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Sardinian plants with antimicrobial potential. Biological screening with multivariate data treatment of thirtysix extracts

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40 Abstract

41 In this paper, thirty-six extracts from Sardinian plants were evaluated in vitro for their antimicrobial 42 activity towards a panel of reference strains, Staphylococcus aureus, S. epidermidis, Klebsiella 43 pneumoniae and Escherichia coli, and for their cytotoxicity on mammalian cells. The biological 44 data, together with total phenolic and flavonoid content of the extracts, were treated by PCA 45 (Principal Components Analysis), which highlighted the positive correlation among total phenolic 46 content and increasing antibacterial activities, and a possible involvement of flavonoids in mitigate 47 the cytotoxicity. Thirteen extracts displayed relevant IC₅₀ values (half maximal inhibitory 48 concentration) on S. aureus (IC₅₀ from 1.4 to 153.6 µg/mL), ten out of them were active also 49 against S. epidermidis (IC₅₀ from 3.9 to 150 µg/mL), seven against K. pneumoniae (IC₅₀ from 28.5 50 to 97.5 µg/mL), and two against E. coli (IC₅₀ 74.9 and 156.3 µg/mL). In particular, three extracts obtained from Pistacia terebinthus ssp. terebinthus, Cytinus hypocistis and Limonium morisianum 51 emerged as promising antibacterial candidates. They exhibited remarkable inhibitory activity 52 53 towards bacterial strains from clinical specimens and presenting different antibiotic-resistance 54 profiles.

55 56

57	Keywords
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58 Antimicrobials; Sardinian plants; *Pistacia terebinthus ssp. terebinthus; Cytinus hypocistis;*

- 59 *Limonium morisianum;* multivariate data treatment.
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66 **1. Introduction**

67 In the current scenario, the clinical use of antibiotics, and therefore the effective treatment of 68 bacterial infections, is under considerable threat due to the emergence of bacteria that have 69 developed resistance to many classes of generally used antibiotics. Antibiotic-resistant bacterial 70 infections are already widespread across the globe and very high rates of resistance have been ever-71 increasingly observed in common bacteria (WHO, 2014). Among Staphylococcus species, the 72 prevalence of methicillin-resistant S. aureus and S. epidermidis (MRSA and MRSE, respectively) 73 infections is growing worldwide and epidemiology is changing overtime. Although S. aureus and S. 74 epidermidis are normal commensals of the skin and mucous membranes, MRSA is a leading cause 75 of nosocomial infections and, more and more frequently, it is associated to community-acquired 76 infections (mainly skin and wound infections) while MRSE has been identified as the most 77 recurrent cause of health-care related bloodstream and device-related infections (Moellering, 2012; 78 Rolo et al., 2012; May et al., 2014). Concerning Gram negative bacteria, high proportions of 79 resistance to cephalosporins and fluoroquinolones have been reported for *Escherichia coli*, a normal 80 inhabitants of the human intestinal microflora, and, of great concern, to carbapenems for Klebsiella 81 pneumoniae, a primarily opportunistic bacterium that can be nosocomial or community acquired. 82 These high reported resistances mean limitations to available treatment, which may be common in 83 the population, such as urinary tract infections and pneumonia (Nordman et al., 2011).

Generally, infections by drug-resistant bacteria have an increased risk of worse clinical outcome and death compared to infections by the respective susceptible strains, and treatments must rely on second-line drugs that are more expensive and, sometimes, they have severe side-effects for which monitoring is advisable, increasing costs even further.

All these remarks have hastened and widened the quest for the discovery of novel agents for thetreatment of bacterial infections.

90 In this context, plants represent a very important resource, producing hundreds of diverse 91 metabolites, with medicinal and nutraceutical potential (Cragg & Newman 2013, Toledo et al., 92 2015; Chen et al., 2014; Fung et al. 2013). Among their bioactivities, plant metabolites were proved 93 also endowed with antimicrobial potential (Coqueiro et al., 2016; Snene et al., 2017; Dikpınar et al., 94 2018; Mahadi et al., 2018). In addition to find new antimicrobial molecules, plant extracts resulted 95 interesting to study also for their non-antimicrobial compounds, which might be essential for the 96 total bioactivity of the extract, improving solubility, absorption and stability of the active 97 metabolites. Moreover, some phytochemicals, despite not being antimicrobial by themselves, 98 showed antibiotic adjuvant activity, due to the inhibition of pathogens resistance mechanisms 99 (Abreu et al., 2016, Abreu et al., 2017).

100 Sardinia (Italy), due to its geographical isolation and high geological and geomorphological 101 diversification, represents a hotspot for biodiversity within the Mediterranean basin (Médail & 102 Quézel, 1997; Médail & Quézel, 1999; Marignani et al., 2017). This Island constitutes an extremely 103 diverse and dynamic environment with wide range of habitats and high degree of endemism (Fois et 104 al., 2017), driving plants to increase and diversify the production of their secondary metabolites in 105 order to adapt, compete and communicate with other species (Jahangir et al., 2008; Wang et al., 106 2005). In fact, Sardinian plants were found generally endowed with peculiar features, both in 107 respect of the phytochemical and genetic profiles (Bobo-Pinilla et al., 2016; Dettori et al., 2016; 108 Marengo et al., 2017; Sanna et al., 2018a; Venditti et al., 2017; Venditti et al. 2018).

However, despite Sardinian endemic plants resulted interesting for their phytochemical and
biological features, yielding also new molecular scaffolds (Cagno et al., 2017; Daino et al., 2018;
Mandrone et al., 2015; Mandrone et al., 2017; Maxia et al., 2015; Ornano et al., 2016; Sanna et al.,
2018b; Venditti et al., 2016), the majority of them remains still poorly investigated.

113 On this basis, thirty-six extracts obtained from Sardinian plants, including twelve endemic species, 114 were evaluated *in vitro* for their antibacterial activity against Gram positive and Gram negative 115 reference bacteria, and selected extracts were assayed on a panel of fifteen clinical isolates

- presenting different antibiotic-resistance profiles. Moreover, cytotoxicity on mammalian epithelialcells was also tested.
- 118 The overall biological data, together with phenolic and flavonoid content, were summarized byprincipal component analysis (PCA).

120 **2. Methods and materials**

121 *2.1. Plant material*

Wild plants were harvested in Sardinia Island (Italy) during 2017 and 2018 and were identified by Dr. Cinzia Sanna and Prof. Andrea Maxia. Vouchers were deposited at the General Herbarium of the Department of Life and Environmental Sciences, University of Cagliari and reported in Table 1, where plants were listed in alphabetical order using the update nomenclature reported in the new checklist of Italian vascular flora (Bartolucci et al., 2018).

