiosis of the gut microbiome, thus reducing the risk of inflammatory diseases (Porras *et al.*, 2017). Moreover, this compound is capable of decreasing the elevated Th17/Treg ratio (Yang *et al.*, 2018), which is considered a pathological event in covid-19, particularly because IL-17 is an inflammatory cytokine that drives the neutrophil recruitment in the lung tissue of severe covid-19 patients, as explained above.

Moreover, quercetin exhibits a strong antiviral activity against picornaviruses, the vesicular stomatitis virus and influenza A subtypes H1N1, H5N2, H7N3, H9N2 (Dinarrello *et al.*, 2013; Cho *et al.*, 2015; Zakaryan *et al.*, 2017). It has previously been demonstrated that quercetin inhibits many serotypes of rhinovirus, echovirus (type 7, 11, 12, and 19), coxackievirus (A21 and B1), and poliovirus (type 1 Sabin) at a MIC of 0.03 to 0.5μg/mL in Hela and WI-38 cells (Itsuka *et al.*, 1982). Quercetin also reduces plaque formation by RNA and DNA viruses [Respiratory Syncytial Virus (RSV), type 1Poliovirus, type 3 Parainfluenza, and Herpes Simplex Virus-1(HSV-1)) (Kaul *et al.*, 1985). Quercetin also inhibits the cell entry of SARS-CoV with an EC_{50}value of 10.6 μM (Yi *et al*., 2004). Rutin, on the other hand, was found to have an antiviral effect against the avian influenza A strain H5N1 (Ding *et al.*, 2018).

In summary, quercetin, with well-known absorption, distribution, metabolism, excretion and toxicity (ADMET) properties, can be considered a good candidate for further optimization and development of new drugs for covid-19 treatment.

Effects of chlorogenic and caffeic acids

Molecular docking and molecular dynamics studies have been performed to test the capacity of caffeic acids, chlorogenic acid and derivatives to bind to proteases and proteins that are essential for virus replication. For example, some studies were performed to find caffeic acid derivatives with capacity of binding SARS-CoV-2 proteins such as Mpro (6LU7), the S2 subunit (6LXT), the Nsp15 endoribonuclease (6VWW), the spike ectodomain open state structure (6VYB), and the spike closed state glycoprotein structure. To do this, a library of 27 caffeic acid derivatives was screened against these proteins. Three derivatives, namely khainaoside C, 6-O-caffeoylarbutin, khainaoside B, khainaoside C and vitexfolin A showed a good affinity for M^{pro}, Nsp15, the coronavirus fusion protein, the spike open state and the closed state structure (Adem *et al.*, 2021).

Caffeic acid and chlorogenic acid have the capacity to bind, with high affinity, to SARS-CoV-2heat shock protein family A (Hsp70) member 5 (HSPA5) substrate-binding domain β (SBDβ) (HSPA5 SBDβ). HSPA5 has been reported to be the recognition site for the SARS-CoV-2 spike protein. These compounds might then affect SARS-CoV-2 attachment to stressed cells (Elfiky, 2021). Moreover, caffeic acid phenethyl ester can bind to SARS-CoV-2Mpro with high affinity, affecting viral replication (Kumar *et al.*, 2021).

The caffeic acid derivatives associated with

metals and ions present virucidal activity against herpes simplex virus (Langland *et al.,* 2018), SFTS (severe fever with thrombocytopenia syndrome) virus (Ogawa *et al.,* 2018), and influenza virus (Utsunomiya *et al.,* 2014).

Effects of catechins

Molecular docking studies have determined that catechin (flavan-3-ol) can bind to amino acid residues located near the receptor binding domain (RBD) site of the S protein of SARS-CoV-2 and causes fluctuation in the amino acid residues of the RBD and its proximities. Catechin can bind to the interface of RBD/ACE2complex causing fluctuation of the α-helices and β-strands of the protein complex. Protein–protein interaction studies carried out in the presence of catechins also corroborated the above-mentioned findings, suggesting the efficacy of these polyphenols in hindering the formation of the Sprotein-ACE2 complex. This computational study predicted, for the first time, the therapeutic potential of two polyphenols against SARS-CoV-2 (Jena *et al.,* 2021). Catechin can also bind severalSARS-CoV-2 proteins such as 3CLpro, cathepsin L, the RBD of the Sprotein, NSP-6, and the nucleocapsid protein, with binding free energies (ΔG_{bind}) that ranged from -5.09 kcal/mol (cathepsin L) to -26.09 kcal/mol (NSP6) (Mishra *et al.,* 2020).

