

Isolated Compounds from *Buddleja Coriacea* with Antibacterial and Anti-Inflammatory Activities in the Urinary Tract



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ABSTRACT

Buddleja coriacea Remy is one of the plant species used by the Bolivian population for the treatment of urinary infections. This study aimed to identify the extract, fractions, and compounds responsible for the antibacterial and anti-inflammatory activities of *B. coriacea* leaves. Bioguided isolation of compounds with antibacterial and anti-inflammatory activities was carried out by measuring the antibacterial effect against specific pathogenic microbial strains, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Serratia marcescens*, and the inhibition of NF- κ B in RM-2 and MM.14Ov cells. Bioassay-guided isolation led to the isolation and characterisation of (4aR,4bS,5S,6aS,6bS,9aR,10aS,10bS)-6b-glycoloyl-5-hydroxy-4a,6a-dimethyl-8-propyl-4a,4b,5,6,6a,6b,9a,10,10a,10b,11,12-dodecahydro-2H-naphtho [2',1':4,5] indeno [1,2-d] [1,3] dioxol-2-one (**1**), 3-[3-(2-dimethylaminoethyl)-1H-indol-5-yl]-N-(4-methoxybenzyl) acrylamide (**2**), and (1 β ,11 β ,12 α -1,11,12-trihydroxy-11,20-epoxypicrasa-3,13(21)-diene-2,16-dione (**3**) by nuclear magnetic resonance and mass spectroscopy. All compounds showed antibacterial activity with minimum inhibitory concentration values of 11.64–11.81, 0.17–0.19, and 0.34–0.36 μ M, respectively, on the tested strains, while the positive control, ofloxacin, had a minimum inhibitory concentration of 27.66 μ M. Finally, all the compounds showed NF- κ B inhibitory activity with IC₅₀ values of 11.25–11.34, 0.15–0.16, and 0.33–0.36 μ M, respectively, in all cell lines, while the positive control, celastrol, had an IC₅₀ of 7.96 μ M. Thus, this study managed to isolate and evaluate for the first time the pharmacological potential of three compounds present in the leaves of *B. coriacea* with antibacterial and anti-inflammatory activities.

ABBREVIATIONS

AcOEt	ethyl acetate
ACT	actinomycin D
CC ₅₀	cytotoxic concentration 50 %
COPD	chronic obstructive pulmonary disease
DCM	dichloromethane
DH ₂ O	distilled water
Et ₂ O	diethyl ether
HEX	<i>n</i> -hexane
IKK $\alpha\beta$	suppresses the degradation of I κ B α and inhibits the translocation of p65 to the nucleus
IL-1 β	interleukin-1 β
IL-6	interleukin-6
LDH	lactate dehydrogenase
LPS	lipopolysaccharides
MIC	minimum inhibitory concentration
NF- κ B	nuclear factor kappa-light-chain-enhancer of activated B cells
QTOF	quadrupole time-of-flight
rt	room temperature
TLR	Toll-like receptor
TMS	tetramethylsilane
UTI	urinary tract infection
ZI	zone of inhibition

Introduction

The excretory system is fundamental for health, as it is responsible for the elimination of all kinds of unnecessary and detrimental substances and waste. In this context, UTIs are one of the most frequent infections among the adult population [1]. However, the increasing prevalence of antibacterial resistance among common uropathogens requires research on new therapeutic treatments [2] given that UTIs are diseases caused by pathogens, predominantly bacteria (such as *Escherichia coli*, *Staphylococcus aureus*, and *Klebsiella pneumoniae*) [3].

Infections begin with periurethral contamination of a pathogen that resides in the intestine that is followed by the pathogen's colonisation of the urethra and bladder. In the bladder, pathogens proliferate and can ascend to the kidneys and reach the vascular system, triggering more serious problems [4]. In this sense, TLRs trigger inflammatory reactions and bind primarily to LPS found in gram-negative bacteria [5], generating bacterial resistance and the chronicity of the disease. The chronicity of the disease implies that the inflammatory response can be prolonged over time, damaging the bladder and mucous membranes, which is detrimental to the patient's life quality [6].

Inflammation is a common physiological process in many diseases that involves tissue damage and is often followed by pain [7]. Inflammation plays a major role in UTIs due to continuous exposure to noxious stimuli in the urine [8]. The protein complex NF- κ B is a cytokine implicated in several inflammatory diseases, such as rheumatoid arthritis and inflammatory bowel disease. In this sense,

NF- κ B cytokine is involved in kidney inflammation caused by UTIs [9].

In response to different cellular stimuli, NF- κ B plays a complex role in different cell types and different disease stages [10]. NF- κ B is activated during infection and promotes the transcription of inflammatory cytokines such as TNF- α and IL-6 [4] that are overexpressed in bladder infections [11]. Another factor to control during an infection is the contact with microorganisms, such as bacteria, fungi, viruses, and parasites. During infection, TLRs detect the presence of bacterial components and activate macrophages that produce a series of proinflammatory cytokines (e. g., TNF- α , IL-1 β , IL-6) that act as a defensive response against the existence of pathogens [12].

Based on the above, the treatment of UTIs can be facilitated by natural products with medicinal properties [13]. Many studies have been conducted on the treatment of urinary problems using natural products with antibacterial and anti-inflammatory action [14]. These medicinal properties can be attributed to the bioactive phytochemical constituents of various chemical families such as alkaloids, essential oils, flavonoids, tannins, diterpenes, and phenolic compounds [14, 15].

In this context, the *Buddleja* genus has been used in traditional Chinese medicine for its anti-inflammatory properties [16]. Within this genus, *Buddleja coriacea* Remy, known as "Kiswara or Quishuar" and belonging to the Loganiaceae family, is native to Bolivia and has been traditionally used in the form of an infusion for the treatment of ulcers, stomach pain, liver problems, urinary tract inflammation, and rheumatism [17–19]. The *B. coriacea* species has been reported to contain flavonoid glycosides, tannins, alkaloids, and diterpenes [20]. Regarding the pharmacological activities of this plant species, it has been reported that the DCM/hydroalcoholic and hydroalcoholic extracts have presented anti-inflammatory activity [20].

This manuscript aims to provide a scientific support for the traditional use of *B. coriacea* as a remedy for UTI treatment. For this purpose, a bioguided phytochemical study has been carried out to identify those compounds present in the aqueous extract of *B. coriacea* with antibacterial and anti-inflammatory activities.

Determination of the antibacterial capacity of the aqueous extract, subextracts, fractions, and compounds of isolates of *B. coriacea* was carried out against *K. pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Serratia marcescens* bacteria. On the other hand, for the determination of the anti-inflammatory capacity has been determined by measuring the inhibition of NF- κ B production in the RM-2 (mouse prostate cell) and MM14.Ov (mouse ovary cell) cell lines.

