Zuccagnia punctata: A Review of its Traditional Uses, Phytochemistry, Pharmacology and Toxicology

María Inés Isla[a,b,*,#], María Alejandra Moreno[a], Gabriela Nuño[a], Fabiola Rodriguez[a], Antonella Carabajal[a], María Rosa Alberto[a,b] and Iris Catiana Zampini[a,b,*]

[a]*Instituto de Química del Noroeste Argentino (INQUINOA, CONICET),* [b]*Facultad de Ciencias Naturales, Universidad Nacional de Tucumán. San Lorenzo 1469. San Miguel de Tucumán. Tucumán. Argentina.*

Both authors - Isla and Zampini - participated equally in this research work.

[#]*misla@tucbbs.com.ar*

Zuccagnia punctata Cav. (Fabaceae, Caesalpiniaceae) is a plant with a long history of use in Argentine traditional medicines; it belongs to a monotypic genus, and is an endemic species of Argentina. This review provides a comprehensive overview of the traditional uses, phytochemistry, pharmacological activity and toxicology of *Z. punctata*. A wide range of traditional uses are cited in the literature such as antibacterial, antifungal, anti-inflammatory, and antitumor, among others. Pharmacological studies to date have demonstrated significant activities that support the traditional uses of this plant. No human clinical trials had been completed up to the time of this review and no toxic effect had been detected in animals. Compounds from different chemical groups have been isolated such as phenolic compounds and essential oils. Plant extracts and phytochemicals isolated exhibit a broad range of activities, anti-inflammatory, antibacterial, antifungal, antigenotoxic, antioxidant, antiulcer, and nematicidal. The main bioactive phytochemicals in the aerial parts (leaf, stem and flower) were identified as 2´, 4´-dihydroxy-3´-methoxychalcone and 2´, 4´-dihydroxychalcone and were proposed as chemical markers. Consequently, standardized dry extracts of aerial parts of *Z. puntacta* could be used in herbal medicinal products. Also, they could be included in phytotherapeutic preparations such as capsules, creams, and gels, and for microencapsulation.

Keywords: *Zuccagnia punctata*, Pharmacological activity, Phytochemistry, Traditional use, Toxicity.

Zuccagnia punctata Cav. (Fabaceae, Caesalpiniaceae), commonly known as jarilla pispito, pus pus, lata, and jarilla macho, belongs to a monotypic genus endemic to Argentina and which is characteristic of xerophytic plants from the Biogeographic Province of Monte [1a-c]. This species is widely distributed in arid and semiarid areas of western Argentina (Jujuy, Salta, Tucumán, Catamarca, La Rioja, San Juan, Mendoza and San Luis) from 700 to 2700 masl [1a-d].

Z. punctata inhabits the same environment as *Larrea cuneifolia* Cav. and *L. divaricata* Cav., with which it forms a natural arid community named "jarillal" [1a]. Other plant species growing in this eco-region are *Bulnesia retama* (Gillies ex Hook. & Arn.) Griseb., *Monttea aphylla* (Miers) Benth. & Hook., *Gochnatia glutinosa* (D. Don) Hook. & Arn., *Plectrocarpa rougesii* Descole, O'Donell & Lourteig, *Mimosa ephedroides* (Gillies ex Hook. & Arn.) Benth., *Bougainvillea spinosa* (Cav.) Heimerl, *Prosopis alba* Griseb; *Prosopis nigra* (Griseb.) Hieron., and *Geoffroea decorticans* (Gillies ex Hook. & Arn.) Burkart [1a-e].

Botanical description: *Z. punctata* is a glutinous and aromatic shrub 1 to 2.5 m high (Figure 1A); pseudo-paripinnate resinous leaves of 3-5 cm with subopposite leaflets (5 to 13 pairs), nanophyll with acuminate apex, rounded base and entire margin (Figure 1B) [2a,b]. Plants bloom from August to March and fructify from November to April [1a]. Its yellow flowers, born in erect racemes, have a funnel-shaped calyx, with 5 sepals, a corolla with 5 free petals and 10 free stamens (Figure 1C) [1a,b,e]. Its fruits are capsule like, indehiscent, leathery, ovoid-acute, oblique, compressed and estipitate [1a,b,e]. Both leaflet epidermal surfaces and the raquis exhibit epidermal cells with straight anticlinal walls, a thick cuticle, cyclocytic stoma, crypt-located sunken capitate glandular trichomes and non-glandular one-celled trichomes arranged on the
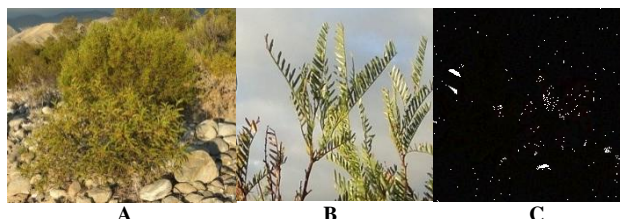

Figure 1: Photographic image of *Zuccagnia punctata* Cav. in a natural setting in Tucumán. Argentina. **A.** General aspect of plant. **B.** Leaves. **C.** Flowers

margins (Figure 2A and B) [2a-d]. In a cross section, leaflets are iso-lateral and amphi-stomatic. The middle vein presents a collateral vascular bundle with sclerenchymatous layers at the phloem pole (Figure 2B). Idioblasts containing druses in the mesophyll are abundant [2b].

Popular use of aerial parts: Infusions and decoctions in water, as well as extracts prepared by maceration in ethanol of *Z. punctata* aerial parts with or without flowers or fruits have been used extensively as a traditional medicine in Argentina as a foot antiseptic and rubefacient and against bacterial and fungal infections, asthma, arthritis, rheumatism, inflammation, and tumors [3a-c]. Stems are used for the building of house roofs [1a]; they are also used to dye leaden-colored wool [3a,b, 4a-c], and the leaf resin was used in the ritual for mummification and preservation of dead ancestors (Institute of Anthropology, UNLaR).