- **Table 1** The table lists all the plants used in this study. The update botanical names, the plant organ
- 129 used and their labels, families, places and dates of collection and voucher numbers were reported.
- 130

Plant name	Plant organ and sample label in brackets	Family	Location of harvesting	Harvesting date	Voucher	
Arbutus unado I	Fruits (AuF)	Fricaceae	Jerzu	December 2017	Herbarium CAG	
Arbuius uneuo L.	Leaves (AuL)		Jerzu	December 2017	878	
Asphodelus ramosus L.	Rhizome (ArRh)	Asphodelaceae	Asphodelaceae	Geremeas	April 2017	Herbarium CAG
subsp <i>ramosus</i>	Leaves (ArL)		Geremeas	April 2017	1405	
Carlina gummifera (L.) Less.	Leaves (CgL)	Asteraceae	Cala Surya (Cardedu)	July 2018	Herbarium CAG 770	
Centaurea calcitrapa L.	Aerial parts (CcA)	Asteraceae	Siliqua	June 2017	Herbarium CAG 781	
<i>Centaurea horrida</i> Badarò*	Aerial parts (ChA)	Asteraceae	Capo Falcone	June 2017	Herbarium CAG 777	
Centaurea napifolia L.	Aerial parts (CnA)	Asteraceae	Uta	June 2017	Herbarium CAG 784	