Results

An aqueous extract was prepared from dry leaves of *B. coriacea* in order to reproduce the traditional use of the plant species [18]. Subsequently, from this aqueous extract, other subextracts were made in solvents of different polarity to study which compounds are responsible for its medicinal potential. In order to know the presence of natural metabolites and the complexity of the initial aqueous extract and subextracts, NMR spectroscopy was used.

The evaluation of the initial aqueous extract revealed a series of aromatic signals with low intensity at around δ_H 6.0–8.0 ppm that indicate the possible presence of compounds containing aromatic rings such as polyphenolic compounds, flavonoids, or alkaloids. Likewise, the presence of a large group of signals between δ_H 0–4.0 ppm (aliphatic region) was observed, which may correspond to protons close to alcohols, ethers, or ketones or the presence of aliphatic chains or essential oils (► Fig. 1).

The 1H NMR spectrum of the HEX subextract showed a pattern of aromatic compounds. Around δ_H 7.0 ppm and δ_H 5.5 ppm, signals of aromatic and vinyl hydrogens were found. Signals were observed at δ_H 3.8 ppm, corresponding to neighbouring hydrogens to a heteroatom such as N-CH₃ or O-CH₃. Finally, around 1.3–0.9 ppm, typical aliphatic signals of simple hydrocarbons were observed that did not overlap with the HEX solvent (► Fig. 1).

Analysing the spectra of the methanolic subextract, we observed that a group of low intensity signals appeared between δ_H 6.7–7.5 ppm, corresponding to aromatic signals. Likewise, signals at δ_H 5.08–5.36 ppm (minisignals) corresponding to the presence of an olefinic bond were observed (► Fig. 1).

In addition, the study carried out by 1H NMR together with the polarity of the used solvents allowed to affirm, based on the ob-

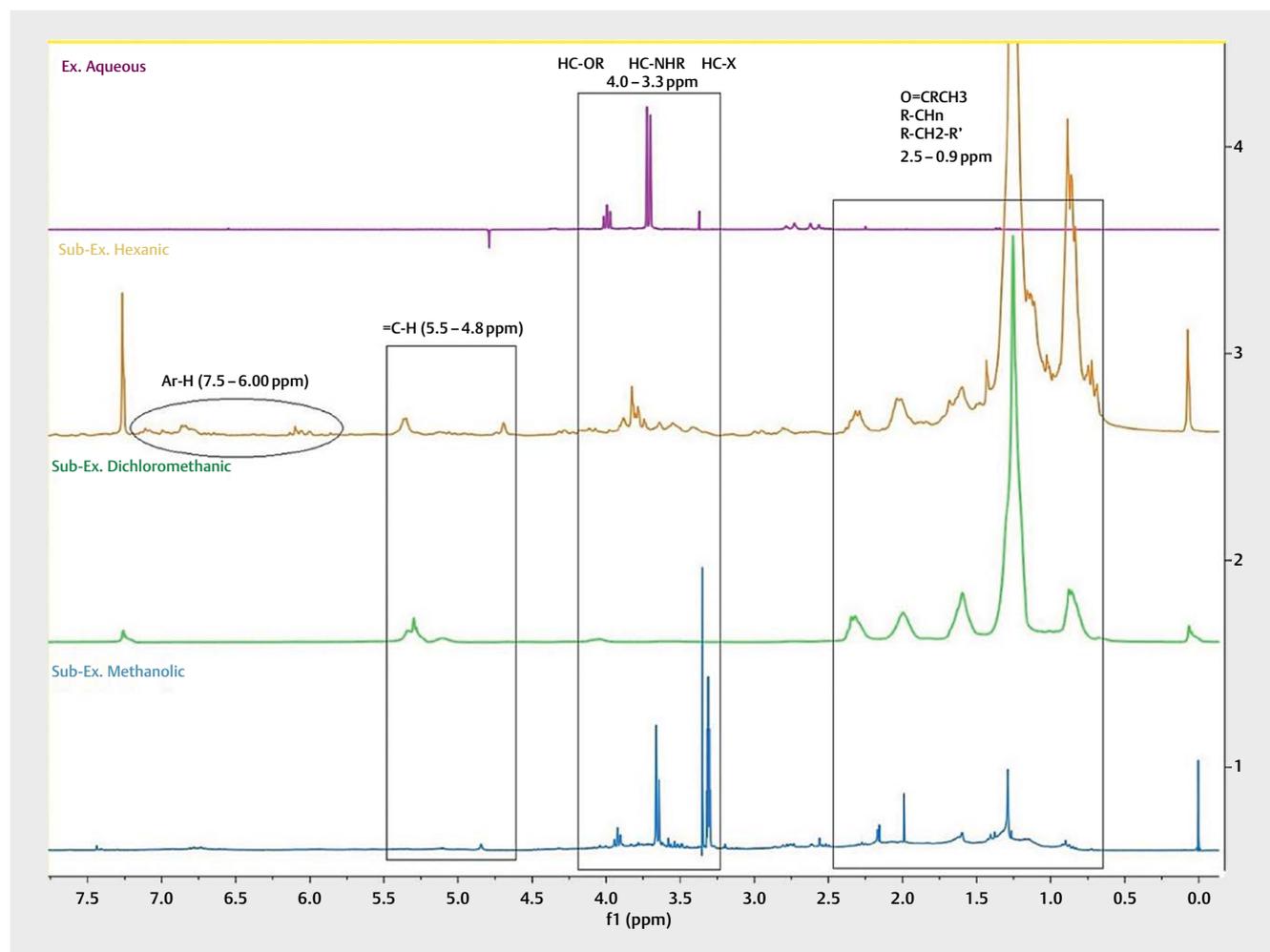
served signals, that the HEX subextract contains more free structures, while the methanolic extract contains more molecules linked to sugars since the signals between δ_H 2.0–4.0 ppm are more intense, which correspond to flavonoid glycosides.

Next, a comparison was made between the spectra of the HEX subextract and the three compounds isolated from it, observing how all the signals present in the compounds are also found in the subextract, although these are of less intensity and are not well defined since the subextract is a complex mixture of compounds (► Fig. 2).