Catechin and (-)-epigallocatechin-3-gallate (EGCG) showed a wide range of antiviral activities against adenovirus, influenza virus, Zika virus, herpesvirus, and hepatitis virus (Weber *et al.,* 2003; Ciesek *et al.,* 2011). EGCG showed inhibitory activity against the SARS-CoV-2 3CLprotease and on the PLpro protein. In the case of the SARS-CoV-2 3CL protease, a dose-dependent response was observed, with a half inhibitory concentration (IC_{50}) value of 7.58 μg/ml (Jang *et al.,* 2020; Wu *et al.,* 2020). Another study confirmed that EGCG and epicatechin (EC) can bind to the S1 ubiquitin-binding site of PLPro, thus inhibiting its protease activity and abrogating the SARS-CoV-2 inhibitory function on the ubiquitin proteasome and the interferon stimulated gene systems (Chourasia *et al.,* 2021).

These results suggest that catechins could be potentially useful to treat covid-19.

Effects of 4-hydroxibenzoic acid

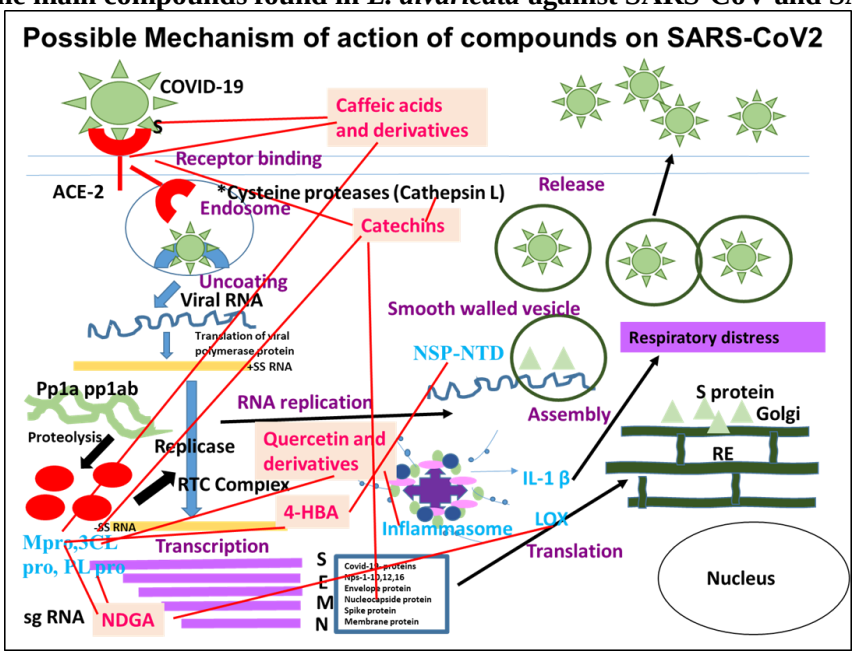
In silico studies have demonstrated that four compounds including 4-hydroxybenzoic acid (4-HBA), benzoic acid, 4-aminobenzoic acid and salicylic acid were effective to inhibit the NSP-NTD (responsible for packing the viral RNA into the crown-like capsid) (Mishra *et al.,* 2020). It has also been demonstrated that benzoic acid derivatives as 4-hydroxybenzoic acid are capable of interacting with the main protease 6WNP, showing a low docking score (Stefaniu *et al.,* 2020). Moreover, 4-hydroxybenzoic acid was reported to have antibacterial (against Gram-positive and Gram-negative bacteria), antifungal, antialgal, antimutagenic and estrogenic activities (Manuja *et al.,* 2013).

Ester derivatives of hydroxybenzoic acid have been reported to be widely used for the treatment of infections caused by hepatitis B virus, human papilloma virus, herpes simplex virus, cervicitis and cervical erosions in human and animals (Ximenes *et al.,* 2010). Luecha *et al.* (2009) reported that 4-HBA isolated from an ethanolic extract of *Vitex glabrata* (Verbenaceae), commonly known as smooth chaste tree, has anti-inflammatory effects.