History: In the 19th century, Hieronymus indicated the use of *Z. punctata* as an anti-rheumatic and antispasmodic [5a]. Lallemant in 1894 [5b] pointed out that healers recommended a hot bath with leaves to treat rheumatism, and a tea made from sticks in cases of flank pain or pneumonia.
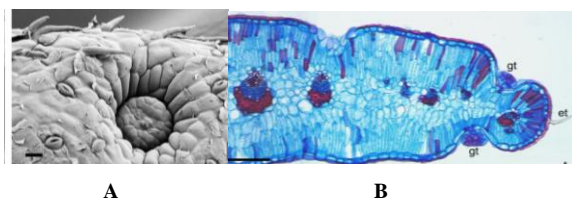
Figure 2: Photographic image of *Zuccagnia punctata* by scanning electron microscopy (A) and light microscopy (B). **A.** Leaflet surface with cyclocytic stomata and sunken capitate glandular trichomes and non-glandular one-celled trichomes arranged on the margins. **B.** Astra blue-safranin, general aspect of glandular (gt) and non-glandular (et) trichomes and palisade mesophyll (pm) by conventional anatomy techniques.

Aerial parts of *Zuccagnia punctata*

Phytochemical composition: Essential oils from the aerial parts of *Z. punctata* were obtained by hydro-distillation and analyzed by GC–FID, GC–MS and ^{13}C NMR spectroscopy. Eighty constituents, mainly oxygenated monoterpenes, were identified representing from 79.0 to 95.2% of the total oils. The main components were identified as linalool and (-)-5, 6-dehydrocamphor [2c]. Thymol and carvacrol were also identified as minor components [2c].

Z. punctata showed a high content of total phenolic compounds (168.0±15 mg GAE/g aerial parts) and flavones/flavonols (16.0 ± 1.0 mg QE/g aerial parts). Thirteen phenolic compounds were isolated from the aerial parts. The isolated compounds were identified as two chalcones, 2´,4´-dihydroxy-3´-methoxychalcone (DHMC) and 2´,4´-dihydroxychalcone (DHC) (Figure 3); five flavones, 3,5,7-trihydroxyflavone (galangin), 3,5-dihydroxy-7-methoxy flavone (izalpinin), 3,5,4´-trihydroxy-7-methoxy flavone (rhamnocitrin), 3-hydroxy-7,8-dimethoxyflavone, and 3,7-dihydroxy flavone (3,7-DHF) (Figure 3); four flavanones, 7-hydroxy flavanone (7-HF), 5,7-dihydroxyflavanone (pinocembrin), 5-hydroxy-7-methoxy flavanone (pinostrobin), and 7-hydroxy-8-methoxyflavanone (Figure 3); and two caffeic acid derivatives, 1-methyl-3-(4´-hydroxyphenyl)-propyl caffeate and 1-methyl-3-(3´,4´-dihydroxyphenyl)-propyl caffeate [3c, 6a-f, 7a-e]. Chalcones were the main constituents of aqueous, ethanolic, methanolic and dichloromethane (DCM) extracts [6c-d, 7d-e].

Recently, Moreno *et al.* [2d] reported the content of total polyphenolic compounds on *Z. punctata* foliar surface (177±13 µg GAE/cm^2). Analysis of the foliar washings revealed the presence of two major constituents, DHC and DHMC. The content of both major compounds (cm^2 of leaf) was 99.2 µg DHC and 73.4 µg DHMC/cm^2. Histochemical analysis (fluorescence microscopy and emission scanning electron microscopy coupled with energy dispersive X-ray spectrometry) revealed on the foliar surface a high accumulation of chalcones. Consequently, the authors have proposed that these could act as a defense mechanism against UV radiation for the protection of photosynthetic tissues.

As a result of pharmacological studies, several pure chalcones isolated from different plants have been approved for clinical trials for the treatment of cancer and cardiovascular disorders or have been included as ingredients in cosmetic preparations [8a,b]. Clinical trials have shown that these compounds reached reasonable plasma concentrations and did not cause toxicity [8 b]. For their potential use, the high content of both chalcones (DHC and DHMC) quantified in *Z. punctata* aerial parts is very interesting. Recently, Buttasi *et al.* [8c] have described the variability of chalcone content in four batches of *Z. punctata* dry extract prepared with samples collected in the same place in different months of a year by using DCM as extraction solvent (96.4-165.4 mg chalcones/g extract) (Table 1).
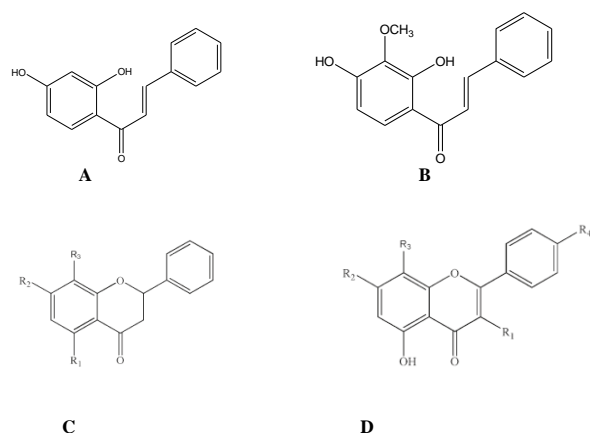
Compound A			
2´,4´-dihydroxychalcone			
Compound B			
2´,4´-dihydroxy-3´-methoxychalcone			
Compounds C	**R$_1$**	**R$_2$**	**R$_3$**
5-hydroxy-7-methoxy flavanone	OH	OCH$_3$	H
5,7-dihydroxyflavanone	OH	OH	H
7-hydroxy flavanone	H	OH	H
7-hydroxy-8-methoxyflavanone	H	OH	OCH$_3$

Compounds D	**R$_1$**	**R$_2$**	**R$_3$**	**R$_4$**
3,5,7-trihydroxyflavone	OH	OCH$_3$	H	H
3,5-dihydroxy-7-methoxy flavone	OH	OH	H	H
3,5,4´-trihydroxy-7-methoxy flavones	OH	OCH$_3$	H	OH
3-hydroxy-7,8-dimethoxyflavone	OH	OCH$_3$	OCH$_3$	H
3,7-dihydroxyflavone	OH	OH	H	H

Figure 3: Chemical structures of isolated phenolic compounds from *Z. punctata*.

Moreno *et al.* [2d] also reported the content of cuticular wax in *Z. punctata* leaves (88 µg/cm^2). In the foliar wax, the main alkane constituents, identified by GC-MS, were *n*-heptacosane (C27), *n*-nonacosane (C29) and *n*-hentriacosane (C31).

Table 1: Quantitative analysis of chalcones in four batches of *Z.punctata* DCM extract from plant material collected in the same place in four months of one year [8c].

Chemical markers	February	May	September	November
	mg compounds/g dry extract			
DHC	163.9±0.5	153.9±0.7	155.1±0.9	165.4±0.10
DHMC	162.2±0.9	96.4±0.6	151.5±0.1	103.7±0.21

Pharmacological activities

*Antibacterial activities***:** The increasing prevalence of multidrug-resistant bacteria has left clinicians with fewer treatment options, often resulting in the use of more costly treatments [9a].