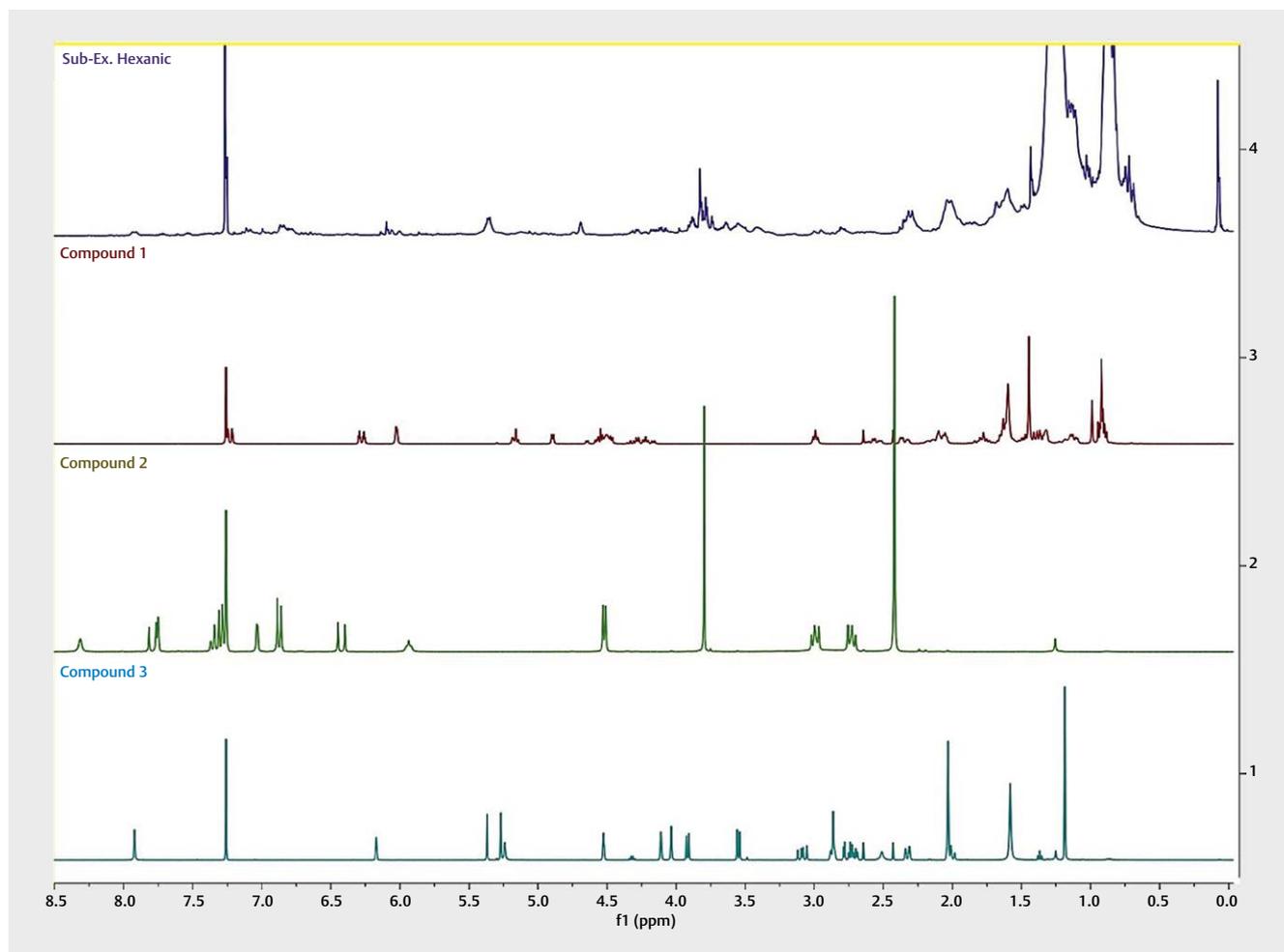
Regarding the antibacterial capacity, the HEX subextract showed an MIC on the *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, and *S. marcescens* strains of 37.51–37.92 $\mu\text{g/mL}$. These results were better (statistically significant, *** $p < 0.001$) than those of the aqueous extract and the other subextracts of *B. coriacea* (► Table 1).

These results were confirmed by the ZI assay where the HEX subextract showed a ZI of 33.39–33.90 mm, which was better (statistically significant, *** $p < 0.001$) than the values of the aqueous extract and the other *B. coriacea* subextracts (► Table 2).

Concerning the cytotoxicity of the *B. coriacea* extracts, the results showed that the initial aqueous extract ($CC_{50} = 91.31\text{--}93.86 \mu\text{g/mL}$) was less cytotoxic ($n^s p = 0.074$) than the negative control (untreated



► Fig. 1 Comparison of 1H NMR spectra of the aqueous extract and subextracts of *B. coriacea*.



► **Fig. 2** Comparison of ^1H NMR spectra of the HEX subextract and the isolated compounds of *B. coriacea*.

cells) in all cell lines (RM-2 and MM14.Ov). This result supports the traditional use of an aqueous infusion. Additionally, the HEX subextract ($\text{CC}_{50} = 86.13\text{--}86.73\ \mu\text{g/mL}$) was less cytotoxic ($p = 0.0637$) than the negative control (untreated cells) (► **Table 3**). Finally, the DCM and MeOH subextracts were discarded for subsequent fractionation because they presented statistically significant higher cytotoxicity ($***p < 0.001$) than the negative control (untreated cells).

Regarding the anti-inflammatory capacity, the results showed that the HEX subextract ($\text{IC}_{50} = 35.29\text{--}36.06\ \mu\text{g/mL}$) presented a higher inhibitory activity of the production of NF- κB ($***p < 0.001$) than the aqueous extract and the other subextracts of *B. coriacea* (► **Table 4**).

In relation to the antibacterial capacity of the fractions obtained from the HEX subextract, fractions **F3** and **F4** showed an MIC of 30.06–30.25 and 30.01–30.52 $\mu\text{g/mL}$, respectively (Table 15, Supporting Information) and values of ZI of 34.03–34.21 and 35.16–35.85 mm, respectively (Table 25, Supporting Information). These values were better ($**p < 0.05$) than those of the other fractions. Regarding the antibacterial activity of the **F1** fraction, it did not present statistically significant differences ($^{ns}p = 0.065$) when compared to the other fractions.

On the other hand, regarding the cytotoxicity of the fractions obtained from the HEX subextract (Table 35, Supporting Information), a trend of increasing cytotoxicity was observed as the fractionation proceeded, the first fractions hardly showed any cytotoxicity while the last fractions turned out to be much more cytotoxic. In this sense, the fractions that have not shown a statistically significant higher cytotoxicity ($^{ns}p = 0.073$) than the negative control (untreated cells) in the LDH assay were **F1** ($\text{CC}_{50} = 86.77\text{--}86.95\ \mu\text{g/mL}$), **F3** ($\text{CC}_{50} = 81.57\text{--}81.66\ \mu\text{g/mL}$), and **F4** ($\text{CC}_{50} = 80.14\text{--}81.11\ \mu\text{g/mL}$).

Finally, the anti-inflammatory capacity of the fractions obtained from the HEX subextract are shown in Table 45, Supporting Information, observing that fractions **F3** ($\text{IC}_{50} = 30.75\text{--}31.44\ \mu\text{g/mL}$) and **F4** ($\text{IC}_{50} = 30.17\text{--}30.41\ \mu\text{g/mL}$) presented higher inhibitory activities of the production of NF- κB in all cell lines than the other fractions ($***p < 0.001$). Regarding fraction **F1** ($\text{IC}_{50} = 39.33\text{--}39.68\ \mu\text{g/mL}$), although it presented an inhibitory activity of NF- κB , this was not higher (not statistically significant, $p = 0.0246$) when compared to the other fractions.