$ \begin{array}{c c} Cistus salviifolius L. \\ Cistus salviifolius L. \\ Cista CA) \\ Cynara cardunculus L. \\ Cynara cardunculus L. \\ Aerial parts \\ (CycA) \\ Asternecea \\ Siliqua \\ April 2018 \\ April 2018 \\ April 2017 \\ Herbarium CAG \\ 1200$	Cistus monspeliensis L.	Aerial parts (CmA)	Cistaceae	Cala Surya (Cardedu)	April 2018	Herbarium CAG 135
$ \begin{array}{c c} Cynara \ cardunculus L. & Arrial parts \\ (CycA) & Asteraceae \\ Cytinus hypocistis (L.) L. & Arrial parts \\ (CycA) & Cytinaceae \\ \hline Cytinus hypocistis (L.) L. & Arrial parts \\ (CycA) & Cytinaceae \\ \hline Cytinus hypocistis (L.) L. & Arrial parts \\ (Fal.) & Arrial parts \\ (Fal.) & Apria cons \\ (Fal.) & Fal. \\ (Fal.) & Fal.$	Cistus salviifolius L.	Aerial parts (CsA)	Cistaceae	Cala Surya (Cardedu)	April 2018	Herbarium CAG 135/C
$ \begin{array}{c c} Cytinus hypocistis (L) L. & Aerial parts (Cytha) & Cytinaceae & Gesturi & May 2017 & Herbarium CAG 1200 \\ \hline \\ Ferula arrigonii Boechieri* & Leaves (FaR) & Apiaceae & Tharros & April 2017 & Herbarium CAG 612/A \\ \hline \\ \hline \\ Farlo & Apiaceae & Jerzu & September 2018 & Herbarium CAG 789 \\ \hline \\ \hline \\ Galactites tomentosa & Aerial parts (GiA) & Asteraceae & Seui & May 2017 & 286 \\ \hline \\ \hline \\ Gesta corsica (Loisel.) & Aerial parts & Lamiaceae & Gennargentu & June 2017 & Herbarium CAG 789 \\ \hline \\ \hline \\ \hline \\ Gesta corsica (Loisel.) & Aerial parts & Lamiaceae & Gennargentu & June 2017 & Herbarium CAG 789 \\ \hline \\ \hline \\ \hline \\ Gesta corsica (Loisel.) & Aerial parts & Lamiaceae & Jerzu & June 2018 & Herbarium CAG 1104 \\ \hline \\ Hypericum hircinum L .ssp & Aerial parts & Hypericaceae & Jerzu & June 2018 & Herbarium CAG 239/C \\ \hline \\ \hline \\ Lavandula stoechas L. & Aerial parts & Hypericaceae & Jerzu & June 2018 & Herbarium CAG 239/C \\ \hline \\ Lavandula stoechas L. & Aerial parts & Hypericaceae & Jerzu & June 2018 & Herbarium CAG 239/C \\ \hline \\ \hline \\ Limonium morisianum & Aerial parts & Myrtaceae & Geal Surya & Cala Surya & December 2017 & Herbarium CAG 909/G \\ \hline \\ \hline \\ Pistacia lentiscus L. & & Aerial parts & Myrtaceae & & Geal Surya & December 2017 & Herbarium CAG 514 \\ \hline \\ \hline \\ Pistacia lentiscus L. & & & & \\ \hline \\ Pistacia lentiscus L. & & & & \\ \hline \\ Pistacia terebinthus L .ssp & Leaves & & \\ (PIL) & & & & \\ \hline \\ Pistacia terebinthus L .ssp & Leaves & & \\ (PIL) & & & \\ \hline \\ Pistacia terebinthus L .ssp & Leaves & & \\ (PIL) & & & \\ \hline \\ Pistacia terebinthus L .ssp & Leaves & & \\ (PA) & & & \\ \hline \\ Pistacia terebinthus L .ssp & Leaves & & \\ (PA) & & & \\ \hline \\ Pitagius floxculosus (L.) & Aerial parts & & \\ (PA) & & & \\ \hline \\ Pistacia terebinthus L .ssp & Leaves & & \\ (PA) & & & \\ \hline \\ Pistacia terebinthus L .ssp & Leaves & & \\ (PA) & & & \\ \hline \\ Pistacia terebinthus L .ssp & & \\ \hline \\ Pistacia terebinthus L .ssp & & \\ \hline \\ Pistacia terebinthus L .ssp & & \\ \hline \\ Pistacia terebinthus L .stp & & \\ \hline \\ Pistacia terebinthus L .stp & & \\ \hline \\ Pistacia terebinthus L .stp & & \\ \hline \\ Pi$	Cynara cardunculus L.	Aerial parts (CycA)	Asteraceae	Siliqua	April 2017	Herbarium CAG 790
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	<i>Cytinus hypocistis</i> (L.) L.	Aerial parts (CyhA)	Cytinaceae	Gesturi	May 2017	Herbarium CAG 1200
$\begin{array}{c c c c c c c c c c c c c c c c c c c $	<i>Ferula arrigonii</i> Bocchieri* _	Leaves (FaL)	Arianna	Tharros	April 2017	Herbarium CAG
		Roots (FaR)	Aplacede	Tharros	April 2017	612/A
	Galactites tomentosa Moench	Aerial parts (GtA)	Asteraceae	Jerzu	September 2018	Herbarium CAG 789
Glechoma sardoa (Bég.) Bég.*Aerial parts (GsA)LamiaceaeGennargentuJune 2017Herbarium CAG 1104Hypericum hircinum L. ssp hircinum*Aerial parts (HHA)HypericaceaeJerzuJune 2018Herbarium CAG 232Hypericum scruglii Bacch., Brullo & Salmeri*Aerial parts (HsA)HypericaceaeJerzuJune 2018Herbarium CAG 239/CLavandula stoechas L.Aerial parts (LsA)LamiaceaeCala Surya (Cardedu)April 2017Herbarium CAG 1067Limonium morisianum Arrigoni*Aerial parts (LmA)PlumbaginaceaeJerzuDecember 2017Herbarium CAG 1067Myrtus communis L.MeriaFruits (MeF)MyrtaceaeCala Surya (Cardedu)December 2018 Poggio dei PiniHerbarium CAG 514Pistacia lentiscus L.Fruits (PIL)AnacardiaceaeCala Surya (Cardedu)December 2017 2017Herbarium CAG 280Pistacia terebinthus L. ssp. terebinthusLeaves (PIL)AnacardiaceaeJerzuJune 2018 2017Herbarium CAG 279Plagius flosculosus (L.) Alavi & Heywood*Aerial parts (PfA)AsteraceaeGairo TaquisaraJune 2018 2017Herbarium CAG 280Pilostemon casabonae (L.) Greuter*Aerial parts (ReA)AsteraceaeGairo TaquisaraJune 2018 2017Herbarium CAG 280Pilostemon casabonae (L.) Greuter*Aerial parts (ReA)AsteraceaeGairo TaquisaraJune 2018 2017Herbarium CAG 280Pilostemon casabonae (L.	Genista corsica (Loisel.) DC*	Aerial parts (GcA)	Fabaceae	Seui	May 2017	Herbarium CAG 286
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Glechoma sardoa (Bég.) Bég.*	Aerial parts (GsA)	Lamiaceae	Gennargentu	June 2017	Herbarium CAG 1104
$ \begin{array}{c c c c c c c c c c c c c c c c c c c $	Hypericum hircinum L. ssp hircinum*	Aerial parts (HhA)	Hypericaceae	Jerzu	June 2018	Herbarium CAG 232
Lavandula stoechas L.Aerial parts (LsA)LamiaceaeCala Surya (Cardedu)April 2017Herbarium CAG 1067Limonium morisianum Arrigoni*Aerial parts (LmA)PlumbaginaceaeJerzuDecember 2017Herbarium CAG 909/GMyrtus communis L.Fruits (McF) Leaves (McL)Fruits (Cardedu)December 2018 Poggio dei PiniDecember 2018 (Cardedu)Herbarium CAG 909/GPistacia lentiscus L.Fruits (PIL)Anacardiaceae (PIL)Cala Surya (Cardedu)December 2017 December 2017Herbarium CAG 514Pistacia terebinthus terebinthusFruits (PIL)Anacardiaceae (PIL)Cala Surya (Cardedu)December 2017 December 2017Herbarium CAG 280Pistacia terebinthus terebinthusLeaves (PIL)Anacardiaceae 	<i>Hypericum scruglii</i> Bacch., Brullo & Salmeri*	Aerial parts (HsA)	Hypericaceae	Jerzu	June 2018	Herbarium CAG 239/C
Limonium morisianum Arrigoni*Aerial parts (LmA)PlumbaginaceaeJerzuDecember 2017Herbarium CAG 909/GMyrtus communis L.Fruits (McF) Leaves (MeL)MyrtaceaeCala Surya (Cardedu)December 2018 Poggio dei PiniHerbarium CAG 	Lavandula stoechas L.	Aerial parts (LsA)	Lamiaceae	Cala Surya (Cardedu)	April 2017	Herbarium CAG 1067
Myrtus communis L.Fruits (McF) Leaves (McL)MyrtaceaeCala Surya (Cardedu)December 2018 Poggio dei PiniHerbarium CAG 514Pistacia lentiscus L.Fruits (ML)Fruits (PIF) 	Limonium morisianum Arrigoni*	Aerial parts (LmA)	Plumbaginaceae	Jerzu	December 2017	Herbarium CAG 909/G
InfridaceLeaves (McL)InfridacedPoggio dei PiniApril 2018514Fruits (PIF) Leaves (PIL)Fruits (PIF)December 2017Herbarium CAG 280Pistacia lentiscus L.Leaves (PIL)AnacardiaceaeCala Surya (Cardedu)December 2017Herbarium CAG 	Mortus communis I	Fruits (McF)	Myrtaceae	Cala Surya (Cardedu)	December 2018	Herbarium CAG
Fruits (PIF) Leaves (PIL)Fruits (PIF) Leaves (PIL)AnacardiaceaeCala Surya 	Myrtus communis E	Leaves (McL)		Poggio dei Pini	April 2018	514
Pistacia tentiscus L.Leaves (PIL)AnacardiaceaeCala Surya (Cardedu)December 2017280Pistacia terebinthus L. ssp. terebinthusLeaves (PtL)AnacardiaceaeJerzuJune 2018Herbarium CAG 279Plagius flosculosus (L.) Alavi & Heywood*Aerial parts 		Fruits (PlF)		Cala Surya (Cardedu)	December 2017	Herbarium CAG
Pistacia terebinthus L. ssp. terebinthusLeaves (PtL)AnacardiaceaeJerzuJune 2018Herbarium CAG 279Plagius flosculosus (L.) Alavi & Heywood*Aerial parts (PfA)AsteraceaeIglesiasJuly 2017Herbarium CAG 743Ptilostemon casabonae (L.) 	Pistacia tentiscus L.	Leaves (PlL)	Anacarchaceae	Cala Surya (Cardedu)	December 2017	280
Plagius flosculosus (L.) Alavi & Heywood*Aerial parts (PfA)AsteraceaeIglesiasJuly 2017Herbarium CAG 743Ptilostemon casabonae (L.) Greuter*Aerial parts (PcA)AsteraceaeGairo TaquisaraJune 2018Herbarium CAG 	Pistacia terebinthus L. ssp. terebinthus	Leaves (PtL)	Anacardiaceae	Jerzu	June 2018	Herbarium CAG 279
Ptilostemon casabonae (L.) Greuter*Aerial parts (PcA)AsteraceaeGairo TaquisaraJune 2018Herbarium CAG 796Rosmarinus officinalis L.Aerial parts (RoA)LamiaceaeAlgheroMay 2017Herbarium CAG 1091Santolina corsica Jord. & Fourr*Aerial parts (ScA)AsteraceaeMonte AlboNovember 2017Herbarium CAG 1091	<i>Plagius flosculosus (</i> L.) Alavi & Heywood*	Aerial parts (PfA)	Asteraceae	Iglesias	July 2017	Herbarium CAG 743
Rosmarinus officinalis L.Aerial parts (RoA)LamiaceaeAlgheroMay 2017Herbarium CAG 1091Santolina corsica Jord. & Fourr*Aerial parts (ScA)AsteraceaeMonte AlboNovember 2017Herbarium CAG 1091	Ptilostemon casabonae (L.) Greuter*	Aerial parts (PcA)	Asteraceae	Gairo Taquisara	June 2018	Herbarium CAG 796
Santolina corsica Jord. & Fourr*Aerial parts (ScA)AsteraceaeMonte AlboNovember 2017Herbarium CAG 732/A	Rosmarinus officinalis L.	Aerial parts (RoA)	Lamiaceae	Alghero	May 2017	Herbarium CAG 1091
	Santolina corsica Jord. & Fourr*	Aerial parts (ScA)	Asteraceae	Monte Albo	November 2017	Herbarium CAG 732/A