For the last years, there has been a renewed interest in research on alternative antimicrobials and more targeted treatment. The first strategy, including therapies used before the advent of antibiotics, was a return to traditional remedies and medicines such as essential oils, herbal and plant extracts. Some of these alternative therapies can be administered systemically, but there are many more that can be administered topically. These new treatments may complement antibiotic therapy and help reduce the further spread and development of resistance in the future. *Z. punctata* is used in Argentine traditional medicine as an antiseptic [3a-c]. Several reports have validated its traditional use through determination of minimal inhibitory concentrations (MIC) and minimal bactericidal concentration (MBC) [9b].

Hydroethanolic extract of *Z. punctata* aerial parts was active against antibiotic-multiresistant Gram-negative bacteria isolated from human lesions such as *Escherichia coli*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter cloacae*, *Serratia marcescens*,

Morganella morganii, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia*, with MIC values ranging from 25 to 200 µg/mL. MBC values were identical to or two-fold higher than the corresponding MIC values [6d]. DHC, a compound isolated from *Z. punctata* leaves, exhibited strong antibacterial activity with MIC values between 0.10 and 1.00 µg/mL for *P. mirabilis*, *E. cloacae*, *S. marcescens*, *M. morganii*, *A. baumannii*, *P. aeruginosa*, and *S. maltophilia* [6d]. These results clearly show that DHC is more active than the whole extract (between 25 and 250 times) (Table 2).

Z. punctata extract, and three major compounds isolated from it (DHC, 7-HF and 3,7-DHF), were also active against Gram-positive bacteria, four different serotypes of *Streptococcus pneumoniae* with MIC values between 50 and 500 µg/mL [6f] (Table 2). Antibacterial activities of different quantities of *Z. punctata* extract, DHC, 3,7-DHF and 7-HF were also examined by using a *S. pneumoniae* infection model in mice. Mice were infected with 10^6 CFU of *S. pneumoniae* and, the following day, the products were orally administered to the infected mice. Treatment with *Z. punctata* extract (1 mg/mice) and 7-HF (1 mg/mice) significantly reduced the number of viable *S. pneumoniae* in lung ($p < 0.01$) while DHC and 3,7-DHF had no effect *in vivo*.

These results suggest that the extract's antibacterial activity on *S. pneumoniae*, *in vivo*, might be related, at least in part, to the action of 7-HF [6f]. The extract and 7-HF could be considered as antibacterial natural drugs against *S. pneumoniae* [6f].

Table 2: Minimal inhibitory concentration (MIC) of *Z. punctata* (*Zp*) extract and isolated flavonoids against bacterial and fungal strains. DHC: 2'4'-dihydroxychalcone.

Microorganisms	*Zp* aerial part extract	*Zp* flower extract	*Zp* root extract	*Zp* fruit extract	DHC	Ref.
	MIC (µg/mL)					
S. pneumoniae	50-400	-	-	-	100	[6f]
E. faecalis	-	250-500	-	-	+	[18]
S. aureus	-	125-250	-	-	+	[18]
Gram negative bacteria	25 -200	-	-	-	0.1 -1	[6d]
Yeasts	62.5-250	60-120	R	62.5-250	8-100	[7b,d, e, 9b, 10a]
Dermatophytes	8-16	--	R	8-16	4-32	[7b,9b, 10a]

Activities on human pathogenic yeasts and dermatophyte fungi: In recent years, the number of infections worldwide by *Candida* species and dermatophytes has increased considerably and resistance to traditional antifungal therapies is also rising. Current therapeutic options appear to be highly toxic and there are a lot of drug interactions. The lack of availability of conventional antifungal agents has encouraged the search for new alternatives such as medicinal plants traditionally used in popular medicine [10a]. Native people from Argentina northwestern have reported the use of *Z. punctata* as an antifungal [3a-c]. The ethnopharmacological use of this plant species was confirmed by several scientific reports. Thus, the effect on human pathogenic yeasts (*Candida albicans*, *Saccharomyces cerevisiae* and *Cryptocccus neoforms*) was demonstrated in light petroleum and DCM extracts. MIC values were between 62.5 and 250 µg/mL [7b,e,10b,c]. Antifungal activity was attributed to DHC [7b,d,e,10b]. The antimicrobial activity and particularly the chalcone antifungal action have been largely attributed to the reactive enone moiety [10d]. As a Michael reaction acceptor the enone unit binds to thiol groups of certain proteins [10d].

Recently, Buttasi *et al*. [8c] have described the synergistic effect of *Z. punctata* DCM extract and *Larrea nitida* DCM extract on *C.*

albicans and *C. glabrata* growth. Taking into account that the most synergistic combinations are those that produce 95% fungal growth inhibition, the authors indicate that each dose (1 mL) of the most synergistic mixture against *C. albicans* should contain 65.96 µg of the whole mixture, composed of 18.84 µg of dry extract from *Z. punctata* aerial parts and 47.12 µg of dry extract from *L. nitida* aerial parts, corresponding to 5.39 µg of flavonoid markers from *Z.punctata* and 23.63 µg of lignan markers from *L.nitida*. In turn, each dose (1 mL) of the most synergistic mixtures against *C. glabrata* should contain 168.23 µg of the whole mixture, composed of 45.47 µg of dry extract from *Z. punctata* aerial parts and 122.76 µg of dry extract from *L. nitida* aerial parts, corresponding to 16.35 µg of flavonoid markers from *Z.punctata* and 53.24 µg of lignan markers from *L. nitida*.

Nuño *et al*. [7d] showed that *Z. punctata* DCM extracts and chalcones isolated from them are effective not only as inhibitors of *Candida* growth but also as inhibitors of biofilm formation, as well as on preformed *Candida* biofilm and yeast germ tube formation in doses lower than MIC values. Chalcone concentration necessary to produce 50% inhibition of yeast germ tube formation (12.5 µg/mL for DHC and 30 µg/mL for DHMC) was similar to that necessary to produce 50 and 87% biofilm inhibition of *C. albicans*, respectively. Furthermore, they are able to inhibit the exoenzymes (phospholipase and hemolysin) responsible for the invasion mechanisms of *Candida* strains. All these effects could moderate colonization, thereby suppressing the pathogen's invasive potential.