Considering these results, we can affirm that the fractions of the HEX subextract are safe for humans and that they present anti-in-

► **Table 1** Minimum inhibitory concentration (MIC) of *B. coriacea* extract and subextracts against different strains of bacteria.

Extracts	MIC ($\mu\text{g/mL}$) at 48 h (CI 95 %, R2)			
	<i>K. pneumoniae</i>	<i>P. mirabilis</i>	<i>P. aeruginosa</i>	<i>S. marcescens</i>
Ofloxacin	10.01 (5.09 to 15.62, 0.9836)	10.01 (5.28 to 15.18, 0.9599)	10.01 (5.74 to 15.38, 0.9523)	10.01 (5.16 to 15.14, 0.9532)
Aq extract	53.54 (48.36 to 58.50, 0.9615)	53.19 (48.93 to 58.05, 0.9968)	53.60 (48.81 to 58.82, 0.9825)	53.84 (48.84 to 58.90, 0.9507)
HEX subextract	37.55 (31.84 to 41.61, 0.9528)	37.51 (31.08 to 41.49, 0.9624)	37.80 (31.43 to 41.56, 0.9576)	37.92 (31.90 to 41.79, 0.9533)
DCM subextract	81.26 (76.92 to 86.86, 0.9853)	81.11 (76.34 to 86.94, 0.9944)	81.89 (76.78 to 86.04, 0.9526)	81.95 (76.58 to 86.31, 0.9649)
MeOH subextract	67.28 (62.20 to 72.63, 0.9672)	67.12 (62.17 to 72.94, 0.9558)	67.40 (62.37 to 72.74, 0.9964)	67.51 (62.36 to 72.21, 0.9529)
MIC was calculated using Prism v9.0.0 (GraphPad Software) using nonlinear regression, dose-response curves. CI 95 %: confidence interval 95 %/Tukey's multiple comparisons test (***) $p < 0.001$				

inflammatory activity, although it is still far from the positive control, celastrol ($\text{IC}_{50} = 3.32\text{--}3.34 \mu\text{g/mL}$). In this sense, fractions **F1**, **F3**, and **F4** were selected for their better antibacterial and anti-inflammatory activities and lower cytotoxicity, focusing on the subsequent isolation and purification of the compounds.

Subsequently, the chromatographic separation of fraction **F1** (obtained from the HEX subextract) was carried out, obtaining a total of six subfractions (**F1a–F1f**), with fraction **F1f** (compound **1**) being the most active (Tables 5S–8S, Supporting Information). Likewise, the chromatographic purification of **F3** and **F4** (obtained from the HEX subextract) was carried out, obtaining five and four subfractions, respectively. Subfractions **F3b** (compound **2**) (Tables 9S–12S, Supporting Information) and **F4c** (compound **3**) (Tables 13S–16S, Supporting Information) were the most active.

The isolated compounds were identified as (4aR,4bS,5S,6aS,6bS,9aR,10aS,10bS)-6b-glycoloyl-5-hydroxy-4a,6a-dimethyl-8-propyl-4a,4b,5,6,6a,6b,9a,10,10a,10b,11,12-dodecahydro-2H-naphtho [2',1':4,5] indeno [1,2-d][1,3] dioxol-2-one (**1**), 3-[3-(2-dimethylaminoethyl)-1H-indol-5-yl]-N-(4-methoxybenzyl) acrylamide (**2**), and (1 β ,11 β ,12 α)-1,11,12-trihydroxy-11,20-epoxypicrasa-3,13(21)-diene-2,16-dione (**3**) (► Fig. 3). Compounds **1** and **2** were described as synthesis products by Ryrfeld et al. [21], and Barf et al. [22], respectively. However, there are no reports on their chemical characterisation. Finally, in relation to compound **3**, it has been isolated from different plant species, such as *Ailanthus altissima*[23] and *Eurycoma longifolia*[24].

► **Table 2** *In vitro* culture plates (agar cup plate method) of *B. coriacea* extract and subextracts showing the zone of inhibition (ZI) concentration against different strains of bacteria.

Extracts	ZI (mm) at 48 h (CI 95 %, R2)			
	<i>K. pneumoniae</i>	<i>P. mirabilis</i>	<i>P. aeruginosa</i>	<i>S. marcescens</i>
Ofloxacin	27.58 (22.92 to 32.71, 0.9833)	27.98 (22.89 to 32.88, 0.9593)	27.58 (22.65 to 32.93, 0.9919)	27.50 (22.89 to 32.81, 0.9991)
Aq extract	64.30 (59.68 to 69.42, 0.9929)	64.55 (59.10 to 69.93, 0.9556)	64.17 (59.93 to 69.43, 0.9663)	64.14 (59.43 to 69.34, 0.9668)
HEX subextract	33.53 (28.92 to 38.35, 0.9828)	33.90 (28.21 to 38.23, 0.9949)	33.50 (28.52 to 38.90, 0.9748)	33.39 (28.50 to 38.90, 0.9743)
DCM subextract	85.88 (80.50 to 90.64, 0.9553)	85.89 (80.97 to 90.90, 0.9801)	85.61 (80.67 to 90.55, 0.9625)	85.06 (80.86 to 90.31, 0.9657)
MeOH subextract	81.17 (76.55 to 86.98, 0.9948)	81.91 (76.51 to 86.06, 0.9714)	81.12 (76.03 to 86.88, 0.9711)	81.10 (76.58 to 86.79, 0.9713)
IC ₅₀ values of the ZI was calculated using Prism v9.0.0 (GraphPad Software) using nonlinear regression, dose-response curves. CI 95 %: confidence interval 95 %/Tukey's multiple comparisons test (***) $p < 0.001$				

Regarding antibacterial activity, the MIC assay showed MIC values of 11.64–11.81 (compound **1**), 0.17–0.19 (compound **2**), and 0.34–0.36 μM (compound **3**). The compounds obtained showed statistically significant higher antibacterial activity (***) $p < 0.001$ compared to the positive control (ofloxacin, $\text{IC}_{50} = 27.58\text{--}27.65 \mu\text{M}$) (► Fig. 4).

However, in the ZI assay, no statistically significant difference ($p = 0.0869$) was observed between the three compounds when compared to the positive control (ofloxacin, ZI = 23.26–24.10 mm) in *K. pneumoniae*, *P. mirabilis*, *P. aeruginosa*, and *S. marcescens* bacteria (► Fig. 5).