Scolymus hispanicus L. subsp. hispanicus	Aerial parts (ShA)	Asteraceae	Sarroch	June 2018	Herbarium CAG 812
Silybum marianum (L.) Gaertn.	Aerial parts (SmA)	Asteraceae	Uta	May 2017	Herbarium CAG 801
Smilax aspera L.	Aerial parts (SaA)	Smilacaceae	Geremeas	May 2017	Herbarium CAG 1414
Stachys glutinosa L.*	Aerial parts (SgA)	Lamiaceae	Gennargentu	June 2017	Herbarium CAG 1099
<i>Tanacetum audibertii</i> (Req.) DC*	Aerial parts (TaA)	Asteraceae	Gennargentu	August 2018	Herbarium CAG 737/A
Thymus herba barona Loisel.	Aerial parts (ThA)	Lamiaceae	Gennargentu	June 2017	Herbarium CAG 1065

131 *Endemic species of Sardinia

132

133 2.2. Chemicals and extracts preparation

134 All solvents and reagents were purchased from Sigma-Aldrich (Milan, Italy), MeOH was an 135 analytical grade ($\geq 99.9\%$).

136 Thirty mg of dried and powdered plant material were extracted by sonication for 30 minutes using 1.5 mL of MeOH/H₂O (1:1). Subsequently, samples were centrifuged (1700 \times g) for 20 min, the 137 138 supernatant was separated from the pellet and dried, firstly in vacuum concentrators (speedVac SPD 139 101b 230, Savant, Italy) for two hours to remove MeOH, then the residual extracts were freeze-140 dried over night to completely remove the residual H₂O finally yielding the crude extracts. For each 141 sample different extracts were produced, in an adequate number to perform all the biological tests 142 in replicates. This extraction procedure is designed to be performed relatively quickly and to 143 prepare little quantity of extracts for *in vitro* bioactivity tests, been ideal for screenings of high 144 number of plants. Moreover, this procedure allows a minimal waste of both solvents and plant 145 material. The choice of a mid-polar solvent system such as aqueous MeOH and the use of 146 sonication are recommended and used by several metabolomics studies (Kim & Verpoorte, 2010; 147 Verpoorte, R. et al., 2007), where MeOH/H₂O (1:1) turned out as the best choice for a first line 148 extraction procedure for general plant material, since it allows to extract a broad spectrum of 149 compounds. This protocol has been also used to compare biological activities of plants to their

phytochemical profile (Mandrone et al, 2018), resulting also suitable to facilitate further
metabolomic studies to identify the active principles of the extracts.

For biological assays, stock solutions were prepared solubilizing extracts in water at 10 mg/mL, centrifuged to remove the pellet if present, and stored at 4°C until use.

154 2.3. Total flavonoid and phenolic assays

155 The assays were performed in Spectrophotometer Jasco V-530 as described by Chiocchio et al. 156 (2018). Briefly, for total phenolic content analysis a calibration curve was constructed using 50 µL 157 of different gallic acid stock solutions prepared in MeOH 80% (from 10 to 200 µg/mL) mixed with 158 250 µL of Folin-Ciocalteau reagent (diluted 1:10) and 500 µL of H₂O. Different stock solutions of 159 extracts were prepared in water (from 0.05 to 0.2 mg/mL) and 50 µL of each stock were mixed with 160 the same reagents as described above. Both calibration curve and samples were incubated at room 161 temperature for 5 min before adding 800 µL of sodium carbonate solution (Na₂CO₃ 20%). After 30 162 min of incubation at 40°C, absorption was recorded at 760 nm. Total phenolic content was 163 calculated by interpolation in the calibration curve and expressed as: mg GAE (gallic acid 164 equivalent)/g of extract (dried weight).

165 Total flavonoid content was determined using rutin to perform the calibration curve. Different stock 166 solutions of extracts were prepared in water (from 0.05 to 0.2 mg/mL) and 50 µL of each one were 167 mixed with 450 µL of methanol and 500 µL of AlCl₃ (2% w/volume of methanol). The absorption 168 at 430 nm was recorded after incubation (15 min) at room temperature. The calibration curve was 169 obtained using 50 µL of different rutin stock solutions prepared in DMSO (from 1 to 100 µg/mL). 170 Total flavonoid content of the extracts was calculated by interpolation in the calibration curve and 171 expressed in terms of mg RE (rutin equivalent)/g of extract (dried weight). Analysis were 172 performed in triplicate.

173 2.4. Multivariate data analysis

For multivariate analyses (PCA), data were subjected to UV (United Variance) scaling and the
model was developed using SIMCA P+ software (v. 15.0, Umetrics, Sweden).

177 Staphylococcus aureus ATCC 25293, Staphylococcus epidermidis (ATCC 12228), Escherichia coli (ATCC 25922) and Klebsiella pneumoniae (ATCC 9591) were obtained from the American Type 178 179 Culture Collection. Subsequently, having defined the antibacterial properties of the extracts, the 180 main active were assayed towards 15 clinical isolates recovered from different clinical specimens, 181 and collected at the Microbiology Unit, St Orsola Malpighi University Hospital, Bologna, Italy. 182 Strains included 5 S. aureus of which 3 methicillin-resistant (MRSA), 5 S. epidermidis of which 3 183 methicillin-resistant (MRSE) and 5 K. pneumoniae of which 2 carbapenemase-producing (KPC-184 producing K. pneumoniae). Species identification and antimicrobial susceptibility testing were 185 performed by Vitek2 semi-automated system (bioMerieux, France), and EUCAST criteria were 186 used for the interpretation of results and for the definition of methicillin and carbapenem resistance.