Very strong activity of polyphenolic extracts of *Z. punctata* on dermatophyte fungi commonly isolated from skin infections (*Microsporum gypseum*, *Tricophyton rubrum* and *T. mentagrophytes*) (MIC values 8-16 µg/mL) was demonstrated [4 h,o,p]. This activity was attributed to DHC (MIC=4-32 µg/mL) and DHMC (MIC=8 µg/mL) [7b,10b,c] (Table 2).

Alvarez *et al*. [2c] have demonstrated that essential oils obtained from *Z. punctata* also show antifungal activity against the dermatophytes *M. gypseum*, *T. rubrum* and *T. mentagrophytes*, with MIC values between 15.6 and 125 µg/mL; the oils were not active against *C. albicans, C. tropicalis, S. cerevisiae* or *C. neoformans*. The major constituents of the oil, linalool and (-)- 5,6-dehydrocamphor, were also tested against the same fungal strains. Linalool showed moderate activity against dermatophytes (MICs = 125–250 µg/ mL), but was inactive against *C. albicans, C. tropicalis* and *C. neoformans*. The other major constituent, (-)-5,6-dehydrocamphor, was also inactive up to 250 µg/mL. According to these results, the main components of the essential oil from *Z. punctata* would not be responsible for its anti-dermatophyte activity; the activity was attributed to minor components such as thymol and carvacrol (MIC values of 15.6-31.2 µg/mL [2c].

Consequently, the polyphenolic extract of aerial parts of *Z. punctata* could be used as a yeast and dermatophyte growth inhibitor, while essential oils could be considered as promising antifungal agents that inhibit dermatophyte growth.

Tangarife-Castaño *et al*. [10a] suggested a classification system for antifungal activity in plant derivatives based on MIC values as strong inhibitors (MIC values of < 500 µg/mL), moderate inhibitors (MIC values of 600-1500 µg/mL), and weak inhibitors (MIC values of > 1600 µg/mL). Therefore, *Z. punctata* polyphenolic extracts and essential oils could be considered as strong antifungal agents.

Activity on phytopathogenic fungi: Commercial crops are usually attacked by fungal infections either during cultivation or during

post- harvest storage, affecting their productivity and commercial quality. Although there is an extensive list of available fungicides, the appearance of resistant strains and the toxicity of the fungicides to humans and animals have emerged as significant problems. For this reason, new anti-fungal agents are still needed.

Several authors have reported activity of *Z. punctata* crude extract against the growth of wood-destroying fungi such as *Lenzites elegans*, *Schizophyllum commune*, *Pycnoporus sanguineus*, and *Ganoderma applanatum*, and plant pathogenic fungi such as *Aspergillus niger, A. fumigatus, A. flavus, Colletotrichum truncatum, Fusarium oxysporum, F. verticillioides, F. graminearum sensu stricto, F. boothii, F. meridionale, F. subglutinans, F. thapsinum, Penicillium notatum*, and *Phomopsis longicolla* [6c,7b,10e,f] with MIC values between 100 and 500 µg/mL. The main compounds with activity on these phytopathogenic fungi were identified as chalcones (DHC and DHMC), 7-HF and 1-methyl-3-(4-hydroxyphenyl)-propyl caffeate with MIC values between 6.25 and 50 µg/mL [6c,7b,10f]. The results obtained up to date indicate the potential of *Z. punctata* extract and some metabolites obtained from it to control fungal species responsible for fungitoxic ear rot diseases, soybean anthracnosis that affects seed quality and yield, and postharvest lemon disease, among others.

Antioxidant activities: The antioxidant capacity of *Z. punctata* hydroethanolic extracts has been demonstrated [7e]. Avila *et al.* and Morán Vieyra *et al.* [7a,11] reported the antioxidant properties and mechanisms of three structurally-related flavonoids isolated from *Z. punctata*, 7-HF, DHC and 3,7-DHF. The scavenging activity of DPPH and ABTS radicals by *Z. punctata* flavonoids in ethanol solution was dominated by a sequential proton-loss electron-transfer (SPLET) mechanism, favored by 10% of the flavonoids being deprotonated. The ABTS and DPPH radical scavenging reactivity trend was DHF>DHC>HF, which was correlated with the electron-donor capacity of the flavonoids (Table 3). However, the $O_2^{\bullet-}$ scavenging in aqueous buffered solution was significantly controlled by the fraction of neutral flavonoids (FOH) through concerted proton-coupled electron-transfer (PCET). In this case, the radical scavenging reactivity trend was DHC>DHF>HF (Table 3). In the case of $^1O_2$ quenching in ethanol solutions, the quenching efficiency was controlled by the electron donor properties of flavonoids [7a,11]. Avila *et al.*, [11] demonstrated that DHC and 7-HF are poor singlet molecular oxygen generators upon direct photoirradiation (near UV) where the DHMC compound does not generate any of the oxidative species.

Table 3: Free radical scavenging activity of flavonoids from *Zuccagnia punctata*

Radicals	SC_{50} (mM)*			Reference
	DHF	DHC	HF	[10c]
ABTS$^{\bullet+}$	0.013	0.59	19	[10c]
DPPH$^\bullet$	0.38	2.1	500	[10c]
$O_2^{\bullet-}$	0.98	0.28	2.30	[10c]

*SC_{50}=Flavonoid concentration necessary to scavenge 50% of free radicals (s.d.10%).

Antiulcer activity: *Z. punctata* is used in popular medicine as a rubefacient and anti-inflammatory. Cytoprotective effects of chalcones from *Z. punctata* [3c,12a] and antiulcer activity of *Z. punctata* extract have been reported [12b]. The methanolic and aqueous extracts (infusion) of *Z. punctata*, as well as DHC and DHMC isolated from them, were able to inhibit 84, 43, 63 and 25% of necrotizing gastric damage, respectively (100 mg/Kg, ip).