In relation to the determination of the cytotoxicity of the compounds, ► Table 5 shows that compound **1** ($\text{CC}_{50} = 88.44\text{--}89.24 \mu\text{M}$) did not present more cytotoxicity ($p = 0.0586$) than the negative control (untreated cells). Although compounds **2** ($\text{CC}_{50} = 69.51\text{--}70.28 \mu\text{M}$) and **3** ($\text{CC}_{50} = 53.95\text{--}55.16 \mu\text{M}$) showed cytotoxicity when compared to the negative control; these cytotoxicity values were lower than the positive control (ACT, $\text{CC}_{50} = 0.008 \mu\text{M}$)

Regarding anti-inflammatory activity, compounds **2** ($\text{IC}_{50} = 0.15\text{--}0.16 \mu\text{M}$) and **3** ($\text{IC}_{50} = 0.33\text{--}0.36 \mu\text{M}$) showed an inhibitory capacity of NF- κ B production that was higher (statistically significant, ***) $p < 0.001$ than that of the positive control (celastrol, $\text{IC}_{50} = 7.41 \mu\text{M}$). Regarding compound **1** ($\text{IC}_{50} = 11.25\text{--}11.34 \mu\text{M}$), its inhibitory activity against the production of NF- κ B was similar to the positive control ($p = 0.0865$) (► Fig. 6).

► **Table 3** CC₅₀ values of the LDH (cytotoxicity) assays calculated for the extract and subextracts from *B. coriacea*.

Extracts	Cytotoxicity (CC ₅₀ µg/mL) at 48 h (CI 95 %, R2)	
	RM-2	MM14.Ov
Untreated cells	97.92 (92.48 to 102.53, 0.9454)	98.35 (93.70 to 103.09, 0.9901)
DMSO	20.15 (15.29 to 25.18, 0.9759)	20.19 (15.44 to 25.80, 0.9872)
Actinomycin D	0.01 (-3.991 to 3.357, 0.9503)	0.01 (-3.725 to 3.233, 0.9844)
Aq extract	91.31 (86.45 to 96.88, 0.9576)	93.86 (88.45 to 98.48, 0.9994)
HEX subextract	86.13 (81.89 to 91.94, 0.9891)	86.73 (81.47 to 91.58, 0.9932)
DCM subextract	77.71 (72.78 to 82.97, 0.9765)	79.37 (74.23 to 84.88, 0.9659)
MeOH subextract	56.68 (51.34 to 61.07, 0.9986)	58.56 (53.55 to 63.85, 0.9712)

CC₅₀ was calculated using Prism v9.0.0 (GraphPad Software) using nonlinear regression, dose-response curves. CI 95%: confidence interval 95%/Tukey's multiple comparisons test (***) p < 0.001

► **Table 4** IC₅₀ values of the inhibition of NF-κB production calculated for the extract and subextracts from *B. coriacea*.

Extracts	Inhibition of NF-κB production (IC ₅₀ µg/mL) at 48 h (CI 95 %, R2)	
	RM-2	MM14.Ov
Untreated cells	19.72 (14.68 to 24.87, 0.9647)	19.84 (14.45 to 24.64, 0.9984)
Celastrol	3.33 (-2.23 to 8.32, 0.9888)	3.34 (-2.47 to 8.90, 0.9868)
Aq extract	53.27 (48.93 to 58.78, 0.9946)	54.16 (49.14 to 59.35, 0.9674)
HEX subextract	35.29 (30.93 to 40.47, 0.9968)	36.06 (31.34 to 41.44, 0.9644)
DCM subextract	90.72 (85.05 to 95.55, 0.9937)	90.76 (85.18 to 95.38, 0.9938)
MeOH subextract	66.79 (61.07 to 71.35, 0.9563)	67.04 (62.17 to 72.05, 0.9789)

IC₅₀ was calculated using Prism v9.0.0 (GraphPad Software) using nonlinear regression, dose-response curves. CI 95%: confidence interval 95%/Tukey's multiple comparisons test (***) p < 0.001

Discussion

NMR spectroscopy has recently emerged as a very powerful and versatile tool for the identification of compounds in plant extracts [25, 26]. Unlike the most widely used characterisation methods, such as chromatography, NMR allows investigations to be carried out both in solids and in solution. Furthermore, in recent decades, NMR hardware, computer technology, and experimental design have been developed simultaneously, thus producing a modern generation of NMR spectrometers capable of probing complex structures. This has resulted in substantial improvements in spectral data processing and visualisation [27]. In this sense, we were able to identify the presence of different compounds in the extracts of *B. coriacea* by means of ¹H NMR spectroscopy, focusing on those

that had a higher concentration, which were (4aR,4bS,5S,6aS,6bS,9aR,10aS,10bS)-6b-glycoloyl-5-hydroxy-4a,6a-dimethyl-8-propyl-4a,4b,5,6,6a,6b,9a,10,10a,10b,11,12-dodecahydro-2H-naphtho [2',1':4,5] indeno [1,2-d][1,3] dioxol-2-one (**1**), 3-[3-(2-dimethylaminoethyl)-1H-indol-5-yl]-N-(4-methoxybenzyl) acrylamide (**2**), and (1β,11β,12α)-1,11,12-trihydroxy-11,20-epoxypicrasa-3,13(21)-diene-2,16-dione (**3**).

Regarding the antibacterial activity of *B. coriacea*, there are no previous reports, however, there are reports on the antibacterial activity of other species of the genus *Buddleja*, e. g., the MeOH extract of *Buddleja saligna* showed an MIC of 5 mg/mL against *K. pneumoniae*, *P. aeruginosa*, and *S. marcescens*. When comparing this to our results, we can confirm the antibacterial potential of the *Buddleja* species against gram-negative bacteria. Moreover, we can also indicate that the HEX subextract of *B. coriacea* is more active than the MeOH extract of *B. saligna*[28]. Likewise, there are reports that indicate that UTIs are usually caused by gram-negative bacteria, which tend to become multiresistant to conventional antibiotics [29, 30], thus confirming the importance of *B. coriacea* extracts as an alternative treatment.