187 2.6. Determination of antibacterial activity

188 The in vitro antibacterial activity of the thirty-six extracts was evaluated against four reference 189 strains and some selected extracts towards clinical isolates by a broth microdilution method 190 (Bonvicini et al., 2014; Bonvicini et al., 2017). The bacterial suspension, prepared in Mueller 191 Hinton broth (Sigma-Aldrich, St. Louis, USA) was incubated with the extracts at 200 µg/mL or 192 serially two-fold diluted from 200 µg/mL depending on the assay. A number of wells was reserved 193 in each microplate for negative (no inoculum added) and positive growth controls. The microplate 194 was incubated at 37°C for 24h, and subsequently the $OD_{630 \text{ nm}}$ was spectrophotometrically measured 195 (Multiskan Ascent microplate reader, Thermo Fisher Scientific Inc., Waltham, USA). Growth 196 percentage values were determined as relative to the positive control. Extracts demonstrating an 197 inhibitory activity superior to 70% at 200 µg/mL were defined as active and their IC₅₀ values 198 corresponding to the sample concentrations giving rise to an inhibition of bacterial growth of 50% 199 were obtained by the interpolation on the dose-response curves. Statistical analysis was carried out 200 by nonlinear regression method using GraphPad Prism version 5.00 for Windows (GraphPad 201 Software, San Diego California, USA). A one-way ANOVA was done for comparison between IC₅₀

values obtained for the reference strains and clinical isolates followed by Dunnett's multiplecomparison test to detect significant differences among groups.

204 2.7. Cell viability assay

205 African green monkey kidney cells (Vero ATCC CCL-81) were cultured in Eagle's Minimal 206 Essential Medium (MEM) (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal 207 bovine serum (FBS) (Carlo Erba Reagents, Milan, Italy), 100 U/mL penicillin, and 100 µg/mL 208 streptomycin at 37°C with 5 % CO₂. For experiments, cells were seeded into 96-well plates at 10⁴ 209 cells/well, and incubated at 37°C for 24h. Cell density and incubation time were previously 210 optimized (Bonvicini et al., 2018). Following washes with PBS (phosphate-buffered saline) to 211 remove floating cells, monolayer was incubated with 100 µL of serially 2-fold dilution of the 212 extract starting from 200 µg/mL, and with standard medium as positive control. The cell viability 213 was assessed by a WST8-based assay according to the manufacturer's instructions (CCK-8, Cell 214 Counting Kit-8, Dojindo Molecular Technologies, Rockville, MD, USA). After 48 h of incubation, 215 culture medium was removed from each well, the monolayer was washed with PBS, and 100 µL of 216 fresh medium containing 10 µL of CCK-8 solution were added and incubated for 2h at 37°C. Cell 217 viability was measured at OD_{450/630 nm} and expressed as the percentage of the cell viability relative to the untreated controls. The CC₅₀ values were obtained by the interpolation of percentage values 218 219 on the dose-response curves.

220 **3. Results and Discussion**

221 3.1. Screening of biological activities and multivariate data analysis

The thirty-six extracts were assayed *in vitro* at 200 μ g/mL to determine their antibacterial activity towards four reference strains and their cytotoxicity on mammalian epithelial cells. Overall data are reported in Tables S1 and S2 in Supplementary Material and Figure 1. Thirteen out of the thirty-six extracts resulted strong inhibitors of one or more bacteria (30% of bacterial growth compared to the extract-free control), as reported in Table 2. In particular, ten extracts inhibited the growth of both *S. aureus* and *S. epidermidis*, while three, PIF, RoA and SaA, showed activity only towards *S.* *aureus*. Regarding the effectiveness on Gram negative bacteria, seven extracts were effective against *K. pneumoniae*. Only two extracts, CyhA and PtL were able to reduce the growth of all bacterial strains below the abovementioned threshold of activity (30%), reducing also *E. coli* activity of 34% and 33%, respectively, which were the lowest values obtained out of the thirty-six extracts tested.

233

Table 2. Bacterial growth of the reference strains treated with the 13 most active extracts at 200 μ g/mL. Data are mean values and standard deviation obtained in two independent experiments performed in triplicate. Percentage values are relative to the positive control (100% of growth).

237

Sampla labla	S. aureus	S. epidermidis	E. coli	K. pneumoniae
Sample lable	ATCC 25293	ATCC 12228	ATCC 25292	ATCC 9591
AuL	16 ± 3	2 ± 3	58±5	29 ± 5
CmA	8± 3	5 ± 5	66 ± 6	18 ± 4
CsA	11 ± 6	3 ± 4	47 ± 4	37 ± 10
CyhA	5 ± 4	3 ± 4	34 ± 14	19 ± 1
LmA	9. ± 4	10 ± 5	69 ± 12	44 ± 6
McF	19 ± 5	12 ± 7	69 ± 7	64 ± 6
McL	5 ± 8	4 ± 6	55 ± 8	26 ± 11
PlF	26 ± 9	49 ± 15	77 ± 8	42 ± 3
PlL	9 ± 8	7 ± 13	47 ± 5	24 ± 7
PtL	4 ± 5	3 ± 3	33 ± 6	17 ± 3
RoA	13 ± 6	74 ± 7	97 ± 6	89 ± 2
SaA	30 ± 11	111 ± 15	73 ± 13	76 ± 4
ThA	13 ± 3	21 ± 15	106 ± 10	90 ± 1

The screening pipeline on the thirty-six extracts included the evaluation of their effects on cell viability and proliferation in order to discriminate between a specific ability to affect bacterial growth or to a general toxic activity on mammalian cells. As depicted in Figure 1, among the thirtysix extracts, eight strongly reduced mammalian cells metabolism below the 30% and, among these

extracts, six were labeled as *active* through the microbiological investigations, thus requiring further
evaluations to specify their safety profile.

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- 246

INSERT FIGURE 1

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To gain comprehensive insights on the biological properties of all tested extracts, principal component analysis model (PCA) was build, using as set of x variables: the bioactivity data against the four bacterial strains (expressed as % of inhibition at 200 μ g/mL), the cytotoxicity data (expressed as % of cell viability at 200 μ g/mL), and total polyphenols and flavonoids content of the extracts, expressed as mg of gallic acid equivalents (GAE)/g of extract and % of rutin equivalents (RE)/g of extract, respectively. These latter phytochemical data are reported in Table S3 of Supplementary Material.

255 As shown by the PCA scatter plot (Figure 2), antibacterial activity (against all strains) and phenolic 256 content followed a similar trend. In fact, extracts shifted on the positive side of the component t[1] 257 (PC1) were generally endowed with high value of both antibacterial activity and phenolic content. 258 Phenolic compounds might be involved in the positive effects observed, since they have been 259 recognized as bioactive molecules with pronounced antimicrobial activity (Gomes et al., 2018; 260 Scavo et al., 2019). Conversely, on the negative side of PC1 axis, the extracts showing no activity 261 on bacteria and an extremely low content of phenolic and flavonoid compounds were grouped. On 262 the positive side of the PC1 and along the negative side of the component t[2] (PC2) were placed 263 the extracts with the highest cytotoxicity on mammalian cells, such as CycA and CcA, and showing 264 only a medium activity against Staphylococci spp. High level of cytotoxicity on Vero cells was 265 shown also by CyhA, AuL and CsA, which followed, in fact, a similar trend along the PC2, shifting 266 toward the lower-right quadrant of the plot. Nevertheless, their strong antibacterial activities made 267 those extracts still interesting for further investigations (IC₅₀ and SI determination), while CycA and

268 CcA were considered not interesting, due to their strong cytotoxicity while scant antibacterial 269 activity.

270 On the upper part of the plot (positive PC2), the extracts with medium antibacterial activity while 271 very low cytotoxicity were clustered. Interestingly, low toxicity on mammalian cells was associated 272 to high flavonoids content, suggesting a possible cytoprotective role of these compounds, which are 273 also renowned antioxidants (Hosseinzadeh & Nassiri-Asl, 2014). Among the samples endowed with 274 high content of flavonoids, a peculiar case was represented by PtL, which, in fact, was identified as 275 an outlier in the PCA model. This extract showed high content of both phenols and flavonoids, high 276 antibacterial activity against all strains tested and very low cytotoxicity.