Anti-inflammatory activity: Inflammatory and pathogenic conditions activate cyclooxygenase (COX) and lipoxygenase (LOX), two key enzymes in the synthesis of prostanoids and eicosonoids from poly-unsaturated fatty acids, which are involved

in various inflammatory and allergic disorders [13a]. Inhibition of COX activity is the mechanism by which non-steroidal anti-inflammatory drugs exert their analgesic, antipyretic, anti-inflammatory, and antithrombotic effects [13b]. The effect of isolated flavonoids from *Z. punctata* aerial parts on COX-2 was assayed by measuring the prostaglandin (PG) production by ELISA. DHC was 14-fold more potent than HF on COX-2. The activity was dose-dependent between 4-190 µM [13c]. Various synthetic chalcones were shown to be active towards one or more inflammatory mediators [8b]. Modifications of the α, β-unsaturated ketone group were observed to cause either a decrease or loss of anti-inflammatory activity, which is consistent with its role as a Michael acceptor to nucleophilic species like gluthatione or cysteine residues on proteins. The presence of a 2'-hydroxy group produced significant anti-inflammatory effects by increasing the electrophilic properties of the α,β-unsaturated ketone group due to hydrogen bonding to the ketone moiety [8b]. Both characteristics are present in DHC. The inhibition of pro-inflammatory enzymes by DHC and its antioxidant capacity support the potential use of *Z. punctata* as a phytomedicine, which could be used to prevent the development of chronic inflammatory pathologies.

Effect on Caenorhabditis elegans: Due to the appearance of drug-resistant worms, new therapeutically efficient and low toxicity drugs are urgently needed. The isolated flavonoids (7-HF, 3,7-DHF and DHC) from *Z. punctata* aerial parts were assayed on the free-living *Caenorhabditis elegans* nematode. Only DHC showed an anthelmintic effect and alteration of egg hatching and larval development processes of *C. elegans*. At 17 µg/mL, DHC was able to kill 50% of adult nematodes [14a]. Therefore, DHC could be proposed as a potential anthelmintic drug.

Effect on the function and expression of ABCB1/P-glycoprotein multidrug transporter (P-gp): Multidrug resistance is a significant challenge in the treatment of infectious diseases and cancer. Antitumor treatments currently in use often fail at some stage of the illness. This is because many types of cancer develop resistance to chemotherapeutic drugs, rendering them unresponsive to treatment. Several studies have been performed to analyze drug resistance mechanisms. One of the major drug resistance mechanisms in cells involves decreased uptake of water soluble drugs, including foliate antagonists, nucleoside analogs, and cisplatin, which require transporters to enter cells. This decrease can be attributed to upregulation of drug transporters, such as P-gp. These transporters can export a range of anticancer drugs from the cells, thus lowering the concentration to below the level required to provide a cytotoxic effect.

Z. punctata hydroethanolic extracts, 3,7-DHF and, to a lesser extent, DHC, were able to modulate activity and expression of the ABC membrane transporters, in particular P-gp, the best known membrane efflux pump involved in drug resistance (Figure 4). P-gp is physiologically expressed in the intestine apical membrane of, liver and kidney cells [15]. The studies were performed in a human proximal tubule cell line (HK-2), and the results suggested an impact of *Z. punctata* extract and some of its flavonoid components on drug pharmacokinetics, which are P-gp substrates, as well as a potential role on multidrug resistance modulation [7c].

Genotoxic/antigenotoxic effect: The hydroethanolic extract of *Z. punctata* and DHC, the major bioactive compound isolated from it, were investigated for genotoxicity/antigenotoxicity in the *in vitro* Comet assay test on human hepatoma HepG2 cells. Results indicated that the extract of *Z. punctata*, under the experimental conditions tested, neither affected cell viability nor induced DNA

damage, and the extract protected HepG2 cells against DNA damage induced by the direct-acting genotoxic compound (4-nitroquinoline-*N*-oxide). The antigenotoxic effect observed could be accounted by the presence of DHC [6e]. This genotoxicological study suggests that the *Z. punctata* antigenotoxic properties are of great pharmacological importance and might be beneficial for cancer prevention.
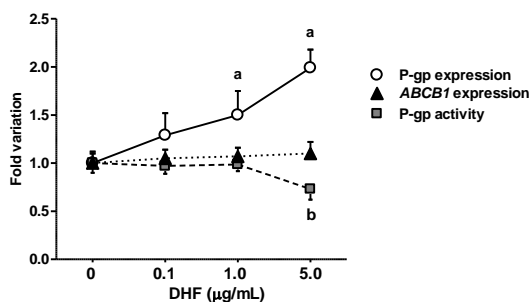

Figure 4: Correlations among the changes in P-gp amount, ABCB1 mRNA amount and P-gp activity in HK-2 cultured for three days in the presence of 3,7-DHF. Values represent the means (expressed as percent of control) ± S.D. of at least three independent experiments. a, b mean significant differences compared with control group.

Toxicity studies in vivo: The possible hepatotoxic and nephrotoxic effects of *Z. punctata* extract (1mg/mice) were analyzed in mice with and without streptococcal infections. In both cases, the activities of alanine transaminase (ALT) and aspartate transaminase (AST) enzymes and the levels of creatinine and urea in blood were not changed by *Z. punctata* extract as compared with the control values. Therefore, the report showed that intake once or twice a day of 1 mg of plant extract for seven days did not result in toxicity [6f].

Zuccagnia punctata as botanical origin of Argentine propolis: It is known that the chemical composition of propolis, the natural product made by bees collecting vegetal resin, depends mainly on the phytogeographic characteristics of the collection propolis site for its botanical origin. *Z. punctata* was assigned as the propolis botanical origin from Amaicha del Valle, Tucuman, Argentina [7b,16a-c].

In summary, *Z. punctata* aerial parts have several pharmacological activities, but to date there appears to be no published research on human clinical trials.

Galenic forms

A) *Decoction* (dry leaves and stems): two spoons of dry plant material in 100 mL of water. Boil for 15 minutes [17a]. According to the Farmacopea Argentina, decoctions are obtained with 5 g of dry plant material/100 mL water [17b].

B) *Hydroethanolic extract or tincture* (dry leaves and stems): two spoons of dry plant material macerated in ethanol for 3 days with stirring at room temperature [17a]. According to the Farmacopea Argentina, tinctures are obtained with 10 g of dry plant material/100 mL ethanol or ethanol-water for 3 days [17b].

In accordance with the Argentine Regulations from "*Administracion Nacional de Medicamentos, de Alimentos y de Tecnología médica*" (ANMAT-Resolution N° 5418) [17c], *Z punctata* could be considered an herbal medicine of traditional use because it was used as a medicinal plant in Argentina for many years by different communities (Art.2). Taking into account EMA guidelines [17d], the dry extracts of *Z. puntacta* aerial parts could

be used in herbal medicinal products as standardized extracts declaring the quantity of main bioactive compounds or marker compounds (chalcones) or as quantitative extracts.
Contraindications: Traditional uses did not cite any contraindications.