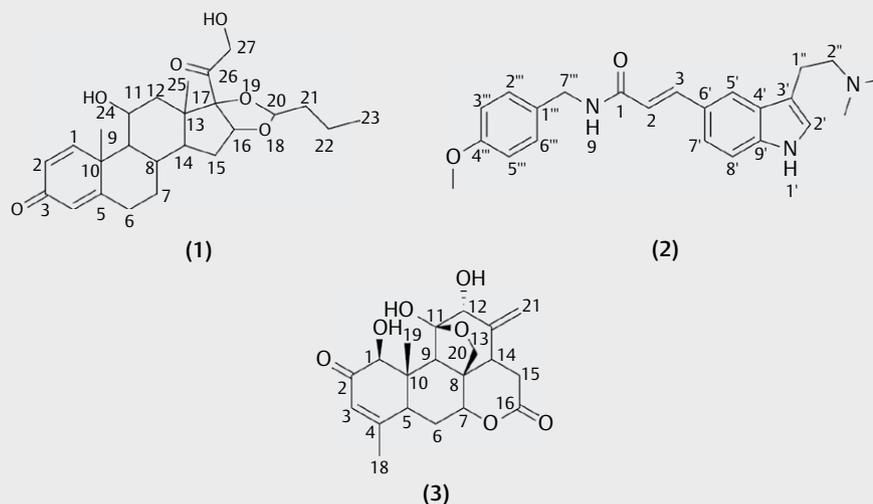
Moreover, although no reports were found on the cytotoxicity of the *B. coriacea* species, it was found that other species of the genus *Buddleja* presented cytotoxicity, e. g., the aqueous extract of *Buddleja globosa* presented cytotoxicity at concentrations higher than 50 µg/mL [31]. Analysing our results, the extracts obtained from *B. coriacea* were less cytotoxic compared to other extracts from other *Buddleja* species.

On the other hand, previous studies have shown that the hydroalcoholic and DCM/hydroalcoholic extracts of *B. coriacea* presented anti-inflammatory activity (inhibition of the production of COX-2) *in vivo* in a range of concentrations around 3 g/kg b.w. [20].

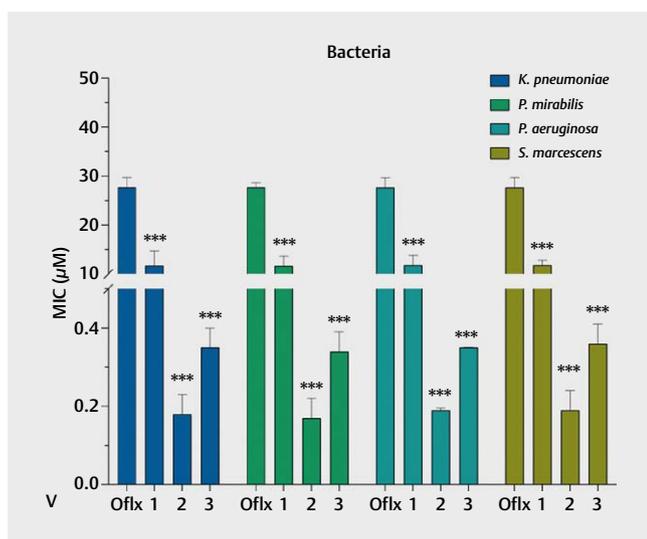
In our study, *in vitro* assays of the HEX subextract showed that *showed what?* after their production of NF-κB is inhibited in the RM-2 and MM14.Ov cell lines. In addition, the use of solvents of different polarity allowed for evaluating compounds of a different nature present in the initial aqueous extract, indicating that in the case of the HEX subextract, there is a higher relationship between its anti-inflammatory activity and its cytotoxicity. For this reason, the HEX subextract was fractionated using HEX/ACoEt as the mobile phase, producing seven fractions that were subjected to antibacterial, cytotoxicity, and anti-inflammatory assays.

In relation to the antibacterial activity of the isolated compounds, results on compound **1** contradict previous studies. Wang et al. [32] reported that compound **1** suppresses the lung antibacterial defence, increasing the infection caused by *P. aeruginosa*. In our study, compound **1** has been reported to have antibacterial activity against gram-negative bacteria such as *E. coli* or *P. aeruginosa* in a concentration range of 0.01-100 µg/mL [33] and against gram-positive bacteria such as *S. aureus* in a concentration range of 16-2000 µg/200 µL. Thus, these results obtained confirm the antibacterial properties of compound **1** against gram-negative bacteria, contradicting the results of Wang et al.

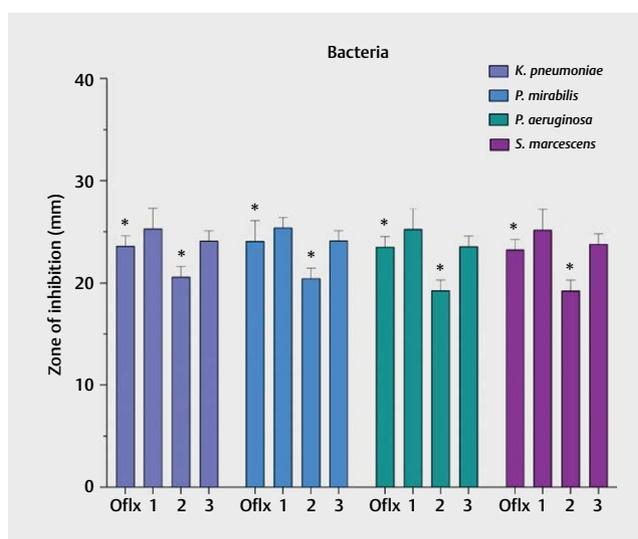
Regarding the antibacterial activity of compounds **2** and **3**, there are no previous studies. However, the three compounds, especially **2** and **3**, have shown better activity than the positive control, which is why they stand as potential alternatives for the eradication of those bacterial strains that cause chronic UTIs.



► **Fig. 3** The chemical structures of compounds from *B. coriacea*: **1** ((4aR,4bS,5S,6aS,6bS,9aR,10aS,10bS)-6b-glycoloyl-5-hydroxy-4a,6a-dimethyl-8-propyl-4a,4b,5,6,6a,6b,9a,10,10a,10b,11,12-dodecahydro-2H-naphtho[2',1':4,5]indeno[1,2-d][1,3]dioxol-2-one), **2** (3-[3-(2-dimethylaminoethyl)-1H-indol-5-yl]-N-(4-methoxybenzyl)acrylamide), and **3** ((1 β ,11 β ,12 α)-1,11,12-trihydroxy-11,20-epoxypicrasa-3,13(21)-diene-2,16-dione).



► **Fig. 4** Minimum inhibitory concentration (MIC) of *B. coriacea* compounds against different strains of bacteria at 48 h. MIC was calculated using Prism v9.0.0 (GraphPad Software) using nonlinear regression, dose-response curves. CI 95%: confidence interval 95%/Tukey's multiple comparisons test (***) $p < 0.001$.



► **Fig. 5** *In vitro* culture plates (agar cup plate method) of *B. coriacea* compounds showing the zone of inhibition (ZI) concentration against different strains of bacteria at 48 h. IC₅₀ values of the ZI were calculated using Prism v9.0.0 (GraphPad Software) using nonlinear regression, dose-response curves. CI 95%: confidence interval 95%/Tukey's multiple comparisons test (***) $p < 0.001$.

Analysing the mechanism of action based on the positive control (ofloxacin), we can indicate that the isolated compounds prevent bacterial division, which is achieved by inhibiting topoisomerases II and IV and DNA gyrase, which are necessary to complete this process [34].