277 The herein described model, providing a graphical overview of all biological data, facilitates also 278 considerations on extracts obtained from plants belonging to the same genus. In particular, samples 279 included three different species of Centaurea genus (C. calcitrapa, C. napifolia and C. horrida), 280 and two different species of Pistacia (P. lentiscus and P. terebinthus ssp. terebinthus), Cistus (C. 281 salvifolius and C. monspeliensis) and Hypericum (H. scruglii and H. hircinum ssp. hircinum). 282 Regarding the three Centaurea species (CcA, CnA and ChA), they yielded very similar results, 283 namely they were proved not active against all pathogens tested and were also poor in phenols and 284 flavonoids. However, while CnA and ChA were also not cytotoxic on Vero cells, CcA was one of 285 the highly cytotoxic extract of the dataset. Regarding the two *Cistus* species, CsA and CmA, they 286 were placed very close in the PCA plot, since they showed a similar trend in both bioactivities and 287 phenolic/flavonoids content. The same behavior was observed for the two species of Hypericum 288 (HsA and HhA), which resulted both rich in flavonoids, not cytotoxic, while endowed with 289 moderate antibacterial activity. Finally, the two Pistacia, PlL and PtL, were both strongly active 290 against bacterial strains, even though PtL was more enriched in flavonoids and less cytotoxic than PlL.

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293

INSERT FIGURE 2

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As shown in Figure 3, the majority of the samples studied were plant leaves or aerial parts, one was constituted by rhizomes (ArRh), one by roots (FaR), and three of them were fruits (PIF, McF and AuF). In case of *Myrtus communis* and *Pistacia lentiscus*, both fruits and leaves extracts were tested and proved to be active and characterized by similar features, appearing very close into the PCA scatter plot. Conversely, only leaves of *Arbutus unedo* (AuL) were active, while fruits (AuF), being not active, were placed on the opposite quadrant of the plot.

- 301
- 302

INSERT FIGURE 3

303

304 *3.2. Antibacterial activity and selectivity*

305 The active subset of the thirteen extracts was further assayed in vitro towards some selected 306 bacterial strains to obtain IC₅₀ values on the specific dose-response curves. Based on data in Table 307 3, some general remarks can be drawn. Of the thirteen extracts inhibiting S. aureus, five displayed 308 potent one-digit μ g/mL IC₅₀ values and CyhA resulted the most effective S. aureus inhibitor (IC₅₀ = 309 1.4 µg/mL); of the ten extracts active towards S. epidermidis four exhibited comparable inhibitory effectiveness, and LmA displayed the highest activity (IC₅₀ = $3.9 \mu g/mL$). Concerning Gram 310 311 negative bacteria, according to generally lower inhibition rates, IC₅₀ values for the active extracts 312 were superior compared to those obtained for Gram positive strains, however worthy of note for 313 raw plant extracts (Cos et al., 2006). The extracts of CyhA and McL resulted the most potent against K. pneumoniae (IC₅₀ = 28.5 μ g/mL and IC₅₀ = 37.0 μ g/mL, respectively) and the first one, 314 315 being active even towards E. coli (IC₅₀ = 74.9 μ g/mL), displayed a broad spectrum antibacterial 316 activity. Differences in susceptibility between Gram positive and Gram negative bacteria are strictly 317 related to the presence of the outer membrane and the lipopolysaccharides in the latter cells; these 318 structures form an additional barrier that account for the Gram negative increased permeability 319 threshold to many molecules.

320

Table 3. Antibacterial activity of the thirteen selected extracts expressed as IC_{50} (µg/mL of extract), defined as the concentration giving rise to an inhibition of growth of 50% compared to the drug-free control. Data are reported as mean values and 95% confidence interval.

324

Samula labla	S. aureus	S. epidermidis	E. coli	K. pneumoniae
Sample lable	ATCC 25293	ATCC 12228	ATCC 25292	ATCC 9591
AuL	31.9 [26.2-38.8]	10.1 [9.3-10.9]	n.d. [§]	93.8 [81.8-107.6]
CmA	5.3 [4.4-6.5]	12.4 [11.1-13.9]	n.d.	64.65 [57.0-73.2]
CsA	9.0 [7.9-10.4]	29.5 [26.4-32.9]	n.d.	97.5 [80.6-118.1]
CyhA	1.4 [0.9-1.9]	8.0 [7.5-8.5]	74.9 [57.9-96.9]	28.5 [22.8-35.6]
LmA	9.2 [6.8-12.3]	3.9 [2.5-6.1]	n.d.	n.d.
McF	15.4 [10.7-21.9]	8.8 [7.5-10.5]	n.d.	n.d.
McL	7.5 [6.0-9.3]	9.7 [8.9-10.9]	n.d.	37.0 [28.3-48.4]
PlF	144.5 [126.0-165.6]	n.d.	n.d.	n.d.
PlL	27.3 [21.6-34.5]	56.8 [48.1-67.2]	n.d.	48.0 [40.6-56.7]
PtL	62.9[48.6-81.4]	103.1 [92.6-109.0]	156.3[138.1-177.0]	49.0 [42.8-56.0]
RoA	99.2 [83.1-118.5]	n.d.	n.d.	n.d.
SaA	153.6 [129.1-182.7]	n.d.	n.d.	n.d.
ThA	63.3 [55.5-72.1]	150.0 [131.0-171.8]	n.d.	n.d.

325 \$ n.d. = not determined

326

327 Dose-effect experiments on Vero cells were finally carried out to establish their safety on non-328 malignant epithelial cells. Table 4 reports the CC_{50} values and the corresponding selectivity index 329 (SI), calculated as CC_{50}/IC_{50} ratio, for the bacterial strain more susceptible to inhibition. Samples 330 obtained from CyhA, LmA and McL presented very high SI in relation to Vero cells on 331 *Staphylococci* spp. and only moderate values were obtained on *K. pneumoniae*, thus suggesting a 332 preferential inhibitory activity towards bacterial cells with respect to eukaryotic cells.