Flowers of *Zuccagnia punctata*

Chemical composition: Flowers were used either directly after harvesting or as dried material by lyophilization. Hydroethanolic extracts of flowers showed a high content of phenolic compounds (166.7 ± 15 mg GAE/g flowers), similar to that of *Z. punctata* aerial parts (168.0 ± 15 mg GAE/g aerial parts). The content of flavones and flavonols was higher in aerial parts (16.0 ± 1.0 mg QE/g) than in flowers (9.0 ± 0.9 mg QE/g flowers). The presence of cinnamic acid, galangin, crysin, DHC and DHMC was demonstrated by TLC, HPLC-DAD, and NMR [2d]. DHMC was the major component of flower extracts, whereas in aerial parts the major component was DHC [18].

Pharmacological activities

Antioxidant capacity: Flower extracts exhibited ABTS$^{\bullet+}$ reducing capacity, with SC_{50} values of 3.8±0.2 µg GAE/mL. In addition, flower extracts were able to protect lipids from oxidation, showing an IC_{50} value of 14.7±0.2 µg GAE/mL [18]. Both identified chalcones would be responsible for antioxidant capacity as demonstrated in previous reports [7a,11].

Anti-inflammatory capacity: The *in vitro* inhibitory activity of two enzymes responsible for the biosynthesis of inflammatory mediators, cyclooxygenase (COX-2) and lipoxygenase (LOX), was used as an indicator of sample anti-inflammatory activity. The phenolic extracts obtained from *Z. punctata* flowers were able to inhibit COX-2 (IC_{50} = 59.7 µg/mL) and LOX (IC_{50} = 49.6 µg/mL) [18].

The author reported that Jarilla flowers could be considered as a new dietary supplement that could help to prevent pathologies associated with oxidative stress, and the polyphenolic extract obtained from them could be considered as a standardized phytotherapeutic product with antioxidant and anti-inflammatory activities [18].

Antimicrobial activity: Flower extracts were active on six *Candida* species, *C. albicans, C. krusei, C. tropicalis, C. glabrata, C. parasilopsis* and *C. guilliermondii* (MIC values between 60 and 120 µg GAE/mL) and on methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin resistant *Staphylococcus* coagulase negative (MRSCN) (MIC values of 250 µg GAE/mL) (Table 2). MIC values against *Enterococcus faecalis* were two-fold higher (MIC values: 500 µg GAE/mL) than those against *S. aureus* (Table 1). The bioautographical assay of *Z. punctata* flower extract on MRSA showed that the extract has two bands with antibacterial and anti-*Candida* activity coincident with the Rfs of chalcones [18].

Toxicity: Flower extracts showed no toxic effect on *Artemia salina.* None of the doses was mutagenic to TA98 or TA100 strains under the conditions used in this assay. This result indicates the absence of mutagens that cause base pair substitution (detected in TA100) and frame-shift (detected in TA98) mutations. The absence of mutagenicity of *Z. punctata* flower extract in the test against *Salmonella* strains indicates that DNA does not seem to be a relevant target [18].

To date there appears to be no published research on human clinical trials of flower extract.

Roots and fruits of *Zuccagnia punctata*

While *Z. punctata* aerial parts have been extensively investigated for phytochemical and pharmacological properties, less research has been undertaken on the flowers, and a single report has been found on biological activity of roots and fruits.

Root extracts in DCM and light petroleum did not exhibit any antifungal activity up to 250 μg/mL, but, in contrast, fruit extracts showed interesting antifungal activities on yeasts such as *C. abicans*, *S. cerevisiae*, and *C. neoformans* (MIC values 62.5-250 μg/mL) and dermatophytes such as *M. gypseum*, *T. rubrum* and *T. mentagrophytes* (MIC values 8-16 μg/mL), [9b], Table 2.

Conclusions: In this review we show a broad medicinal potential of *Zuccagnia punctata* extracts as antifungal, antibacterial, antioxidant, antitumor, nematicidal and anti-inflammatory products, as well as their characterization from a chemical point of view and the determination of main bioactive constituents. *Z. punctata* could be used as dry or liquid extracts or incorporated into other herbal preparations or as an active in pharmaceutical forms (gels, creams, microencapsulates, capsules). While progress has been made in the knowledge of this species, much has still to be done, especially in human clinical trials.

Methodology: This review was carried out by using a systematic literature search on *Zuccagnia punctata* Cav. Searches were conducted via the databases PubMed (http://www.ncbi.nlm.nih.gov/pubmed), Scopus (http://www.scopus.com), Scirus (http://www.scirus.com), Google Scholar (http://scholar.google.com), Science Direct (http://www.sciencedirect.com) and http://www.theplantlist.org/; Flora Argentina and Flora del Conosur. Searches were also made using key word combinations: Jarilla, *Zuccagnia punctata*, biological activities, phytochemicals, toxicity, 2´,4´-dihydroxy chalcone, 2´,4´-dihydroxy-3´-methoxychalcone, phytomedicine, antibacterial, antifungal, antioxidant, antibacterial, anti-inflammatory, chemopreventive, antiulcer, between others. Publications were considered up to the end of December 2015.

References

[1]   (a) Ulibarri EA. (**2005**) *Zuccagnia punctata* (Leguminosae): nuevo o viejo endemismo argentino? *Darwiniana*, **43**, 212-215; (b) Zuloaga FO, Morrone O. (**1999**) Catálogo de las Plantas Vasculares de la República Argentina II. Fabaceae-Zygophyllaceae (Dicotyledoneae). *Monography Systematic Botany. Missouri Botany Garden*, **74**, 623-1269. (c) Cabrera AL. (**1976**) Regiones Fitogeográficas Argentinas. In *Enciclopedia Argentina de Agricultura y Jardinería*. Segunda edición. Kugler WF. (Ed). ACME, Bs. As., Argentina, pp. 85; (d) Cabrera AL, Willink A (**1973**) *Biogeografía de América Latina*. O.E.A. Serie de Biología, monografía. Washington, D.C., EUA, pp. 120; (e) Burkart R, Bárbaro NO, Sánchez RO, Gómez DA. (**1999**) *Eco-regiones de la Argentina*. Secretaría de Recursos Naturales y Desarrollo Sustentable, Bs. As., pp. 42.