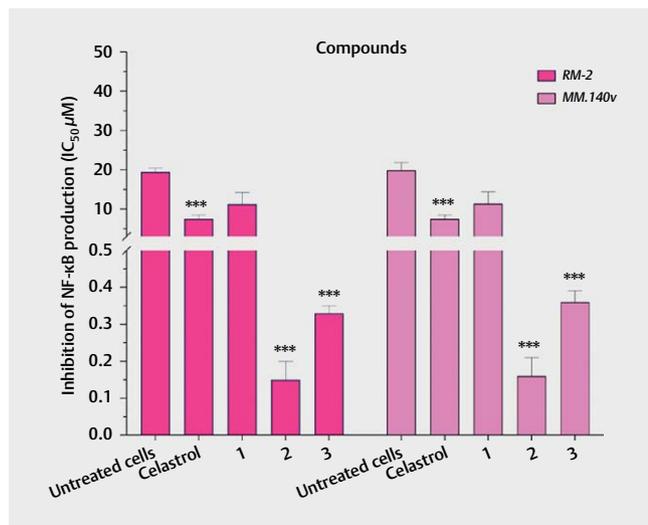
Regarding the cytotoxicity activity of compounds isolated from *B. coriacea*, through an *in silico* study, it has been reported that compound **1** does not present significant toxicity [35]. Our results sup-

port that this compound is not cytotoxic. Concerning compound **2**, no previous cytotoxicity studies have been found. However, the results obtained did not show significant cytotoxicity when compared with untreated cells (98.45–98.97 μ M). Finally, in relation to compound **3**, there are reports on its cytotoxicity against different tumour cell lines, with an IC₅₀ values of 0.25–0.52 μ M [36]. This result is not confirmed in this study. Yet, these contradictory results

► **Table 5** CC₅₀ values of the LDH (cytotoxicity) assays calculated for the compounds from *B. coriacea*.

Compounds	Cytotoxicity (CC ₅₀ /μM) at 48 h (CI 95%, R2)	
	BEAS-2B	IMR-90
Untreated cells	98.45 (93.90 to 103.35, 0.9783)	98.97 (93.75 to 103.84, 0.9635)
DMSO	10.05 (5.75 to 15.08, 0.9634)	10.05 (5.36 to 15.56, 0.9506)
Actinomycin D	0.008 (-4.867 to 4.338, 0.9625)	0.008 (-4.642 to 4.675, 0.9764)
Compound 1	88.44 (83.43 to 93.39, 0.9897)	89.24 (84.74 to 94.96, 0.9827)
Compound 2	70.28 (65.71 to 75.54, 0.9607)	69.51 (64.94 to 74.45, 0.9522)
Compound 3	53.95 (48.45 to 58.37, 0.9856)	55.16 (50.75 to 60.46, 0.9562)

CC₅₀ was calculated using Prism v9.0.0 (GraphPad Software) using nonlinear regression, dose-response curves. CI 95%: confidence interval 95%/Tukey's multiple comparisons test (***) p < 0.001.



► **Fig. 6** IC₅₀ values of the inhibition of NF-κB production, calculated for the compounds from *B. coriacea* at 48 h. IC₅₀ was calculated using Prism v9.0.0 (GraphPad Software) using nonlinear regression, dose-response curves. CI 95%: confidence interval 95%/Tukey's multiple comparisons test (***) p < 0.001.

can be due to the fact that the studies have been carried out in different cell lines [37].

Finally, regarding the anti-inflammatory activity of the isolated compounds, compound **1** is a glucocorticoid with powerful anti-inflammatory effects [38] that is used to treat chronic inflammatory diseases [39]. However, although compound **1** was isolated as a mixture of diastereoisomers, they do not show significant structural differences, so the activity of both structures can be assumed to be similar. Moreover, there are previous studies on diastereoisomeric mixtures with anti-inflammatory activity [40]. It should be noted that glucocorticoids are used in the treatment of lung diseases such as asthma and COPD because they inhibit the gene ex-

pression dependent on NF-κB [33] and suppress the production of proinflammatory cytokines. [32].

On the other hand, the mechanism of action of compounds **2** and **3** on the NF-κB pathway is unknown since there are no previous reports showing anti-inflammatory activity. A possible mechanism of action may be similar to the one of celestrol (positive control), which is based on the suppression of phosphorylation of IKKαβ (it suppresses the degradation of IκBα and inhibits the translocation of p65 to the nucleus) [34]. However, this is only a hypothesis since there are no other studies that analyse the mechanism of action of the isolated compounds.

Thus, we can conclude that the isolated compounds have anti-inflammatory properties. Additionally, by predicting the potential pharmacokinetic properties of the three compounds using *SwissADME*, it was determined that all three compounds have high gastrointestinal absorption and are soluble. These results support the traditional use of the aqueous extract due to its pharmacokinetic properties.

From *in vitro* assays, it has been confirmed that the three compounds isolated from *B. coriacea* leaves have antibacterial and anti-inflammatory activities, and they are not cytotoxic, being partly responsible for the ethnopharmacological activity (treatment of urinary infections) of the plant. This is the first time that compounds **1**, **2**, and **3** have been studied for the treatment of UTIs due to their antibacterial and anti-inflammatory properties.

With the present work, it has been established that the isolated compounds could have potential use for the development of oral treatments for UTIs, offering an alternative to those already present on the market. In addition, the isolated compounds may be useful in the future as structures for the synthetic development of libraries of compounds that may have better activity. However, the continuation of bioguided isolation in search of new compounds cannot be ruled out, since hardly any previous studies were found that report the chemical composition of the species *B. coriacea*.

Material and Methods

Bacteria, cell lines, biochemicals, and chemicals

Gram-negative bacteria, *K. pneumoniae* (BAA-3079), *P. mirabilis* (BAA-3087), *P. aeruginosa* (CRM-9027), and *S. marcescens* (25419), were purchased from the ATCC. Animal cell lines RM-2 (CRL-3311) and MM14.Ov (CRL-6383) were purchased from the ATCC.

Cells were cultured in media DMEM (Sigma-Aldrich). FBS (Summit Biotechnology) and PBS (SAFC Biosciences, Inc.) were used as culture mediums. L-Glutamine was obtained from Applichem. Penicillin and streptomycin were purchased from Fisher Scientific. For both the cytotoxicity and activity assays, the compounds were dissolved in DMSO (Merck) at a concentration of 10 mM, while extracts and fractions were dissolved at 20 mg/mL in DMSO. The incubation condition for all cells was in an atmosphere of 95% air and 5% CO₂ at 37 °C.

Samples were separated by column chromatography with silica gel (20–45 μm and 40–63 μm; Merck). TLC (Merck Silica gel 60-F₂₅₄ plates) was used for the compounds visualisation by means of UV light [Spectroline E-Series UV lamp with one longwave (365 nm)

and one shortwave (254 nm) tube, 230 V] and/or by staining the plate with a 5 % phosphomolybdic acid (Merck) solution in EtOH (Merck) followed by heat application. All organic solvents that were used for isolating the compounds were purchased from Merck.