The antimicrobial effect of *L. divaricata* extracts, as well as its majority compound NDGA had been widely demonstrated. The antiviral activity of the extracts inhibiting RNA virus replication and the high content of NDGA make the plant a good candidate to be assayed against SARS-CoV-2. Moreover, the extracts antibacterial and antifungal activities could be useful in superinfections produced during Covid-19 as coadjuvant of antibiotics.

Covid-19 is not the only cause of deaths in this pandemic, a usual complication of viral infections is secondary superimposed bacterial infection or a superinfection. In fact, high incidence of severe infection and mortality in Covid-19 patients is attributed to these infections. Patients who have severe form of the disease and those requiring prolonged stay in intensive care units (ICUs) are more prone to developing super added infection by nosocomial pathogens. The most common type of infection observed among Covid-19 patients is ventilator-associated pneumonia (VAP), followed by bacteremia with sepsis and urinary tract infections (UTIs) (Nag & Kaur, 2021). It is important to note that not only NDGA contribute to the antimicrobial effect of the plant but also flavonoids and caffeylquinic acids. These compounds together can synergize the antimicrobial effects but also mitigate adverse effects.

Figure N. 5
Effects of the main compounds found in *L. divaricata* against SARS-CoV and SARS-CoV-2



Catechins as potential inhibitors of the structural proteins protein S, 3CL-Pro, PLPro, cathepsin and the RBD/ACE2-complex. Caffeic acid and derivatives as inhibitors of ACE-2, S protein and MPro. Quercetin and derivatives as inhibitor of MPro, 3CLPro, PLPro and the inflammasome. 4-HBA as inhibitor of 3CLPro and NSP-NTD. NDGA as inhibitor of MPro, PLPro and LOX

The aqueous extract of *L. divaricata* not only demonstrated to be safe in animal models but also has many polyphenol compounds that demonstrated to exert a variety of activities such as anti-inflammatory, immunomodulatory and antiviral activity. These compounds have high affinity for SARS-CoV-2 and SARS-CoV proteases and proteins involved in virus replication and have affinity to spike protein counteracting binding of the virus to ACE2. It is generally accepted that relatively polar extracted fractions contain higher levels of bioactive and antimicrobial compounds, as compared to their non-polar counterparts (Han *et al.*, 2007; Tian *et al.*, 2009; Wigmore *et al.*, 2016). This phenomenon allows suggesting that highly polar glycosylated compounds have a higher antiviral activity than non-glycosylated ones (Choi *et al.*, 2009).

Together with antimicrobial activity, it is important to emphasize the anti-inflammatory activity of the aqueous extract and its capacity to modulate the immune system. These activities could be useful to counteract the inflammation produced during Covid-19, which mainly affects the lungs, by modulating macrophages activation. On macrophages, the extract can both induce the innate defense by inducing oxidative stress and blunt the pro-inflammatory cytokines production. In addition, the aqueous extract is able to induce a good specific immune response in presence of different microorganisms.

During Covid-19 infection, a great oxidative stress is produced which induces lipid peroxidation and cell death. The aqueous extract of the plant possesses antioxidant activity not only modulating the level of antioxidant enzymes such as catalase, peroxidase and superoxide dismutase but also exerting simil enzymatic activity. Moreover, the extract is capable to modulate nitric oxide production inducing a balance between cell apoptosis and proliferation.

How could the compounds, present in the aqueous extract, be involved in the action of the extract? Catechins could act as potential inhibitors of the structural proteins protein S, 3CL-Pro, PLPro, cathepsin and the RBD/ACE2-complex; Caffeic acid and derivatives as inhibitors of ACE-2, S protein and MPro; Quercetin and derivatives as inhibitor of MPro, 3CLPro, PLPro and the inflammasome; 4-HBA as inhibitor of 3CLPro and NSP-NTD and NDGA as inhibitor of MPro, PLPro and

lipooxigensae (LOX). All these effects could be involved in the inhibition of virus replication, transcription and binding to host cells.

CONCLUSIONS

Taking into account these evidences, *L. divaricata* and, principally, its aqueous extract could be considered promising agents to be assessed for the treatment of Covid-19 as a source of antiviral compounds to be used either isolated or as a crude extract. In the latter option, synergistic effects could also be achieved.

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