[2]   (a) Lersten NR, Curtis JD. (**1996**) Survey of leaf anatomy, especially secretory structures, of tribe Caesalpinieae (Leguminosae, Caesalpinioideae). *Plant Systematic Evolution*, **200**, 1-39; (b) Mercado MI, Ruiz AI, Zampini IC, Nuño G, Cuello S, Isla MI, Ponessa GI. (**2013**) Arquitectura y morfoanatomía foliar y caulinar de *Zuccagnia punctata* (Fabaceae). Histolocalización de compuestos bioactivos. *Lilloa*, **50**, 58-68; (c) Álvarez SL, Cortadi A, Juárez MA, Petenatti E, Tomi F, Casanova J, van Baren CM, Zacchino S, Vila R. (**2012**) (−)-5,6-Dehydrocamphor from the antifungal essential oil of *Zuccagnia punctata*. *Phytochemistry Letters*, **5**, 194-199; (d) Moreno MA, Nuño G, Cuello S, Zampini IC, Mercado M, Ponessa G, Sayago JE, Isla MI. (**2015**) Histochemical localization and characterization of chalcones in foliar surface of *Zuccagnia punctata* Cav. Insight into their physiological role. *Phytochemistry Letters, 13,* 134-140.

[3]   (a) Ratera EL, Ratera MO. (**1980**) Plantas de la flora argentina empleadas en medicina popular. Hemisferio Sur, Buenos Aires, Argentina, pp. 189; (b) Toursarkissian M. (**1980**) Plantas medicinales de la Argentina; sus nombres botánicos, vulgares, usos y distribución geográfica. Hemisferio Sur, S.A. (Ed), Buenos Aires, Argentina, pp. 178; (c) Ortega CA, María AOM, Gianello JC. (**2000**) Chemical components and biological activity of *Bidens subalternans*, *B. aurea* (Asteraceae) and *Zuccagnia puntacta* (Fabaceae). *Molecules*, **5**, 465-470.

[4]   (a) Barboza GE, Cantero JJ, Nuñez C, Pacciaroni A, Ariza Espinar L. (**2009**) Medicinal plants: a general review and a phytochemical and ethnopharmacological screening of the native Argentine Flora. *Kurtziana*, **32**, 7-365; (b) Burkart A. (**1952**) Las Leguminosas argentinas, silvestres y cultivadas. Segunda edición. ACME, Bs. As., Argentina, pp. 590; (c) Del Vitto LA, Petenatti EM, Petenatti ME. (**1997**) Recursos herbolarios de San Luis (República Argentina) Primera parte: Plantas Nativas. *Multequina*, **6**, 49-66.

[5]   (a) Hieronymus J. (**1982**) Plantae diaforicae florae argentinae. *Boletín Academisco Nacional de Ciencias*, **4**, 200-598; (b) Leonforte J (**1894**) Ligeros apuntes de la flora puntana. La agricultura. Buenos Aires, Argentina.

[6]   (a) Pederiva R, Kavka J, D'Arcangelo AT. (**1975**) Chalconas y flavanonas aisladas de *Larrea nitida* Cav. *Annales de la Asociación Química Argentina*, **63**, 85-90; (b) Pederiva R, Giordano O. (**1984**) 3,7-Dihydroxy-8-methoxyflavone from *Zuccagnia punctata*. *Phytochemistry*, **23**, 1340-1341; (c) Svetaz L, Tapia A, López S, Furlán R, Petenatti E, Pioli R, Schmeda- Hirschmann G, Zacchino S. (**2004**) Antifungal chalcones and new caffeic acid esters from *Zuccagnia punctata* acting against soybean infecting fungi. *Journal of Agricultural and Food Chemistry*, **52**, 3297-3300; (d) Zampini IC, Vattuone MA, Isla MI. (**2005**) Antibacterial activity of *Zuccagnia punctata* Cav. ethanolic extracts. *Journal of Ethnopharmacology*, **102**, 450-456; (e) Zampini IC, Villarini M, Moretti M, Dominici L, Isla MI. (**2008**) Evaluation of genotoxic and antigenotoxic effects of hydroalcoholic extracts of *Zuccagnia punctata* Cav. *Journal of Ethnopharmacology, 115*, 330-335; (f) Zampini IC, Villena J, Salva S, Herrera M, Isla MI, Alvarez S. (**2012**) Potentiality of standardized extract and isolated flavonoids from *Zuccagnia punctata* for the treatment of respiratory infections by *Streptococcus pneumoniae*: *In vitro* and *in vivo* studies. *Journal of Ethnopharmacology, 140*, 287-292.

[7]   (a) Morán Vieyra F, Boggetti H, Zampini I, Ordoñez R, Isla M, Alvarez R, De Rosso V, Mercadante A, Borsarelli C. (**2009**) Singlet oxygen quenching and radical scavenging capacities of structurally related flavonoids present in *Zuccagnia punctata* Cav. *Free Radical Research*, **43**, 553-564; (b) Agüero MB, González M, Lima B, Svetaz L, Sanchez M, Zacchino S, Feresin G, Schmeda-Hirschmann G, Palermo J, Wunderlin D, Tapia A. (**2010**) Argentinean propolis from *Zuccagnia punctata* Cav. (Caesalpinieae) exudates: Phytochemical characterization and antifungal activity. *Journal of Agricultural and Food Chemistry*, **58**, 194-201; (c) Chieli E, Romiti N, Zampini IC, Garrido G, Isla MI. (**2012**) Effects of *Zuccagnia punctata* extracts and their flavonoids on the function and expression of ABCB1/P-glycoprotein multidrug transporter. *Journal of Ethnopharmacology*, **144**, 797-801; (d) Nuño G, Alberto M, Zampini I, Cuello S, Ordoñez R, Sayago J, Baroni V, Wunderlin D, Isla MI. (**2014**) The effect of *Zuccagnia punctata* Cav, an Argentina medicinal plant, on virulence factors from *Candida* species. *Natural Product Communications*, **9**, 933-936; (e) Nuño G. (**2015**) Doctoral thesis. Aislamiento y caracterización de biomoléculas producidas por especies vegetales que crecen en ecosistemas semiáridos. Universidad Nacional de Tucumán.

[8]   (a) Ni L, Meng CQ, Sikorski JA. (**2004**) Recent advances in therapeutic chalcones. *Expert Opinion on Therapeuthic Patents*, **14**, 1669-1691; (b) Batovska DI, Todorova IT. (**2010**) Trends in utilization of the pharmacological potential of chalcones. *Current Clinical Pharmacology, 5*, 1-29; (c) Butassi E, Svetaz LA, Ivancovich JJ, Feresin GE, Tapia A, Zacchino SA. (**2015**) Synergistic mutual potentiation of antifungal activity of *Zuccagnia punctata* Cav. and *Larrea nitida* Cav. extracts in clinical isolates of *Candida albicans* and *Candida glabrata*. *Phytomedicine*, **22**, 666-578.