Table 4. Cytotoxicity of active extracts against Vero cells and Selectivity Indexes (SI). CC_{50} is defined as the concentration giving rise to an inhibition of cell metabolism of 50% compared to the drug-free control. Data are reported as mean values and 95% confidence interval. SI = selective index corresponding to the ratio between CC_{50} and IC_{50} .

338

Sample lable	CC ₅₀ (µg/mL)	SI
AuL	41.7 [35.0-49.7]	4.1 (S. epidermidis)
CmA	88.2 [69.6-11.7]	16.5 (S. aureus)
CsA	53.7 [43.5-66.3]	5.9 (S. aureus)
CyhA	90.3 [75.2-108.3]	64.7 (S. aureus); 3.2 (K. pneumoniae)
LmA	>200	>51.0 (S. epidermidis)
McF	>200	>22.6 (S. epidermidis)
McL	120.2 [92.9-155.6]	16.1 (S. aureus); 3.3 (K. pneumoniae)
PlF	>200	>1.4 (S. aureus)
PIL	84.2 [74.2-95.5]	3.1 (<i>S. aureus</i>)
PtL	>200	4.1 (K. pneumoniae)
RoA	>200	>2.0 (S. aureus)
SaA	>200	>1.3 (S. aureus)
ThA	>200	>3.2 (S. aureus)

339

340 *3.3. Clinical isolates*

341 The three extracts selectively inhibiting bacterial growth were assayed also towards a broad array of 342 relevant multi-resistant pathogens recovered from biological specimens. In particular, CyhA, LmA 343 and PtL were assayed against S. aureus, S. epidermidis and K. pneumoniae strains, respectively. 344 Data are reported in Table 5. Remarkably, the extracts proved to be active towards all the isolates 345 and no statistically significant differences (ANOVA followed by Dunnett's Multiple comparison) 346 were highlighted comparing IC₅₀ values of isolates, regardless their antibiotic resistance profile (see 347 Tables S4, S5 and S6 in the Supplementary Material), and reference strains. This is clinically 348 relevant considering that isolates may present phenotypic and genetic heterogeneity compared to 349 laboratory reference strains thus some differences in susceptibility may occur.

Table 5. IC₅₀ values of the three selected extracts towards clinical isolates. Data are reported as

352 mean values and 95% confidence interval.

\mathbf{r}	-	\mathbf{r}
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J	J	J

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CyhA Vs S. aureus	IC ₅₀ (μg/mL)	Antibiotic-resistance profile
ATCC 25293	1.4 [0.9-1.9]	
MSSA 1	1.6 [1.3-1.9]	CM ^S , E ^S , GMN ^S , LVX ^S , OX ^S , P ^R , TE ^S , SXT ^S
MSSA 2	2.8 [2.1-3.9]	CM ^R , E ^R , GMN ^S , LVX ^S , OX ^S , P ^S , TE ^S , SXT ^S
MRSA 1§	2.6 [1.9-3.6]	GMN ^S , LVX ^R , OX ^R , P ^R , TE ^S , TEC ^S , SXT ^S , VA ^S
MRSA 2 [§]	3.2 [2.4-4.4]	GMN ^S , LVX ^R , OX ^R , P ^R , TE ^S , TEC ^S , SXT ^S , VA ^S
MRSA 3 [§]	1.9 [1.6-2.2]	CM ^R , E ^R , GMN ^S , LVX ^R , OX ^R , P ^R , TEC ^S , TE ^S , SXT ^S , VA ^S
LmA Vs S. epidermidis		
ATCC 12228	3.9 [2.5-6.1]	
MSSE 1	2.6 [1.0-6.7]	CM ^S , E ^R , GMN ^S , LVX ^S , OX ^S , TE ^S , SXT ^S
MSSE 2	4.2 [2.1-8.3]	CM ^S , E ^S , GMN ^S , LVX ^S , OX ^S , P ^R , TE ^S , SXT ^S
MRSE 1§	3.0 [2.1-8.4]	CM ^S , E ^R , GMN ^S , LVX ^S , OX ^R , P ^R , TE ^S , TEC ^S , SXT ^R
MRSE 2§	6.7 [3.9-11.5]	CM ^S , E ^S , GMN ^S , LVX ^S , OX ^R , TE ^S , SXT ^R , VA ^S , TEC ^S
MRSE 3§	3.7 [1.8-7.8]	CM ^S , DA ^S , E ^I , GMN ^S , LVX ^R , OX ^R , TE ^S , SXT ^S , VA ^S , TEC ^S
PtL Vs K. pneumoniae		
ATCC 9591	49.0 [42.8-56.0]	
Kp 1	48.7 [42.0-56.5]	AK ^s , AMC ^R , CTX ^R , CFZ ^R , CIP ^R , FOS ^s , GMN ^s , TZP ^s , SXT ^R
Кр 2	46.1 [37.5-56.6]	AK ⁸ , AMC ⁸ , CTX ^s , CFZ ⁸ , CIP ⁸ , FOS ^s , GMN ^s , TZP ^s , SXT ⁸
Кр 3	45.5 [34.7-59.7]	AK ⁸ , AMC ⁸ , CTX ^s , CFZ ⁸ , CIP ⁸ , FOS ^R , GMN ^s , TZP ^s , SXT ⁸
KDC Kn 1*	53 0 [42 2 66 5]	AK ^R , AMC ^R , AMP ^R , CFZ ^R , CIP ^R , EPM ^R , GMN ^S , MEM ^R , TZP ^R ,
кі С-кр і	55.0 [42.2-00.5]	SXT ^R , TGC ^I , CS ^S
KPC_Kn 2*	47 3 [44 0-56 9]	AK ^S , AMC ^R , AMP ^R , CFZ ^R , CIP ^R , EPM ^R , GMN ^R , MEM ^I , TZP ^R ,
м с-кр 2	נ. ידן נ. יד	SXT^{R} , TGC^{S} , CS^{S}

354

355	AK = Amikacin; AMC = Amoxicillin/Clavulanic Acid; AMP = Ampicillin; CM = Clindamicyn; CTX = Cefotaxime;
356	CFZ = Ceftazidime; CIP = Ciprofloxacin; CS = Colistin; EPM = Ertapenem; E = Erythromycin; FOS = Fosfomycin;
357	GMN = Gentamicin; LVX = Levofloxacin; MEM = Meropenem; OX = Oxacillin; P = Penicillin; SXT =
358	Trimethoprim/Sulfamethoxazole; TE = Tetracycline; TEC = Teicoplanin; TZP = Piperacillin/Tazobactam, TGC =
359	Tigecycline; VA = Vancomycin
260	

360

361 R = Resistant; S = Susceptible; I = Intermediate, as defined following the EUCAST guidelines

362 *Staphylococcus* species resistant to oxacillin were declared, by convention, methicillin-resistant.