Plant material

The aerial parts of *B. coriacea* were collected from the Pucarani town, Los Andes province, La Paz, Bolivia (16°24'13.5"S 68°28'37.7"W), in June 2019, at an altitude of 3865 m. The botanical identification was confirmed by the National Herbarium of Bolivia (No. 14021).

Extraction and isolation

The air-dried leaves (1 kg) from *B. coriacea* were extracted by decoction (30 min at boiling point) with 2 L of DH₂O. The resulting aqueous extract was frozen in glass containers at a temperature of -38°C and then lyophilised (freeze dryer, Christ alpha 1e2 LD plus) at -50°C. Subsequently, 45 g of the aqueous extract were extracted by repeated maceration (3 times/24 h/rt) with 500 mL of different solvents, increasing the polarity: HEX, DCM, and MeOH. Next, the subextracts were filtered, and the respective solvents were removed by vacuum rotary evaporation at rt (25°C). As a result, three subextracts of 1.38, 1.77, and 2.67 g, respectively, were obtained.

The HEX subextract (1.77 g) was selected because it was the most active one, and was subsequently fractionated using a chromatographic column (2 × 30 cm) with Si-60 Silica gel (40–63 μm; Merck) as a stationary phase and a gradient of HEX/AcOEt (49:1 → 5:1) as the eluent. A total of seven fractions (F1–F7) were obtained: F1 (0.204 g), F2 (0.047 g), F3 (0.114 g), F4 (0.045 g), F5 (0.112 g), F6 (0.074 g), and F7 (0.149 g).

Based on the biological activity data, we could then conclude that fractions F1, F3, and F4 had lower cytotoxicity. A second separation of F1 was carried out by using a chromatographic column (1 × 20 cm) with Si-60 Silica gel (40–63 μm; Merck) as a stationary phase and HEX/AcOEt (2:1) as the mobile phase. A total of six subfractions (F1.a–F1.f) were obtained: F1.a (2.8 mg), F1.b (3.9 mg), F1.c (4.7 mg), F1.d (1.1 mg), F1.e (1.8 mg), and F1.f (4.8 mg), which was compound 1.

A third separation of F3 was carried out by using a chromatographic column (1 × 20 cm) with Si-60 Silica gel (40–63 μm; Merck) as the stationary phase and Et₂O/DCM (1:6) as the mobile phase. A total of five subfractions (F3.a–F3.e) were obtained: F3.a (5.4 mg), F3.b (1.4 mg), which was compound 2, F3.c (1.0 mg), F3.d (3.7 mg), and F3.e (6.8 mg).

Finally, a separation on a silica gel microcolumn was carried out to separate F4 using DCM/AcOEt (1:2). A total of four subfractions (F4.a–F4.d) were obtained: F4.a (2.4 mg), F4.b (1.1 mg), F4.c (1.2 mg, compound 3), and F4.d (1.8 mg).

NMR analysis

NMR spectroscopy experiments were carried out on Bruker Advance DRX 300 and 500 spectrometers operating at 300 MHz, 500 MHz (¹H), or 75 MHz, and 126 MHz (¹³C) with TMS as the reference solvent (δ_H 0 ppm). Spectra were calibrated by assignment of the residual solvent peak to δ_H 7.26, δ_H 3.31, and δ_C 77.16, δ_C 49.00 for CDCl₃ (≥ 99.8%; Sigma-Aldrich, CAS Number 865-49-6) and MeOD (≥ 99.8%; Sigma-Aldrich, CAS Number 811-98-3), respec-

tively. The complete assignment of protons and carbons was done by correlated ¹H-¹H COSY, HSQC, and HMBC spectra analysis.

MS analysis

HR-El-MS analyses were performed using a mass spectrometer with a hybrid QTOF analyser, model MAXIS II from Bruker, S.A. Samples were analysed using the electrospray ionisation technique, by direct infusion at a flow of 3 μL/min, using MeOH (Merck) with 0.1 % formic acid (Merck) as the ionising phase. The source parameters were as follows: end plate offset: 500 V; capillary: 3500; nebuliser: 0.2 bar; dry gas: 2.0 L/min; dry temp.: 250 °C; and mass range of 50–3000 Da.

Broth microdilution method

The MIC values of the samples against the bacterial strains were determined using the microdilution method in 96-well plates following the procedure described by Apaza et al. [34]. Ofloxacin (≥ 98%; Sigma-Aldrich, CAS Number 82419-36-1) was used as a positive control (of 10.01 μg/mL, equivalent to 27.67 μM for the compounds). Each MIC determination was carried out in triplicate.

Agar well diffusion method

Agar well diffusion method was carried out in accordance with the procedure described by Apaza et al. [41]. Ofloxacin (≥ 98% Sigma-Aldrich; CAS Number 82419-36-1) was used as a positive control (of 10.01 μg/mL, equivalent to 27.67 μM for the compounds). ZI of each bacterial strain was measured with a calibrated digital Vernier caliper in triplicate.

Cytotoxicity assay

The samples were tested in a panel of two cell lines (RM-2 and MM14.Ov) by means of the LDH assay in accordance with the procedure described by Apaza et al. [34]. ACT (≥ 95%; Sigma-Aldrich, CAS Number 50-76-0) was used as a positive control at a concentration of 0.01 μg/mL (equivalent to 0.008 μM for the compounds).

Nuclear factor kappa-light-chain-enhancer of activated B cells inhibition assay

In vitro anti-inflammatory activity (NF-κB inhibition assay) was carried out following the procedure described by Apaza et al. [34]. Celestrol (≥ 98%; Sigma-Aldrich, CAS Number 34157-83-0) was used as a positive control at a concentration of 3.34 μg/mL (equivalent to 7.41 μM for the compounds).

Statistical analysis

Cytotoxic concentration 50 % (CC₅₀) and inhibitory concentration 50 % (IC₅₀) values were determined by nonlinear regression. All experiments were performed in triplicate. One-way ANOVA statistical analysis (Tukey's multiple comparisons test, ** p < 0.05, *** p < 0.001) was performed to evaluate the significant differences among values. All analyses were performed using 1994–2020 GraphPad Prism Software LLC version 9.0.0. (86) for Mac (producer Dennis Radashev www.graphpad.com).

Supporting information

¹H- and ¹³C- NMR, ¹H-¹H COSY, HSQC, HMQC, HMBC, and MS spectra for the different fractions assayed in this study and for the iso-

lated compounds (Figs. 55–335) as well as activity data of the *B. coriacea* fractions from the different samples (Tables 15–165) are provided as Supporting Information.

Acknowledgments

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Conflict of Interest

The authors declare they have no conflict of interest.

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