[9]   (a) Cooke J. (**2004**) Infectious diseases- the need for new antibiotics. *Hospital Pharmacist*, **11**, 265-268; (b) Clinical and Laboratory Standards Institute, CLSI (**2006**) Performance standards for antimicrobial susceptibility testing; 17th Informational Supplement M100-S17 Wayne, USA.

[10]   (a) Tangarife-Castaño V, Correa-Royero J, Zapata-Londono B, Durán C, Stanshenko E, Mesa-Arango AC. (**2011**) Anti *Candida albicans* activity, cytotoxicity and interaction with antifungal drugs of essential oils and extracts from aromatic and medicinal plants. *Infection*, **15**, 160-167; (b) Svetaz L, Agüero MB, Alvarez S, Luna L, Feresin G, Derita M, Tapia A, Zacchino S. (**2007**) Antifungal activity of chalcones from *Zuccagnia punctata* Cav. acting against clinically important fungi and studies of mechanism of action. *Planta Medica*, **73**, 1074-1080; (c) Svetaz L, Zuljan F, Derita M, Petenatti E, Tamayo G, Cáceres A, Cechinel Filho V, Giménez A, Pinzón R, Zacchino SA, Gupta M. (**2010**) Value of the ethnomedical information for the discovery of plants with antifungal properties. A survey among seven Latin American countries. *Journal of Ethnopharmacology*, **127**, 137-158; (d) Lahtchev KL, Batovska DI, Parushev StP, Ubiyvovk VM, Sibirny AA. (**2008**) Antifungal activity of chalcones: A mechanistic study using various yeast strains. *European Journal of Medicinal Chemistry*, **43**, 2220-2228; (e) Quiroga EN, Sampietro AR, Vattuone MA. (**2001**) Screening antifungal activities of selected medicinal plants. *Journal of Ethnopharmacology*, **74,** 89-96; (f) Jimenez CM, Sampietro DA, Sgariglia MA, Soberón JR, Vattuone MA. (**2014**) Isolation, identification and usefulness of antifungal compounds from *Zuccagnia punctata* for control of toxigenic ear rot pathogens. *Natural Product Communications*, **9**, 1461-1464.

[11]   Avila V, Bertolotti SG, Criado S, Pappano N, Debattista N, García NA. (**2001**) Antioxidant properties of natural flavonoids: Quenching and generation of singlet molecular oxygen. *Journal of Food Science and Technology*, **36**, 25-33.

[12]   (a) De la Rocha N, María AOM, Gianello JC, Pelzer L. (**2003**) Cytoprotective effects of chalcones from *Zuccagnia punctata* and melatonin on gastroduodenal tract in rats. *Pharmacology Research*, **48**, 97-99; (b) Falcao HS, Mariath IR, Diniz MFFM, Batista LM, Barbosa-Filho JM. (**2008**) Plants of the American continent with antiulcer activity. *Phytomedicine*, **15**, 132-146.

[13]   (a) Martin D, Rojo A, Salinas M, Díaz R, Gallardo G, Alam J, Ruiz de Galarreta CM, Cuadrado A. (**2004**) Regulation of heme oxygenase-1 expression through the phosphatidylinositol 3-kinase/akt pathway and the nrf-2 transcription factor in response to the antioxidant phytochemical carnosol. *Journal of Biological Chemistry*, **279**, 8919-8929; (b) Lee JL, Mukhtar H, Bickers D R, Kopelovich L, Athar M. (**2003**) Cyclooxygenases in the skin: pharmacological and toxicological implications. *Toxicology and Applied Pharmacology*, **192**, 294-306; (c) Alberto MR, Nieva Moreno MI, Zampini IC, Isla MI. (**2007**) Anti-inflammatory activity of structurally related natural flavonoids. *Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas*, **6**, 308-309.

[14]   (a) D'Almeida RE, Alberto MR, Morgan P G, Sedensky MM, Isla MI. (**2015**) Effect of structurally related flavonoids from *Zuccagnia punctata* Cav. on *Caenorhabditis elegans*. *Acta Parasitologica*, **60**, 164-172.

[15]   Alvarez AI, Real R, Pérez M, Mendoza G, Prieto JG, Merino G. (**2010**) Modulation of the activity of ABC transporters (P-glycoprotein, MRP2, BCRP) by flavonoids and drug response. *Journal of Pharmaceutical Sciences*, **99**, 598-617.

[16]   (a) Solórzano E, Vera N, Cuello S, Ordoñez R, Zampini C, Maldonado L, Bedascarrasbure E, Isla MI. (**2012**) Chalcones in bioactive Argentine propolis collected in arid environments. *Natural Product Communications*, **7**, 879-882; (b) Isla MI, Nieva Moreno MI, Zampini IC, Solórzano E, Danert F, Vera N, Sayago JE, Bedascarrabure E, Maldonado L, Ordoñez R. (**2013**) Argentine propolis: Its flavonoid and chalcone content and its relation with the functional properties. In *Beneficial effects of propolis on human health and chronic diseases*. Farooqui T, Farooqui A. (Eds). Nova Science Publisher, **8**, 161-169; (c) Salas A, Mercado MI, Zampini IC, Ponessa GI, Isla MI. (**2016**) Determination of botanical origin of propolis from Monte region of Argentina by histological techniques and chemical methods. *Natural Product Communications*, **11**, 627-630.

[17]   (a) Alonso J, Desmarchelier C. (**2005**) Plantas medicinales autóctonas argentinas. Bases científicas para su aplicación en atención primaria de la salud. Editorial LOLA. Argentina, pp. 466-469; (b) Farmacopea Argentina. Codex Medicamentarius Argentino (**2003**), *1*, 7ta ed. Buenos Aires, Argentina; (c) Resolución N° 5418 ANMAT *Administracion Nacional de Medicamentos, Alimentos y Tecnología Médica*; (d) EMA (**2010**) Guideline on declaration of herbal substances and herbal preparations in herbal medicinal products/traditional herbal medicinal products. Available at http://www.ema.europa.eu/docs/en_ GB/document_library/Scientific_ guideline/ 2009/09/WC500003272.pdf. Accesed in December 2015.

[18]   Moreno A, Nuño G, Cuello S, Sayago J, Alberto MR, Zampini C, Isla MI. (**2015**) Anti-inflammatory, antioxidant and antimicrobial activity characterization and toxicity studies of flowers of "Jarilla", a medicinal shrub from Argentina. *Natural Product Communications*, **6**, 991-994.