363 *Carbapenemase-producing *K. pneumoniae*.

365 *3.3 Traditional uses, bioactivities and phytochemical data of the three selected plants.*

The effectiveness of these selected extracts validates the Sardinian plants *Cytinus hypocistis*, *Pistacia terebinthus ssp. terebinthus* and *Limonium morisianum* as important source of antimicrobial compounds. These plants might be interesting for the development of food supplements and herbal products with antibacterial activity. Moreover, since *Limonium morisianum* is an endemic plant of Sardinia, the obtained results might contribute also to valorize the biodiversity of the territory and the development of local industries.

Cytinus hypocistis is a parasitic plant belonging to Cytinaceae family that grows on roots of *Cistus spp.* It has been used in Sardinian traditional medicine as astringent, tonic and haemostatic (Loi et al., 2002), to soften corns and hard skin, and to sooth epidermal inflammations (Ballero et al., 1997). Despite this wealth of traditional uses, its chemical composition is largely unknown. Hydrolysable tannins were previously identified as the main components (Magiatis et al., 2001), confirming the high phenolic content of CyhA extract observed in this study, and among them, isoterchebin, belonging to the ellagitannin class, was characterized (Schildknecht et al., 1985).

Given the well-known antimicrobial properties of hydrolysable tannins (Buzzini et al., 2008) it is likely that these compounds might be responsible for the observed antibacterial activity of CyhA. Recently, Zucca et al. (2015) found antimicrobial activity of *C. hypocistis* but using an extraction procedure different from the one performed in this work. Chiocchio et al. (2018) reported also the anti-elastase and anti-tyrosinase activities of this plant. Moreover, antimalarial and antitumor properties of this plant have also been described (Fokialakis et al., 2007; Magiatis et al., 2001).

Pistacia terebinthus ssp. terebinthus (Anacardiaceae), commonly known as terebinth or turpentine tree, is a small deciduous tree widely distributed in the Middle East and Southern Europe. In Sardinia, it grows only on a calcareous restricted area of east coast (Usai et al. 2006). The consumption of *P. terebinthus ssp. terebinthus* in the Mediterranean countries traced back to ancient times. For instance, leaves of this plant have been used for the treatment of burns and the branch 390 resin for bronchitis and other respiratory afflictions, as well as for anti-inflammatory and antipyretic 391 properties (Topcu et al., 2007). The mature fruits were used as a diuretic and for urinary 392 inflammations, stomachache (Cakilcioglu et al., 2010), stomach ulcers (Polat et al., 2013), 393 antiseptic, hypotensive and for headache (Agelet and Vallès 2003). The resin is used as a chewing 394 gum and as food additive (Schoina et al., 2015). In Sardinia the decoction has been used to treat 395 catarrhal cough (Bruni et a., 1997), while the resin as expectorant, diaphoretic, analgesic, tonic and 396 to obtain an ointment used for the treatment of bladders (Atzei 2003). P. terebinthus ssp. 397 terebinthus has been reported to be rich in essential oil, proteins, organic acids, sugars, flavonoids, 398 tannins and resinous substances (Couladis et al., 2003; Marengo et al., 2018; Ozcan, 2004; Ozcan et 399 al., 2009; Piras et al., 2017; Pulaj et a., 2016; Usai et al., 2006). Several studies highlighted 400 remarkable differences in the essential oil composition of this plant, attributable to geographic and 401 climatic features (Couladis et al., 2003; Dhifi et al., 2013; Duru et al., 2003; Ismail et al., 2013; 402 Marengo et al., 2018; Piras et al., 2017; Ulukanli et al., 2014; Pulaj et al., 2016). P. terebinthus ssp. 403 terebinthus is reported to be active as: antibacterial, antifungal, antioxidant, cytotoxic, 404 neuroprotective, antinflammatory and insecticidal agent (Dhifi et al., 2013; Duru et al., 2003; Orhan 405 et al., 2012; Ismail et al., 2013; Kavak et al., 2010; Kordali et al., 2003; Piras et al., 2017; Ulukanli 406 et al., 2014; Pulaj et al., 2016; Topcu et al., 2007).

407 Limonium morisianum (Plumbaginaceae) is a dwarf frutex endemic and exclusive of calcareous 408 mountains of Sardinia. To the best of our knowledge, no information on its use in Sardinian 409 traditional medicine is available, since it is a very rare species. Limonium spp. are reported to 410 contain several classes of active components, such as hydrolysable and condensed tannins, 411 alkaloids, flavonoids, sterols, terpenes, saponins, coumarins, and amino acids (Blainski et al. 2013; 412 Medini et al. 2014; Gadetskaya et al. 2015; Medini et al. 2015; de Oliveira Caleare et al. 2017). 413 Moreover, myricetin, myricetin 3-O-rutinoside, myricetin-3-O-(6"-galloyl)- β -D-galactopyranoside, 414 (-)-epigallocatechin 3-O-gallate, tryptamine, ferulic and phloretic acids have been identified from 415 its aerial parts (Sanna et al., 2018. Definitely, L. morisianum has been slightly studied both 416 phytochemically and biologically. Recently, the antiviral activity has been reported against HIV-1 417 and Ebola viruses (Sanna et al., 2018c; Daino et al., 2018), as well as the ability to inhibit tyrosinase 418 and elastase enzymes (Chiocchio et al., 2018). No information on antimicrobial and cytotoxic 419 activities has been previously reported for any extract of this plant.

420 4. Conclusions

This work reports the antimicrobial activity of some plants growing spontaneously in Sardinia (Italy). Thirty-six extracts were assayed *in vitro* towards four reference bacterial strains and evaluated for their cytotoxicity on mammalian epithelial cells.

The results of the biological screening, together with total phenolic and flavonoid content of the extracts, were processed through Principal Component Analysis (PCA), which highlighted the positive correlation among total phenolic content and increasing antibacterial activities, and a possible involvement of flavonoids in mitigate the cytotoxicity against eukaryotic cells.

428 A significant activity was observed for thirteen extracts at non-cytotoxic concentration, and among 429 them three emerged for their selective and potent inhibitory effect on bacterial growth; Cytinus 430 hypocistis proved to be a broad spectrum antibacterial extract, mainly active towards S. aureus (IC50 431 1.4 µg/mL), Limonium morisianum exhibited a potent anti-staphylococcal properties and Pistacia 432 terebinthus ssp. terebinthus resulted the extracts with the highest SI on K. pneumoniae. These 433 extracts, when tested towards isolates obtained from biological specimens and with different 434 antibiotic-resistance profiles, confirmed their effectiveness to inhibit bacterial growth, thus 435 validating their potential as antimicrobial agents.

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- 439 **Declarations of interest**
- 440 None
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